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In wie ferne kann man das Holz als ein isotroper Körper betrachten?

VON

Prof. Dr. D. Kitao.

Wir wollen jetzt die Componenten der elastischen Druckkräfte + mittlst der Neumann'schen Methode*

$$X_x Y_y Z_z \quad X_z Y_x Z_y$$

also Functionen der Verschiebungscomponenten u, v, w darzustellen suchen und dabei untersuchen, wie ein krystallinischer Körper, wie Holz als ein isotroper Körper betrachtet werden kann.

Die Kräfte welche die Theilchen eines dilatirenden Körpers in ihre Gleichgewichtlage zurückzuführen streben und als Drückkräfte wirken haben ihren Grund in den Kräften, welche zwischen den Theilchen wirken und so beschaffen sind, dass sie nur dann wirken, wenn die Entfernung der Theilchen klein ist und die Theilchen selbst aus der natürlichen Gleichgewichtlage verschoben worden sind.

Unter dem natürlichen Zustand eines Körpers verstehen wir den Zustand, dass ohne Einwirkung äusserer Kräfte alle im Inneren des Körpers thätige Kräfte sich gegenseitig aufheben.

Es seien die Coordinaten eines Massentheilchen dm x, y, z , die des zweiten Massentheilchen dm' aber $x+\xi, y+\eta, z+\zeta$, und es wirke auf den Punkt (xyz) die Kraft

$$dm \, dm' f(\rho)$$

längs der Linie

$$\rho = \sqrt{\xi^2 + \eta^2 + \zeta^2}$$

die Resultate aller auf dm wirkenden Kräfte ist

$$= dm \int f(\rho) \, dm'$$

wo das Integral so weit zu erstrecken ist, als $f(\rho)$ nicht verschwindet. Es ist sonach im natürlichen Zustande

* Franz Neumann: Theorie der Elasticität pag. '82.

$$o = dm \int dm' f(\rho) \frac{\xi}{\rho} = dm \int dm' f(\rho) \frac{\eta}{\rho} = dm \int dm' f(\rho) \frac{\xi}{\rho}$$

Nun erleiden die Theilchen (xyz) und $n + \xi$, $y + \eta$, $z + \zeta$ relative Verrückung, deren Componenten durch

$$\xi' \quad \eta' \quad \zeta'$$

bezeichnet werden mögen. Die Componenten der auf dm ausgeübten Molecularkräfte sind

$$\begin{aligned} dm \int A \, dm' &= dm \int dm' f(\rho + \rho') \frac{\xi + \xi'}{\rho + \rho'} \\ dm \int B \, dm' &= dm \int dm' f(\rho + \rho') \frac{\eta + \eta'}{\rho + \rho'} \\ dm \int \Gamma \, dm' &= dm \int dm' f(\rho + \rho') \frac{\xi + \xi'}{\rho + \rho'} \end{aligned}$$

wo ρ' durch

$$\rho + \rho' = \sqrt{(\xi + \xi')^2 + (\eta + \eta')^2 + (\zeta + \zeta')^2}$$

definiert ist, oder mit Vernachlässigung unendlich kleiner Grösse höherer Ordnung

$$\rho \rho' = \xi \xi' + \eta \eta' + \zeta \zeta'$$

mit demselben Grade der Annäherung geschrieben werden kann

$$\begin{aligned} f(\rho + \rho') \left(\frac{\xi + \xi'}{\rho + \rho'} \right) &= (f(\rho) + \rho' f'(\rho)) \left(\frac{\xi + \xi'}{1 + \frac{\rho'}{\rho}} \right) \\ &= \left(f(\rho) + \rho' f'(\rho) \right) (\xi + \xi') \left(1 - \frac{\rho'}{\rho} \right) \\ &= \frac{\rho}{\xi} f(\rho) + \frac{f(\rho)}{\rho} \xi' + \frac{1}{\rho} \left(f(\rho) - \frac{f(\rho)}{\rho^2} \right) \rho' \xi' \\ &= \frac{\xi}{\rho} f(\rho) + \frac{f(\rho)}{\rho} \xi' + F(\rho) \xi' \end{aligned}$$

Wo

$$F(\rho) = \frac{1}{\rho} \frac{df(\rho)}{d\rho}$$

gesetzt worden ist, so folgt.

$$\begin{aligned} dm \int A \, dm' &= dm \int dm' \left(\frac{\xi}{\rho} f(\rho) + \frac{f(\rho)}{\rho} \xi' + \xi F(\rho) \rho \rho' \right) \\ dm \int B \, dm' &= dm \int dm' \left(\frac{\eta}{\rho} f(\rho) + \frac{f(\rho)}{\rho} \eta' + \eta F(\rho) \rho \rho' \right) \end{aligned}$$

In wie fern kann man das Holz.

$$dm \int \Gamma dm' = dm \int dm' \left(\frac{\xi}{\rho} f(\rho) + \frac{f(\rho)}{\rho} \zeta' + \zeta F(\rho) \rho \rho' \right)$$

Es werde ferner auf die Oberfläche des Körpers ein Druck ausgeübt, dessen Componenten für die Flächenheit

$$\Xi H Z$$

heissen. Es seien ferner $\partial u \partial v \partial w$ unendlich kleine virtuellen Verrückungen $\bar{\partial} u \bar{\partial} v \bar{\partial} w$ ihre Werthe an der Oberfläche, deren Elemente $d\omega$ sein möge, das Princip der virtuellen Verrückung giebt.

$$\begin{aligned} \int (\Xi \bar{\partial} v + H \bar{\partial} v + Z \bar{\partial} w) d\omega \\ + \int dm \int dm' A \partial u \\ + \int dm \int dm' B \partial w \\ + \int dm \int dm' \Gamma \partial w = 0 \end{aligned}$$

Mit dem Integral

$$\int dm \int dm' A \partial u$$

nehmen wir eine wichtige Umformung vor. In diesem Integral kommt die Wirkung des dm auf dm' als diejenige des dm' auf dm vor, und beide Wirkungen unterscheiden sich im Vorzeichen. Die Coordinaten

$$x + \xi \quad y + \eta \quad z + \zeta$$

verwandeln nach der Verschiebung u, v, w in

$$\begin{aligned} x + \xi + u + \frac{\partial u}{\partial x} \xi + \eta \frac{\partial u}{\partial y} \zeta + \frac{\partial u}{\partial z} \\ y + \eta + v + \frac{\partial v}{\partial x} \xi + \eta \frac{\partial v}{\partial y} \zeta + \frac{\partial v}{\partial z} \\ z + \zeta + w + \frac{\partial w}{\partial x} \xi + \eta \frac{\partial w}{\partial y} + \zeta \frac{\partial w}{\partial z} \end{aligned}$$

Da $\xi \eta \zeta$ unendlich klein sein sollen, die Grössen

$$\begin{aligned} \xi \frac{\partial u}{\partial x} + \eta \frac{\partial u}{\partial y} + \zeta \frac{\partial u}{\partial z} \\ \xi \frac{\partial v}{\partial x} + \eta \frac{\partial v}{\partial y} + \zeta \frac{\partial v}{\partial z} \end{aligned}$$

$$\xi \frac{\partial \tau w}{\partial x} + \eta \frac{\partial \tau w}{\partial y} + \zeta \frac{\partial \tau w}{\partial z}$$

sind nichts anderes, als die Componenten der Verschiebung, welche die beiden Punkte $x y z$, und $x + \xi y + \eta, z + \zeta$ nach der stattgehabten Verschiebung gegen einander erhalten haben d. h.

$$\xi' = \xi \frac{\partial u}{\partial x} + \eta \frac{\partial u}{\partial y} + \zeta \frac{\partial u}{\partial z}$$

$$\eta' = \xi \frac{\partial v}{\partial x} + \eta \frac{\partial v}{\partial y} + \zeta \frac{\partial v}{\partial z}$$

$$\zeta' = \xi \frac{\partial \tau w}{\partial x} + \eta \frac{\partial \tau w}{\partial y} + \zeta \frac{\partial \tau w}{\partial z}$$

Es ist so dann

$$\int dm' f(\rho) \rho' = \int dm' f \frac{(\rho)}{\rho} \left(\xi \frac{\partial u}{\partial x} + \eta \frac{\partial v}{\partial y} + \zeta \frac{\partial \tau w}{\partial z} + \xi \eta \left(\frac{\partial u}{\partial z} + \frac{\partial v}{\partial y} \right) + \eta \zeta \left(\frac{\partial v}{\partial z} + \frac{\partial \tau w}{\partial y} \right) + \zeta \xi \left(\frac{\partial \tau w}{\partial x} + \frac{\partial u}{\partial z} \right) \right)$$

oder indem wir setzen

$$\lambda_{11} = \int dm' \xi^2 \frac{f(\rho)}{\rho}$$

$$\lambda_{12} = \int dm' \xi \eta \frac{f(\rho)}{\rho}$$

$$\lambda_{13} = \int dm' \xi \zeta \frac{f(\rho)}{\rho}$$

$$\lambda_{21} = \int dm' \eta \xi \frac{f(\rho)}{\rho}$$

$$\lambda_{22} = \int dm' \eta^2 \frac{f(\rho)}{\rho}$$

$$\lambda_{23} = \int dm' \eta \zeta \frac{f(\rho)}{\rho}$$

$$\lambda_{31} = \int dm' \zeta \xi \frac{f(\rho)}{\rho}$$

$$\lambda_{32} = \int dm' \zeta \eta \frac{f(\rho)}{\rho}$$

$$\lambda_{33} = \int dm' \zeta^2 \frac{f(\rho)}{\rho}$$

Wo

$$\lambda_{12} = \lambda_{21} \quad \lambda_{13} = \lambda_{31} \quad \lambda_{23} = \lambda_{32}$$

sind, so folgt

Dehnt man das Integral über den ganzen Körper aus, so ist es offenbar dass wenn wir den Coordinatenzuwachs für dm mit abc , denjenigen für dm' mit $a' b' c'$ bezeichnen

$$\begin{aligned} \int dm \int dm' \Lambda \delta u &= \frac{1}{2} \iiint dm' dm \Lambda (\partial a - \partial a') = \frac{1}{2} \iiint dm dm' \Lambda \partial (a - a') \\ &= \frac{1}{2} \iiint dm dm' \Lambda (\partial \xi') \end{aligned}$$

da ja

$$\xi' = a - a' \quad \eta' = b - b' \quad \zeta' = c - c'$$

sind, wir erhalten somit

$$\begin{aligned} \int d\tau (\Xi \delta u + H \delta v + Z \delta w) \\ + \frac{1}{2} \int dm \int dm' \Lambda \partial \xi' \\ + \frac{1}{2} \int dm \int dm' B \partial \eta' \\ + \frac{1}{2} \int dm \int dm' \Gamma \partial \zeta' \end{aligned}$$

oder in dem wir für $\Lambda B \Gamma$ ihre Ausdrücke einsetzen

$$\begin{aligned} \int (\Xi \overline{\delta u} + H \overline{\delta v} + Z \overline{\delta w}) d\tau \\ + \frac{1}{2} \iiint dm dm' \frac{f(\rho)}{\rho} (\xi \partial \xi' + \eta \partial \eta' + \zeta \partial \zeta') \\ + \frac{1}{2} \iiint dm dm' \frac{f(\rho)}{\rho} (\zeta' \partial \xi' + \eta \partial \eta' + \xi \partial \zeta') \\ + \frac{1}{2} \iiint dm dm' \Gamma(\rho) \rho \rho' (\xi \partial \xi' + \eta \partial \eta' + \zeta \partial \zeta') = 0 \end{aligned}$$

Da nun aber

$$\partial(\rho \rho') = \xi \partial \xi' + \eta \partial \eta' + \zeta \partial \zeta' = \rho \partial \rho'$$

so folgt

$$\begin{aligned} \int d\tau (\Xi \overline{\delta u} + H \overline{\delta v} + Z \overline{\delta w}) \\ + \frac{1}{2} \iiint dm dm' \frac{f(\rho)}{\rho} \rho \rho' \\ + \frac{\partial}{4} \iiint dm dm' \frac{f(\rho)}{\rho} (\xi'^2 + \eta'^2 + \zeta'^2) \end{aligned}$$

$$+\frac{\delta}{4}\iint dm dm' F(\rho)\rho^2\rho'^2=0$$

Es sind die Verschiebungscomponenten des Punktes $x y z, u v w$ sodass seine Coordinaten nach der Verschiebung

$$x+u \quad y+v \quad z+w.$$

sein werden. Es gelangt der Punkt in

$$\begin{aligned} x+u+\xi' \\ y+v+\eta' \\ z+w+\zeta' \end{aligned}$$

Es ist so dann

$$\begin{aligned} \int dm f(\rho)\rho' &= \lambda_{11}\frac{\partial u}{\partial x} + \lambda_{12}\frac{\partial u}{\partial y} + \lambda_{13}\frac{\partial u}{\partial z} \\ &+ \lambda_{21}\frac{\partial v}{\partial x} + \lambda_{22}\frac{\partial v}{\partial y} + \lambda_{23}\frac{\partial v}{\partial z} \\ &+ \lambda_{31}\frac{\partial w}{\partial x} + \lambda_{32}\frac{\partial w}{\partial y} + \lambda_{33}\frac{\partial w}{\partial z} \end{aligned}$$

Da ferner

$$\begin{aligned} \int dm' \frac{f(\rho)}{\rho} (\xi'^2 + \eta'^2 + \zeta'^2) &= \int dm' \frac{f(\rho)}{\rho} \left(\xi^2 \left(\frac{\partial u}{\partial x} \right)^2 + \left(\frac{\partial v}{\partial x} \right)^2 + \left(\frac{\partial w}{\partial x} \right)^2 \right. \\ &+ 2\xi\eta \left(\frac{\partial u}{\partial x} \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \frac{\partial v}{\partial y} + \frac{\partial w}{\partial x} \frac{\partial w}{\partial y} \right) \\ &+ \eta^2 \left(\left(\frac{\partial u}{\partial y} \right)^2 + \left(\frac{\partial v}{\partial y} \right)^2 + \left(\frac{\partial w}{\partial y} \right)^2 \right) \\ &+ 2\eta\zeta \left(\frac{\partial u}{\partial y} \frac{\partial u}{\partial z} + \frac{\partial v}{\partial y} \frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \frac{\partial w}{\partial z} \right) \\ &+ \zeta^2 \left(\left(\frac{\partial u}{\partial z} \right)^2 + \left(\frac{\partial v}{\partial z} \right)^2 + \left(\frac{\partial w}{\partial z} \right)^2 \right) \\ &+ 2\xi\zeta \left(\frac{\partial u}{\partial z} \frac{\partial u}{\partial x} + \frac{\partial v}{\partial z} \frac{\partial v}{\partial x} + \frac{\partial w}{\partial z} \frac{\partial w}{\partial x} \right) \\ &= \lambda_{11} \left(\left(\frac{\partial u}{\partial x} \right)^2 + \left(\frac{\partial v}{\partial x} \right)^2 + \left(\frac{\partial w}{\partial x} \right)^2 \right) \\ &+ \lambda_{12} \left(\left(\frac{\partial u}{\partial y} \right)^2 + \left(\frac{\partial v}{\partial y} \right)^2 + \left(\frac{\partial w}{\partial y} \right)^2 \right) \\ &+ \lambda_{13} \left(\left(\frac{\partial u}{\partial z} \right)^2 + \left(\frac{\partial v}{\partial z} \right)^2 + \left(\frac{\partial w}{\partial z} \right)^2 \right) \\ &+ 2\lambda_{21} \left(\frac{\partial u}{\partial x} \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \frac{\partial v}{\partial y} + \frac{\partial w}{\partial x} \frac{\partial w}{\partial y} \right) \\ &+ 2\lambda_{13} \left(\frac{\partial u}{\partial z} \frac{\partial u}{\partial x} + \frac{\partial v}{\partial z} \frac{\partial v}{\partial x} + \frac{\partial w}{\partial z} \frac{\partial w}{\partial x} \right) \end{aligned}$$

$$+ 2\lambda_{23} \left(\frac{\partial u}{\partial y}, \frac{\partial u}{\partial s} + \frac{\partial v}{\partial y}, \frac{\partial v}{\partial s} + \frac{\partial \tau v}{\partial y}, \frac{\partial \tau w}{\partial s} \right)$$

da ferner

$$\begin{aligned} (\rho \rho')^2 = & \xi^2 \left(\xi^2 \left(\frac{\partial u}{\partial x} \right)^2 + \eta^2 \left(\frac{\partial u}{\partial y} \right)^2 + \zeta^2 \left(\frac{\partial u}{\partial s} \right)^2 + 2 \frac{\partial u}{\partial x}, \frac{\partial u}{\partial y} \xi \eta \right. \\ & + 2 \frac{\partial u}{\partial x}, \frac{\partial x}{\partial s} \eta \eta' + 2 \frac{\partial u}{\partial y}, \frac{\partial u}{\partial s}, \eta \zeta \left. \right) \\ & + \eta^2 \left(\xi^2 \left(\frac{\partial v}{\partial x} \right)^2 + \eta^2 \left(\frac{\partial v}{\partial y} \right)^2 + \zeta^2 \left(\frac{\partial v}{\partial s} \right)^2 + 2 \frac{\partial v}{\partial x}, \frac{\partial v}{\partial y}, \xi \eta \right. \\ & + \left(2 \frac{\partial v}{\partial x}, \frac{\partial v}{\partial s} \xi \eta + 2 \frac{\partial v}{\partial y}, \frac{\partial v}{\partial s}, \eta \zeta \right) \\ & \left. \zeta^2 \left(\xi^2 \left(\frac{\partial \tau v}{\partial x} \right)^2 + \eta^2 \left(\frac{\partial \tau v}{\partial y} \right)^2 + \xi^2 \left(\frac{\partial \tau w}{\partial s} \right)^2 + 2 \frac{\partial \tau w}{\partial x}, \frac{\partial \tau w}{\partial y}, \right. \right. \\ & + 2 \frac{\partial \tau w}{\partial x}, \frac{\partial v}{\partial s}, \zeta \xi + 2 \frac{\partial \tau w}{\partial y}, \frac{\partial \tau w}{\partial s}, \eta \zeta \left. \right) \\ & + 2 \xi \eta \left(\xi^2 \frac{\partial u}{\partial x} \frac{\partial v}{\partial x} + \xi \eta \frac{\partial u}{\partial y}, \frac{\partial v}{\partial x} + \zeta \xi \frac{\partial u}{\partial s}, \frac{\partial v}{\partial s} \right. \\ & + \xi \eta \frac{\partial u}{\partial x}, \frac{\partial v}{\partial y} + \eta^2 \frac{\partial u}{\partial y}, \frac{\partial v}{\partial y} + \eta \xi \frac{\partial v}{\partial y}, \frac{\partial u}{\partial s} \left. \right) \\ & + \zeta \xi \frac{\partial u}{\partial x}, \frac{\partial v}{\partial s} + \eta \zeta \frac{\partial u}{\partial y}, \frac{\partial v}{\partial y} + \xi^2 \frac{\partial u}{\partial s}, \frac{\partial v}{\partial s} \left. \right) \\ & + 2 \xi \zeta \left(\xi^2 \frac{\partial u}{\partial x}, \frac{\partial u}{\partial x} + \xi \eta \frac{\partial u}{\partial y} \frac{\partial w}{\partial x} + \xi \zeta \frac{\partial u}{\partial s} \frac{\partial v}{\partial s} \right) \\ & + \xi \eta \frac{\partial u}{\partial x}, \frac{\partial \tau v}{\partial y} + \eta^2 \frac{\partial u}{\partial y} \frac{\partial \tau w}{\partial y} + \eta \zeta \frac{\partial u}{\partial y} \frac{\partial \tau w}{\partial s} \left. \right) \\ & + \zeta \xi \frac{\partial u}{\partial x}, \frac{\partial \tau w}{\partial s} + \eta \xi \frac{\partial u}{\partial y}, \frac{\partial \tau v}{\partial s} + \xi^2 \frac{\partial u}{\partial s}, \frac{\partial \tau w}{\partial s} \left. \right) \\ & + 2 \xi \zeta \left(\xi^2 \frac{\partial v}{\partial x}, \frac{\partial \tau w}{\partial x} + \xi \eta \frac{\partial v}{\partial y}, \frac{\partial \tau w}{\partial x} + \xi \zeta \frac{\partial v}{\partial s}, \frac{\partial \tau w}{\partial s} \right. \\ & + \xi \eta \frac{\partial v}{\partial x}, \frac{\partial \tau v}{\partial x} + \xi \eta \frac{\partial v}{\partial y}, \frac{\partial \tau v}{\partial x} + \xi \zeta \frac{\partial v}{\partial s}, \frac{\partial \tau v}{\partial s} \left. \right) \\ & + \eta \xi \frac{\partial v}{\partial s}, \frac{\partial \tau w}{\partial x} + \eta^2 \frac{\partial v}{\partial y} \frac{\partial \tau w}{\partial y} + \eta \zeta \frac{\partial v}{\partial y}, \frac{\partial \tau v}{\partial s} \left. \right) \\ & \left. \zeta \xi \frac{\partial v}{\partial x}, \frac{\partial \tau v}{\partial s} + \eta \zeta \frac{\partial v}{\partial y}, \frac{\partial \tau w}{\partial s} + \xi^2 \frac{\partial v}{\partial s}, \frac{\partial \tau w}{\partial s} \right) \end{aligned}$$

indem man die Multiplication ausführt, und nach dem ξ^4 und η^4 und etc ordnet.

$$\rho \rho' = \xi^4 \left(\frac{\partial u}{\partial x} \right)^2 + \eta^4 \left(\frac{\partial v}{\partial y} \right)^2 + \xi^4 \left(\frac{\partial \tau w}{\partial s} \right)^2$$

$$\begin{aligned}
& + \xi^3 \eta^2 \left(\left(\frac{\partial u}{\partial y} \right)^2 + \left(\frac{\partial v}{\partial y} \right)^2 + 2 \frac{\partial v}{\partial y}, \frac{\partial v}{\partial y} + 2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial y} \right) \\
& + \xi^2 \eta^2 \left(\left(\frac{\partial u}{\partial z} \right)^2 + \left(\frac{\partial v}{\partial x} \right)^2 + 2 \frac{\partial u}{\partial z}, \frac{\partial v}{\partial z} + 2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial z} \right) \\
& + \eta^2 \xi^2 \left(\left(\frac{\partial v}{\partial z} \right)^2 + \left(\frac{\partial v}{\partial y} \right)^2 + 2 \frac{\partial v}{\partial z}, \frac{\partial v}{\partial y} + 2 \frac{\partial v}{\partial y}, \frac{\partial v}{\partial z} \right) \\
& + \xi^3 \eta \left(2 \frac{\partial u}{\partial x}, \frac{\partial u}{\partial y} + 2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial x} \right) \\
& + \xi^3 \xi \left(2 \frac{\partial u}{\partial z}, \frac{\partial u}{\partial z} + 2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial x} \right) \\
& + \eta^3 \xi \left(2 \frac{\partial v}{\partial y}, \frac{\partial v}{\partial y} + 2 \frac{\partial v}{\partial z}, \frac{\partial v}{\partial z} \right) \\
& + \xi^3 \xi \left(2 \frac{\partial v}{\partial x}, \frac{\partial v}{\partial z} + 2 \frac{\partial u}{\partial z}, \frac{\partial v}{\partial z} \right) \\
& + \xi^3 \eta \left(2 \frac{\partial v}{\partial y}, \frac{\partial v}{\partial z} + 2 \frac{\partial v}{\partial z}, \frac{\partial v}{\partial z} \right) \\
& + \xi^2 \xi \eta \left(2 \frac{\partial u}{\partial y}, \frac{\partial u}{\partial z} + 2 \frac{\partial u}{\partial z}, \frac{\partial v}{\partial x} + 2 \frac{\partial u}{\partial y}, \frac{\partial v}{\partial z} + 2 \frac{\partial v}{\partial x}, \frac{\partial v}{\partial z} \right. \\
& \quad \left. + 2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial z} + 2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial y} \right) \\
& + \eta^2 \xi \xi \left(2 \frac{\partial v}{\partial x}, \frac{\partial v}{\partial z} + 2 \frac{\partial v}{\partial y}, \frac{\partial u}{\partial z} + 2 \frac{\partial u}{\partial y}, \frac{\partial v}{\partial y} + 2 \frac{\partial u}{\partial y}, \frac{\partial v}{\partial y} \right. \\
& \quad \left. + 2 \frac{\partial u}{\partial y}, \frac{\partial v}{\partial x} + 2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial y} \right) \\
& + \xi^2 \xi \eta \left(2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial y} + 2 \frac{\partial u}{\partial z}, \frac{\partial v}{\partial z} + 2 \frac{\partial v}{\partial y}, \frac{\partial v}{\partial z} + 2 \frac{\partial u}{\partial z}, \frac{\partial v}{\partial y} \right. \\
& \quad \left. + 2 \frac{\partial u}{\partial x}, \frac{\partial v}{\partial z} + 2 \frac{\partial v}{\partial z}, \frac{\partial v}{\partial x} \right)
\end{aligned}$$

Setze man ferner

$$\begin{aligned}
2a_1 &= \int dm' F(\rho) \xi^4 & 2a_2 &= \int dm' F(\rho) \eta^4 & 2a_3 &= \int dm' F(\rho) \xi^4 \\
2a_4 &= \int dm' F(\rho) \xi^3 \eta & 2a_5 &= \int dm' F(\rho) \eta^3 \xi & 2a_6 &= \int dm' F(\rho) \xi^3 \xi \\
2a_7 &= \int dm' F(\rho) \xi \eta^3 & 2a_8 &= \int dm' F(\rho) \xi^2 \eta & 2a_9 &= \int dm' F(\rho) \xi^3 \xi \\
2a_{10} &= \int dm' F(\rho) \xi^2 \eta^2 & 2a_{11} &= \int dm' F(\rho) \xi^2 \xi^2 & 2a_{12} &= \int dm' F(\rho) \xi^3 \eta^2
\end{aligned}$$

$$\begin{aligned}
 2\alpha_{13} &= \int dm' F(\rho) \xi' \eta' \zeta' & 2\alpha_{14} &= \int dm' F(\rho) \xi' \eta'^3 \zeta' & 2\alpha_{15} &= \int dm' F(\rho) \xi' \eta' \zeta'^2 \\
 \int dm' F(\rho) \rho^2 \rho'^2 &= 2\alpha_1 \left(\frac{\partial u}{\partial x} \right)^2 + 2\alpha_2 \left(\frac{\partial v}{\partial y} \right)^2 + 2\alpha_3 \left(\frac{\partial w}{\partial z} \right)^2 \\
 &+ 4\alpha_4 \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \frac{\partial u}{\partial x} + 4\alpha_5 \frac{\partial v}{\partial y} \left(\frac{\partial w}{\partial y} + \frac{\partial v}{\partial z} \right) + 4\alpha_6 \frac{\partial w}{\partial x} \left(\frac{\partial u}{\partial z} + \frac{\partial w}{\partial x} \right) \\
 &+ 4\alpha_7 \left(\frac{\partial v}{\partial y} \left(\frac{\partial v}{\partial y} + \frac{\partial v}{\partial x} \right) \right) + 4\alpha_8 \left(\frac{\partial w}{\partial x} \right) \left(\frac{\partial w}{\partial y} + \frac{\partial v}{\partial z} \right) + 4\alpha_9 \frac{\partial u}{\partial x} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \\
 &+ 2\alpha_{10} \left(\left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right)^2 + 2 \frac{\partial v}{\partial y} \frac{\partial w}{\partial z} \right) + 2\alpha_{11} \left(\left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right)^2 + 2 \frac{\partial u}{\partial x} \frac{\partial w}{\partial z} \right) \\
 &+ 2\alpha_{12} \left(\left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right)^2 + 2 \frac{\partial u}{\partial x} \frac{\partial v}{\partial y} \right) \\
 &+ 4\alpha_{13} \left(\left(\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} \right) \left(\frac{\partial u}{\partial z} + \frac{\partial w}{\partial x} \right) + \frac{\partial u}{\partial x} \left(\frac{\partial w}{\partial y} + \frac{\partial v}{\partial z} \right) \right) \\
 &+ 4\alpha_{14} \left(\left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \left(\frac{\partial w}{\partial y} + \frac{\partial v}{\partial z} \right) + \frac{\partial v}{\partial y} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \right) \\
 &+ 4\alpha_{15} \left(\left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) + \frac{\partial w}{\partial z} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \right)
 \end{aligned}$$

Man setze ferner

$$\partial P = \partial \int dm \int dm' f(\rho) \rho'$$

$$\partial Q = \partial \int dm \int dm' f \frac{(\rho)}{\rho} (\xi'^2 + \eta'^2 + \zeta'^2)$$

$$\partial R = \partial \int dm \int dm' f(\rho) \rho'^2$$

so erhält man

$$\begin{aligned}
 \partial P &= \int dm \left(\lambda_{11} \frac{\partial \bar{\delta} u}{\partial x} + \lambda_{12} \frac{\partial \bar{\delta} u}{\partial y} + \lambda_{13} \frac{\partial \bar{\delta} u}{\partial z} \right. \\
 &\quad + \lambda_{21} \frac{\partial \bar{\delta} v}{\partial x} + \lambda_{22} \frac{\partial \bar{\delta} v}{\partial y} + \lambda_{23} \frac{\partial \bar{\delta} v}{\partial z} \\
 &\quad \left. + \lambda_{31} \frac{\partial \bar{\delta} w}{\partial x} + \lambda_{32} \frac{\partial \bar{\delta} w}{\partial y} + \lambda_{33} \frac{\partial \bar{\delta} w}{\partial z} \right)
 \end{aligned}$$

Setzt man ferner

$$u_1 = \lambda_{11} \frac{\partial u}{\partial x} + \lambda_{12} \frac{\partial u}{\partial y} + \lambda_{13} \frac{\partial u}{\partial z}$$

$$u_2 = \lambda_{21} \frac{\partial u}{\partial x} + \lambda_{22} \frac{\partial u}{\partial y} + \lambda_{23} \frac{\partial u}{\partial z}$$

$$u_3 = \lambda_{31} \frac{\partial u}{\partial x} + \lambda_{32} \frac{\partial u}{\partial y} + \lambda_{33} \frac{\partial u}{\partial z}$$

$$v_1 = \lambda_{11} \frac{\partial v}{\partial x} + \lambda_{21} \frac{\partial v}{\partial y} + \lambda_{13} \frac{\partial v}{\partial z}$$

$$v_2 = \lambda_{21} \frac{\partial v}{\partial x} + \lambda_{22} \frac{\partial v}{\partial y} + \lambda_{23} \frac{\partial v}{\partial z}$$

$$v_3 = \lambda_{31} \frac{\partial v}{\partial x} + \lambda_{32} \frac{\partial v}{\partial y} + \lambda_{33} \frac{\partial v}{\partial z}$$

$$\tau w_1 = \lambda_{11} \frac{\partial \tau w}{\partial x} + \lambda_{12} \frac{\partial \tau w}{\partial y} + \lambda_{13} \frac{\partial \tau w}{\partial z}$$

$$\tau w_2 = \lambda_{21} \frac{\partial \tau w}{\partial x} + \lambda_{22} \frac{\partial \tau w}{\partial y} + \lambda_{23} \frac{\partial \tau w}{\partial z}$$

$$\tau w_3 = \lambda_{31} \frac{\partial \tau w}{\partial x} + \lambda_{32} \frac{\partial \tau w}{\partial y} + \lambda_{33} \frac{\partial \tau w}{\partial z}$$

So erhält man

$$\begin{aligned} \delta Q = \int dm' \left(u_1 \frac{\partial \delta u}{\partial x} + u_2 \frac{\partial \delta u}{\partial y} + u_3 \frac{\partial \delta u}{\partial z} \right. \\ \left. + v_1 \frac{\partial \delta v}{\partial x} + v_2 \frac{\partial \delta v}{\partial y} + v_3 \frac{\partial \delta v}{\partial z} \right. \\ \left. + \tau w_1 \frac{\partial \delta \tau w}{\partial x} + \tau w_2 \frac{\partial \delta \tau w}{\partial y} + \tau w_3 \frac{\partial \delta \tau w}{\partial z} \right) \end{aligned}$$

Setzt man endlich

$$\begin{aligned} \Omega_{11} = a_1 \frac{\partial u}{\partial x} + a_{12} \frac{\partial v}{\partial y} + a_{11} \frac{\partial \tau w}{\partial z} + a_{13} \left(\frac{\partial v}{\partial x} + \frac{\partial \tau w}{\partial y} \right) + a_9 \left(\frac{\partial \tau w}{\partial x} + \frac{\partial u}{\partial z} \right) \\ + a_4 \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial z} \right) \end{aligned}$$

$$\begin{aligned} \Omega_{22} = a_{12} \frac{\partial u}{\partial x} + a_2 \frac{\partial v}{\partial y} + a_{10} \frac{\partial \tau w}{\partial z} + a_5 \left(\frac{\partial v}{\partial z} + \frac{\partial \tau w}{\partial y} \right) + a_{13} \left(\frac{\partial \tau w}{\partial x} + \frac{\partial u}{\partial z} \right) \\ + a_7 \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial z} \right) \end{aligned}$$

$$\begin{aligned} \Omega_{33} = a_{11} \frac{\partial u}{\partial x} + a_{10} \frac{\partial v}{\partial y} + a_{23} \frac{\partial \tau w}{\partial z} + a_8 \left(\frac{\partial v}{\partial z} + \frac{\partial \tau w}{\partial y} \right) + a_6 \left(\frac{\partial \tau w}{\partial x} + \frac{\partial u}{\partial z} \right) \\ + a_{15} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial z} \right) \end{aligned}$$

$$\begin{aligned} \Omega_{13} = \Omega_{22} = a_{13} \frac{\partial u}{\partial x} + a_{15} \frac{\partial v}{\partial y} + a_3 \frac{\partial \tau w}{\partial z} + a_{10} \left(\frac{\partial v}{\partial z} + \frac{\partial \tau w}{\partial y} \right) + a_{15} \left(\frac{\partial \tau w}{\partial x} + \frac{\partial u}{\partial z} \right) \\ + a_{13} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial z} \right) \end{aligned}$$

$$\begin{aligned}\Omega_{13} = \Omega_{31} &= a_9 \frac{\partial u}{\partial x} + a_{14} \frac{\partial v}{\partial y} + a_6 \frac{\partial w}{\partial z} + a_{15} \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) + a_{11} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \\ &\quad + a_{13} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \\ \Omega_{12} = \Omega_{21} &= a_4 \frac{\partial u}{\partial x} + a_7 \frac{\partial v}{\partial y} + a_{15} \frac{\partial w}{\partial z} + a_{14} \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) + a_{13} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \\ &\quad + a_{12} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right)\end{aligned}$$

So erhält man

$$\begin{aligned}\delta R = \int dm' \left(\Omega_{11} \frac{\partial \delta u}{\partial x} + \Omega_{12} \frac{\partial \delta u}{\partial y} + \Omega_{13} \frac{\partial \delta u}{\partial z} \right. \\ \left. + \Omega_{21} \frac{\partial \delta v}{\partial x} + \Omega_{22} \frac{\partial \delta v}{\partial y} + \Omega_{23} \frac{\partial \delta v}{\partial z} \right. \\ \left. + \Omega_{31} \frac{\partial \delta w}{\partial x} + \Omega_{32} \frac{\partial \delta w}{\partial y} + \Omega_{33} \frac{\partial \delta w}{\partial z} \right)\end{aligned}$$

Wir erhalten somit als Gleichgewichtsbedingung

$$\int (\Xi \delta u + H \delta v + Z \delta w) + \frac{1}{2} \delta P + \frac{1}{2} \delta Q + \frac{1}{2} \delta R = 0$$

Wir untersuchen die letzten drei Integrale besonderes, indem wir setzen

$$dm = \mu dx dz dz = \mu d\tau$$

und schreiben in dem Integrale

$$\begin{aligned}\iiint \mu d\tau \lambda_{11} \frac{\partial \delta u}{\partial x} \\ \mu \lambda u \frac{\partial \delta u}{\partial x} = \frac{\partial \mu \lambda_{11} \delta u}{\partial x} - \frac{\partial \mu \lambda_{11}}{\partial x} \delta u\end{aligned}$$

sodass

$$\int \mu \lambda_{11} \frac{\partial \delta u}{\partial x} d\tau = \int \partial \tau \frac{\partial \mu \lambda_{11} \delta u}{\partial x} - \int d\tau \frac{\partial \mu \lambda_{11} \delta u}{\partial x}$$

in dem wir das erste Integral nach dem Gaussischen Satze transformieren, so erhalten wir

$$\int \mu \lambda \mu \frac{\partial \delta u}{\partial x} d\tau = - \int \mu \lambda_{11} \delta u \cos nx - \int d\tau \frac{\partial \mu \lambda_{11} \delta u}{\partial x}$$

so mit allen anderen Integralen

$$\begin{aligned}\delta P = - \int d\omega \mu \left(\overline{\lambda_{11}} \cos nx + \overline{\lambda_{12}} \cos ny + \overline{\lambda_{13}} \cos nz \right) \overline{\delta u} \\ + \left(\overline{\lambda_{21}} \cos nx + \overline{\lambda_{22}} \cos ny + \overline{\lambda_{23}} \cos nz \right) \overline{\delta v}\end{aligned}$$

$$\begin{aligned}
& + \left(\overline{\lambda}_{31} \cos nx + \overline{\lambda}_{32} \cos ny + \overline{\lambda}_{33} \cos nz \right) \overline{\delta\tau v} \\
& - \int d\tau \left\{ \frac{\partial \mu \lambda_{11}}{\partial x} + \frac{\partial \mu \lambda_{12}}{\partial y} + \frac{\partial \mu \lambda_{13}}{\partial z} \right\} \delta u \\
& + \left\{ \frac{\partial \mu \lambda_{11}}{\partial x} + \frac{\partial \mu \lambda_{22}}{\partial y} + \frac{\partial \mu \lambda_{33}}{\partial z} \right\} \delta v \\
& + \left\{ \frac{\partial \mu \lambda_{31}}{\partial x} + \frac{\partial \mu \lambda_{32}}{\partial y} + \frac{\partial \mu \lambda_{33}}{\partial z} \right\}
\end{aligned}$$

Wobei das Oberflächenintegral über die ganze Oberfläche des Körpers auszudehnen ist.

Die Grossen λ sind im Inneren des Körpers constant. Wir können sie aber nicht unmittelbar an der Oberfläche constant vorstellen, da in dem Integrale λ um so mehr Glieder fehlen, je näher der Punkt xyz an der Oberfläche liegt. Die Grösse λ muss also so beschaffen sein, dass sie, da der Wirkungskreis der Molecularkräfte sehr klein ist, zuerst sehr rasch variirt mit wachsender Entfernung von der Oberfläche rasch gegen eine constante Grenze convergirt. Das Raumintegral braucht daher nur über die der Oberfläche unendlich nahe liegende Theile ausgedehnt zu werden, wenn der Körper homogen ist. Das Raumintegral denken wir uns in zwei Teile geteilt, eins über den Theil des Körpers ausgedehnt, innerhalb dessen λ Constant ist, das andere über den übrig bleibenden Teil in dem letzten Integral setzen wir

$$d\tau = d\omega \times dn$$

$$d\tau = d\omega \times dn$$

wo dn ein Element der Normale n bedeutet, $\mu\lambda$ ist demnach eine Function von n , ω . Bei der Kleinheit der Grenzen von n können wir wohl ohne Bedenken, mit Neumann annehmen* dass $\mu\lambda$ eine Function von n allein sei, eine Annahme, die nichts anderes aussagt, also dass $\mu\lambda$ in derselben Tiefe unter der Oberfläche überall denselben Werth habe. Wir können, in sofern dieses erlaubt ist, setzen

$$\frac{\partial \mu \lambda}{\partial x} = \frac{\partial \mu \lambda}{\partial n} \cos(nx)$$

so dass wir schreiben können

$$\int d\tau \frac{\partial \mu \lambda_{11}}{\partial x} \delta u = \int_0^n d\omega \int dn \frac{\partial \mu \lambda_{11}}{\partial n} \delta u \cos nx.$$

* Theorie der Elasticität 96.

wo $n=0$ der äusseren Oberfläche entspricht. Particuläre Integration ergibt

$$= - \int d\omega \delta u \mu \lambda_{11} \cos (nx) - \int d\omega \int dn \mu \lambda_{11} \frac{d\delta \cos nx}{\partial x} \\ + \int d\omega \delta u \mu \lambda_{11} \cos (nx)$$

Mithin erhalten wir

$$\delta P = - \int d\omega \mu \left(\lambda_{11} \cos nx + \lambda_{12} \cos ny + \lambda_{13} \cos (nz) \right) \delta u \\ + \left(\lambda_{21} \cos nx + \lambda_{22} \cos ny + \lambda_{23} \cos (nz) \right) \delta v \\ + \left(\lambda_{31} \cos nx + \lambda_{32} \cos ny + \lambda_{33} \cos (nz) \right) \delta w \\ + \int d\omega \int dn \mu \left(\lambda_{11} \frac{d\delta u \cos nx}{dn} + \lambda_{12} \frac{d\delta u \cos ny}{dn} + \lambda_{13} \frac{d\delta u \cos (nz)}{dn} \right) \\ + \lambda_{21} \frac{d\delta v \cos nx}{dn} + \lambda_{22} \frac{d\delta v \cos ny}{dn} + \lambda_{23} \frac{d\delta v \cos (nz)}{dn} \\ + \lambda_{31} \frac{d\delta w \cos nx}{dn} + \lambda_{32} \frac{d\delta w \cos ny}{dn} + \lambda_{33} \frac{d\delta w \cos (nz)}{dn} \\ - \int d\tau \left(\lambda_{11} \frac{\partial \mu}{\partial x} + \lambda_{12} \frac{\partial \mu}{\partial y} + \lambda_{13} \frac{\partial \mu}{\partial z} \right) + \text{etc}$$

Die Dichtigkeit μ kann innerhalb der Molecularentfernung sich ändern. Wir wollen den Körper so beschaffen vorstellen, dass eine Volumeneinheit überall dieselbe Menge Körper enthalte, d. h. so ist die mittlere Dichtigkeit überall constant und der Körper ist homogen gebaut da ferner λ in dem ersten und dritten Integral wesentlich constant = 0, so verschwinden die Integrale. Wenn wir ferner die Annahme machen, dass man von dem Zuständen der Theilchen die unendlich dünne Schichte der Körperoberfläche absehen könne, dass die Theilchen dort überall dieselbe Verrückung erleiden, als wenn die besagte Schichte starr wäre. So ist die Grösse wie $\delta u \cos (nx)$ von n unabhängig, so verschwindet das zweite Integral. Mithin ist überall

$$\delta P = 0$$

in so fern als der Körper homogen ist, und man von den Zuständen der Theilchen in der Oberfläche abstrahiren kann. Selbst aber, wenn der Körper heterogen ist, so verschwindet δP , da ω Grössen λ_{11} etc in dem inneren Punkt verschwinden, wenn es nur von den Vorgängen in der Oberflächen-

schicht abgesehen werden kann. Für den Ruhezustand des Körpers in seinem natürlichen Gleichgewichte d. h. ohne Äussere Kräfte muss δP unter allen Umständen verschwinden, dann wenn

$$\Xi = 0 \quad H = 0 \quad Z = 0$$

so müssen auch

$$u = 0 = v = \tau v = 0$$

da nun δu δv , $\delta \tau v$ ganz willkürliche Functionen sind, so wohl im Inneren als an der Oberfläche des Körpers, so müssen

$$\lambda_{11} \frac{\partial \mu}{\partial x} + \lambda_{12} \frac{\partial \mu}{\partial y} + \lambda_{13} \frac{\partial \mu}{\partial z} = 0$$

etc

$$\lambda_{11} \cos (nx) + \lambda_{32} \cos (ny) + \lambda_{13} \cos (nz) = 0$$

etc

d. h. aus den letzten drei Gleichungen folgt, allgemein

$$\lambda_{11} = \lambda_{12} = \lambda_{13} = \lambda_{21} = \lambda_{22} = \lambda_{23} = \lambda_{31} = \lambda_{32} = \lambda_{33} = 0$$

wodurch auch die drei ersten Gleichungen befriedigt werden.

Um δQ zu untersuchen nehmen wir dieselbe Transformation vor, in dem wir setzen

$$\mu u_1 \frac{\partial \delta u}{\partial x} = \frac{\partial \mu u_1}{\partial x} \delta u - \frac{\partial \mu u_1}{\partial x} \delta u$$

Mithin

$$\begin{aligned} \delta Q = & - \int \mu d\omega \left(\bar{u}_1 \cos nx + \bar{u}_2 \cos ny + \bar{u}_3 \cos (nz) \right) \delta \bar{u} \\ & + \left(\bar{v}_1 \cos nx + \bar{v}_2 \cos ny + \bar{v}_3 \cos (vz) \right) \delta \bar{v} \\ & + \left(\bar{w}_1 \cos nx + \bar{w}_2 \cos ny + \bar{w}_3 \cos (nz) \right) \delta \bar{w} \\ & - \int d\tau \left(\frac{\partial \mu u_1}{\partial x} + \frac{\partial \mu u_2}{\partial y} + \frac{\partial \mu u_3}{\partial z} \right) \delta u \\ & + \left(\frac{\partial \mu v_1}{\partial x} + \frac{\partial \mu v_2}{\partial y} + \frac{\partial \mu v_3}{\partial z} \right) \delta v \\ & + \left(\frac{\partial \mu w_1}{\partial x} + \frac{\partial \mu w_2}{\partial y} + \frac{\partial \mu w_3}{\partial z} \right) \delta \tau v \end{aligned}$$

das Raumintegral braucht nur über die der Oberfläche unendlich nahe Schichte ausgedehnt zu werden, da u_1 u_2 u_3 etc. für jeden innern Punkt verschwinden.

Wir denken uns an der Oberfläche eine unendliche dünne Schichte von der Dicke n innerhalb deren λ von σ sich unterscheidet, und aus dieser Schichte ein Prisma ausgeschnitten, dessen Grundfläche $d\omega$ unendlich klein, aber unendlich gross gegen die Höhe ist, dieses Prisma soll in seinem Theilchen überall dieselbe Verrückung erleiden d. h. wie ein starrer Körper sich verhalten. Wir bilden dieses Integral über dieses Prisma und vernachlässigen dabei die vier Seitenflächen gegen die beiden Grundflächen da nun $\lambda\mu$ nur von n abhängen soll und in jedem Theil des Prismas u v w dieselben Werthe haben, so hängt μu etc nur von n ab. Es ist daher

$$\begin{aligned}\frac{\partial \mu u_1}{\partial x} &= \frac{\partial \mu u_1}{\partial n} \cos (nx) \\ \frac{\partial \mu u_2}{\partial y} &= \frac{\partial \mu u_2}{\partial n} \cos (ny) \\ \frac{\partial \mu u_3}{\partial z} &= \frac{\partial \mu u_3}{\partial n} \cos (nz)\end{aligned}$$

Und wenn wir wieder setzen

$$d\tau = d\omega \, dn$$

erhalten wir

$$\begin{aligned}\int d\tau \left(\frac{\partial \mu u_1}{\partial x} + \frac{\partial \mu u_2}{\partial y} + \frac{\partial \mu u_3}{\partial z} \right) \delta u \\ = \int d\omega \int_0^n (dn) \left(\frac{d\mu u_1}{dn} \cos nx + \frac{d\mu u_2}{dn} \cos ny + \frac{d\mu u_3}{dn} \cos nz \right) \delta u \\ - \int_0^n d\omega \bar{\mu} \left(u_1 \cos nx + u_2 \cos ny + u_3 \cos nz \right) \delta u \\ - \int d\omega \bar{\mu} \left(u_1^2 \cos nx + u_2 \cos ny + u_3 \cos nz \right) \delta u\end{aligned}$$

das erste Glied bezieht sich auf die Oberfläche das zweite aber auf die innere Grenze, weil weder \cos etc noch δu δv δw von n abhängen soll.

Bildet man weiter auf dieselbe Weise die Ausdrücke

$$\begin{aligned}\int \tau \left(\frac{\partial \mu v_1}{\partial x} + \frac{\partial \mu v_2}{\partial y} + \frac{\partial \mu v_3}{\partial z} \right) \delta v \\ \int \tau \left(\frac{\partial \mu w_1}{\partial x} + \frac{\partial \mu w_2}{\partial y} + \frac{\partial \mu w_3}{\partial z} \right) \delta w\end{aligned}$$

so erhält man

$$\begin{aligned} \delta Q = & \int \bar{\mu} d\omega \left(\bar{u}_1 \cos nx + \bar{u}_2 \cos ny + \bar{u}_3 \cos nz \right) \delta u \\ & + \int \bar{\mu} d\omega \left(\bar{v}_1 \cos nx + \bar{v}_2 \cos ny + \bar{v}_3 \cos nz \right) \delta v \\ & + \int \bar{\mu} d\omega \left(\bar{w}_1 \cos nx + \bar{w}_2 \cos ny + \bar{w}_3 \cos nz \right) \delta w \end{aligned}$$

Wo die Grössen sich auf die Punkte beziehen, die sich auf die innere Grenze der Oberflächenschichte beziehen, da aber λ an dieser Grenze verschwinden, und mithin auch u , so folgt

$$\delta Q = 0$$

da dieses in jedem beliebig liegenden Elementarprisma stattfindet, so muss dies auch dann stattfinden, wenn die Flächenintegrale über die ganze Ausdehnung des Körpers ausgedehnt wird. Mithin

$$\delta Q = 0$$

überall, in so fern man von den Vorgängen in der äusseren Oberflächenschichte absehen kann.

Das dritte Integrale δR wandeln wir auf dieselbe Weise um, in dem wir setzen

$$\mu \Omega_{11} \frac{\partial \delta u}{\partial x} = \frac{\partial \mu \Omega_{11}}{\partial x} \delta u - \frac{\partial \mu \Omega_{11}}{\partial x} \delta u$$

so folgt

$$\begin{aligned} \delta R = & - \int \mu d\omega \left(\overline{\Omega}_{11} \cos nx + \overline{\Omega}_{12} \cos ny + \overline{\Omega}_{13} \cos nz \right) \delta \overline{u} \\ & + \left(\overline{\Omega}_{21} \cos nx + \overline{\Omega}_{22} \cos ny + \overline{\Omega}_{23} \cos nz \right) \delta \overline{v} \\ & + \left(\overline{\Omega}_{31} \cos nx + \overline{\Omega}_{32} \cos ny + \overline{\Omega}_{33} \cos nz \right) \delta \overline{w} \\ & - \int \mu d\tau \left(\frac{\partial \mu \Omega_{11}}{\partial x} + \frac{\partial \mu \Omega_{12}}{\partial y} + \frac{\partial \mu \Omega_{13}}{\partial z} \right) \delta u \\ & + \left(\frac{\partial \mu \Omega_{21}}{\partial x} + \frac{\partial \mu \Omega_{22}}{\partial y} + \frac{\partial \mu \Omega_{23}}{\partial z} \right) \delta v \\ & + \left(\frac{\partial \mu \Omega_{31}}{\partial x} + \frac{\partial \mu \Omega_{32}}{\partial y} + \frac{\partial \mu \Omega_{33}}{\partial z} \right) \delta w \end{aligned}$$

Wo in $\Omega_{11} \Omega_{12}$ etc in Allgemeinen die Factoren von $\frac{\partial u}{\partial x} \frac{\partial v}{\partial y}$ etc andere Werthe haben werden als in $\overline{\Omega}_{11} \overline{\Omega}_{12}$ etc da die Grössen $\alpha_1 \alpha_2$ etc im Inneren

constant sind, aber an der Oberfläche im allgemeinen Variabel sein müssen. Wir zerlegen das Raumintegrale in zwei, das eine ausgedehnt über die Oberflächenhülle innerhalb deren $a_1 a_2$ etc variabel sind. Und das andere über den übrig bleibenden Raumtheil. In dem ersten Integral setzen wir wieder

$$d\tau = d\omega dn$$

und

$$\frac{d\mu}{dx} \frac{\Omega_{11}}{dn} = \frac{d\mu}{dn} \frac{\Omega_{11}}{\cos nx} \frac{\partial \mu}{\partial y} \frac{\Omega_{12}}{dm} = \frac{\partial \mu}{dm} \frac{\Omega_{12}}{\cos ny}$$

etc

da nach der eingeführten Annahme jeder Punkt des aus der Grenzhülle ausgeschnittenen Elementarprismas als starr betrachtet werden kann und die Grössen $a_1 a_2$ etc von n allein abhängig sein sollen. Das in der Rede stehende Integral, wird, $\partial u \cos nx$ etc von n etc nicht abhängig sind,

$$\begin{aligned} & \int d\omega \mu \left((\Omega_{11} \cos nx + \Omega_{12} \cos ny + \Omega_{13} \cos nz) \right) \delta u \\ & + \left((\Omega_{21} \cos nx + \Omega_{22} \cos ny + \Omega_{23} \cos nz) \right) \delta v \\ & + \left((\Omega_{31} \cos nx + \Omega_{32} \cos ny + \Omega_{33} \cos nz) \right) \delta w \\ & - \int d\omega \bar{\mu} \left((\bar{\Omega}_{11} \cos nx + \bar{\Omega}_{12} \cos ny + \bar{\Omega}_{13} \cos nz) \right) \delta \bar{v} \\ & + \left((\bar{\Omega}_{21} \cos nx + \bar{\Omega}_{22} \cos ny + \bar{\Omega}_{23} \cos nz) \right) \delta \bar{v} \\ & + \left((\bar{\Omega}_{32} \cos nx + \bar{\Omega}_{32} \cos ny + \bar{\Omega}_{33} \cos nz) \right) \delta \bar{v} \end{aligned}$$

durch die Einsetzung dieses in ∂R heben sich die Integrale auf, in denen $\bar{\Omega}_{11} \bar{\Omega}_{12}$ etc treten so dass

$$\begin{aligned} \partial R = & - \int d\omega \mu \left((\Omega_{11} \cos nx + \Omega_{12} \cos ny + \Omega_{13} \cos nz) \right) \delta u \\ & + \left((\Omega_{21} \cos nx + \Omega_{22} \cos ny + \Omega_{23} \cos nz) \right) \delta v \\ & + \left((\Omega_{31} \cos nx + \Omega_{32} \cos ny + \Omega_{33} \cos nz) \right) \delta w \end{aligned}$$

$$\begin{aligned}
 & - \int d\tau \left(\frac{\partial \mu \Omega_{11}}{\partial x} + \frac{\partial \mu \Omega_{12}}{\partial y} + \frac{\partial \mu \Omega_{13}}{\partial z} \right) \delta u \\
 & + \left(\frac{\partial \mu \Omega_{21}}{\partial x} + \frac{\partial \mu \Omega_{22}}{\partial y} + \frac{\partial \mu \Omega_{23}}{\partial z} \right) \delta v \\
 & + \left(\frac{\partial \mu \Omega_{31}}{\partial x} + \frac{\partial \mu \Omega_{32}}{\partial y} + \frac{\partial \mu \Omega_{33}}{\partial z} \right) \delta \tau v
 \end{aligned}$$

Wir erhalten als Gleichgewichtsbedingung

$$\int (\Xi \delta u + H \delta v + Z \delta \tau v) + \delta R = 0$$

da an der Grenzhülle

$$(\delta u = \bar{\delta u} \quad \delta v = \bar{\delta v} \quad \delta \tau v = \bar{\delta \tau v})$$

Wir haben somit als die Bedingung für die Gleichgewichtslage

$$\begin{aligned}
 \mu \Omega_{11} \cos nx + \mu \Omega_{12} \cos ny + \mu \Omega_{13} \cos nz &= \Xi \\
 \mu \Omega_{21} \cos nx + \mu \Omega_{22} \cos ny + \mu \Omega_{23} \cos nz &= H \\
 \mu \Omega_{31} \cos nx + \mu \Omega_{32} \cos ny + \mu \Omega_{33} \cos nz &= Z
 \end{aligned}$$

$$\frac{\partial \mu \Omega_{11}}{\partial x} + \frac{\partial \mu \Omega_{12}}{\partial y} + \frac{\partial \mu \Omega_{13}}{\partial z} = 0$$

$$\frac{\partial \mu \Omega_{21}}{\partial x} + \frac{\partial \mu \Omega_{22}}{\partial y} + \frac{\partial \mu \Omega_{23}}{\partial z} = 0$$

$$\frac{\partial \mu \Omega_{31}}{\partial x} + \frac{\partial \mu \Omega_{32}}{\partial y} + \frac{\partial \mu \Omega_{33}}{\partial z} = 0$$

Vergleicht man mit der Gleichgewichtsbedingung

$$\frac{\partial Xx}{\partial x} + \frac{\partial Xy}{\partial y} + \frac{\partial Xz}{\partial z} = 0$$

$$\frac{\partial Yx}{\partial x} + \frac{\partial Yy}{\partial y} + \frac{\partial Yz}{\partial z} = 0$$

$$\frac{\partial Zx}{\partial x} + \frac{\partial Zy}{\partial y} + \frac{\partial Zz}{\partial z} = 0$$

so erhält man unmittelbar

$$Xx = \mu \Omega_{11} \quad Xy = \mu \Omega_{12} \quad Xz = \mu \Omega_{13}$$

$$Yx = \mu \Omega_{21} \quad Yy = \mu \Omega_{22} \quad Yz = \mu \Omega_{23}$$

$$Zx = \mu \Omega_{31} \quad Zy = \mu \Omega_{32} \quad Zz = \mu \Omega_{33}$$

$$\begin{aligned}
 Xx = \mu \left(a_1 \frac{\partial u}{\partial x} + a_1 \frac{\partial \tau v}{\partial y} + a_{11} \frac{\partial \tau v}{\partial z} + a_{13} \left(\frac{\partial \tau v}{\partial z} + \frac{\partial \tau v}{\partial y} \right) + a_9 \left(\frac{\partial \tau v}{\partial x} + \frac{\partial u}{\partial z} \right) \right. \\
 \left. + a_4 \left(\frac{\partial u}{\partial y} + \frac{\partial \tau v}{\partial x} \right) \right)
 \end{aligned}$$

$$Xy = \mu \left(a_4 \frac{\partial u}{\partial x} + a_7 \frac{\partial v}{\partial y} + a_{15} \frac{\partial w}{\partial z} + a_{14} \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) + a_{13} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \right. \\ \left. + a_{12} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \right)$$

$$Xz = \mu \left(a_9 \frac{\partial u}{\partial x} + a_{14} \frac{\partial v}{\partial y} + a_6 \frac{\partial w}{\partial z} + a_{15} \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) + a_{11} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \right. \\ \left. + a_{13} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \right)$$

$$Yx = \mu \left(a_4 \frac{\partial u}{\partial x} + a_7 \frac{\partial v}{\partial y} + a_{15} \frac{\partial w}{\partial z} + a_{14} \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) + a_{13} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \right. \\ \left. + a_{12} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \right)$$

$$Yy = \mu \left(a_{12} \frac{\partial u}{\partial x} + a_2 \frac{\partial v}{\partial y} + a_{10} \frac{\partial w}{\partial z} + a_5 \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) + a_{14} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \right. \\ \left. + a_7 \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \right)$$

$$Yz = \mu \left(a_{13} \frac{\partial u}{\partial x} + a_{15} \frac{\partial v}{\partial y} + a_8 \frac{\partial w}{\partial z} + a_{10} \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) + a_{13} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \right. \\ \left. + a_{14} \left(\frac{\partial v}{\partial x} + \frac{\partial w}{\partial y} \right) \right)$$

$$Zx = \mu \Omega_{31}$$

$$Yy = \mu \Omega_{22}$$

$$Zz = \mu \Omega_{33}$$

$$Zz = \mu \left(a_{11} \frac{\partial u}{\partial x} + a_{10} \frac{\partial v}{\partial y} + a_3 \frac{\partial w}{\partial z} + a_8 \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) + a_6 \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) \right. \\ \left. + a_{15} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \right)$$

die 15 Coefficienten a sind die sogenannten elastischen Coefficienten, und sind Constanten ausgenommen die Punkte der unendlich dünnen Oberflächenschichte.

Diese Functionen lassen sich anderes schreiben, in dem wir überall einführen

$$\frac{\partial u}{\partial x} = x_x \quad \frac{\partial v}{\partial y} = y_y \quad \frac{\partial w}{\partial z} = z_z \quad \frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} = y_z \quad \frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} = x_z \\ \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} = y_x$$

$$-X_x = \mu (a_1 x_x + a_{12} y_y + a_{11} z_z + a_{13} z_y + a_9 z_x + a_4 y_x)$$

$$Y_y = \mu(a_{12} x_x + a_2 y_y + a_{10} z_z + a_5 y_z + a_{14} x_z + a_7 y_x)$$

$$Z_z = \mu(a_{11} x_x + a_{10} y_y + a_3 z_z + a_8 y_z + a_6 x_z + a_{15} y_x)$$

$$Z_y = Y_z = \mu(a_{13} + a_{15} y_y + a_8 z_z + a_{10} z_y + a_{15} x_z + a_{14} y_x)$$

$$X_z = Z_x = \mu(a_9 x_x + a_{14} y_y + a_6 z_z + a_{15} z_y + a_{11} x_z + a_{13} y_x)$$

$$Y_x = X_y = \mu(a_4 x_x + a_7 y_y + a_{15} z_z + a_{14} z_y + a_{12} x_z + a_{12} y_x)$$

Man bilde

$$\begin{aligned} P = & \frac{1}{2}(C_{11}x_x^2 + 2C_{12}x_x y_y + 2C_{13}x_x z_z + 2C_{15}x_x x_z + 2C_6 x_x y_x \\ & + C_{22}y_y^2 + 2C_{23}y_y z_z + 2C_{24}y_y x_x + 2C_{25}y_y x_z + 2C_{26}y_y y_x \\ & + C_{33}z_z^2 + 2C_{34}z_z z_y + 2C_{35}z_z x_z + 2C_{36}z_z y_x \\ & + C_{44}z_y^2 + 2C_{45}z_y x_z + 2C_{46}z_y y_x \\ & + C_{55}x_z^2 + 2C_{56}x_z y_x \\ & + C_{66}y_x^2) \end{aligned}$$

und differentirt man nach den 6 Argumenten

$$\begin{aligned} & x_x \quad y_y \quad z_z \quad x_z \quad y_x \quad x_z \\ \frac{\partial P}{\partial x_x} = & (C_{11}x_x + C_{12}y_y + C_{13}z_z + C_{14}z_y + C_{15}x_z + C_{16}y_x) \\ \frac{\partial P}{\partial y_y} = & (C_{12}x_x + C_{22}y_y + C_{23}z_z + C_{24}z_y + C_{25}x_z + C_{26}y_x) \\ \frac{\partial P}{\partial z_z} = & (C_{13}x_x + C_{23}y_y + C_{33}z_z + C_{34}z_y + C_{35}x_z + C_{36}y_x) \\ \frac{\partial P}{\partial z_y} = & (C_{14}x_x + C_{24}y_y + C_{34}z_z + C_{44}z_y + C_{45}x_z + C_{46}y_x) \\ \frac{\partial P}{\partial x_z} = & (C_{15}x_x + C_{25}y_y + C_{35}z_z + C_{45}z_y + C_{55}x_z + C_{56}y_x) \\ \frac{\partial P}{\partial y_x} = & (C_{16}x_x + C_{26}y_y + C_{36}z_z + C_{46}z_y + C_{56}x_z + C_{66}y_x) \end{aligned}$$

Wenn wir hierin setzen

$$\begin{aligned} a_1 = C_{11} \quad a_4 = C_{16} \quad a_7 = C_{26} \\ a_2 = C_{22} \quad a_5 = C_{24} \quad a_8 = C_{34} \\ a_3 = C_{33} \quad a_6 = C_{35} \quad a_9 = C_{15} \\ a_{10} = C_{23} = C_{44} \quad a_{13} = C_{14} = C_{15} \\ a_{11} = C_{13} = C_{35} \quad a_{14} = 2C_{25} = C_{46} \\ a_{12} = C_{12} = C_{66} \quad a_{15} = C_{26} = C_{45} \end{aligned}$$

so folgt

$$X_x = \mu \frac{\partial P}{\partial x_x} \quad Y_y = \mu \frac{\partial P}{\partial y_y} \quad Z_z = \mu \frac{\partial P}{\partial z_z}$$

$$Z_y = Y_z = \mu \frac{\partial P}{\partial x_y} \quad X_z = Z_x = \mu \frac{\partial P}{\partial x_z}$$

$$Y_x = X_y = \mu \frac{\partial P}{\partial y_x}$$

d. h. die Componenten der Druckkräfte haben also ein Potential. Die 15 Constanten lassen sich im Allgemeinen theoretisch auf eine geringere Anzahl nicht zurückführen. Sie lassen sich, wohl, wenn der Körper eine gewisse Symmetrie in seiner Struktur besitzt d. h. ein Krystall ist, der nicht dem triklinischen System angehört.

Wir nehmen zunächst an, die Struktur des Körpers sei symmetrisch in Bezug auf eine Ebene, nehmen wir diese zu $(x \ y)$ Ebene so verschwinden von dem Constanten a diejenigen in denen y in ungerader Potenz, vorkommen, wie

$$2 a_4 = \int dm' F(\rho) \xi^3 y$$

So ist in diesem

$$a_4 = 0 \quad a_5 = 0 \quad a_7 = 0 \quad a_8 = 0 \quad a_{13} = 0 \quad a_{15} = 0$$

Mithin

$$C_{16} = 0 \quad C_{21} = C_{23} = C_{24} = 0 \quad C_{11} = 0 \quad C_{36} = 0 \quad C_{35} = 0 \quad C_{45} = 0$$

die Funktion P wird in diesen Fall

$$P = \frac{1}{2} (C_y x_x^2 + 2C_{12} x_x y_y + 2C_{43} x_x z_z + 2C_{15} x_y x_z$$

$$+ C_{22} y_y^2 + 2C_{23} y_y z_z + 2C_{25} x_y z_z$$

$$+ C_{33} z_z^2 + 2C_{35} z_z x_z$$

$$+ C_{44} y_y z_y + C_{46} z_y y_x$$

$$+ C_{55} x_z^2$$

$$+ C_{66} y_x^2$$

In der That bleibt diese Funktion unverändert. Wenn man hierin statt $yv - y - v$ setzt, denn es werden

$$z_y = -\left(\frac{\partial v}{\partial x} + \frac{\partial z v}{\partial y}\right) \quad y_x = -\left(\frac{\partial v}{\partial x} + \frac{\partial v}{\partial y}\right)$$

die Anzahl der Constanten reducirt sich auf 9. Ist der Körper noch um eine Ebene etwa $y \ z$ Ebene symmetrisch gebaut, so verschwinden alle von den a in denen x in ungerader Potenz auftritt. Es werden noch

$$a_6 = a_7 = a_9 = a_{14} = 0$$

$$C_{35} = C_{16} = C_{15} = C_{27} = C_{46} = 0$$

die Funktion P wird in diesem Fall

$$\begin{aligned}
 P = \frac{1}{2} & (C_{11}x_x^2 + 2C_{12}x_x y_y + C_{13}x_x z_z \\
 & + C_{22}y_y + 2C_{23}y_y z_z \\
 & + C_{33}z_z^2 + C_{44}z_y^2 \\
 & + C_{55}x_z^2 \\
 & + C_{66}y_z^2)
 \end{aligned}$$

Wobei

$$C_{21} = C_{44} \quad C_{15} = C_{33} \quad C_{12} = C_{66}$$

Mithin

$$\begin{aligned}
 P = \frac{1}{2} & (C_{11}^2 x_x + C_{22} y_y + C_{33} z_z^2 + C_{13} (2x_x z_z + x_z^2) \\
 & + C_{12} (2x_x y_y + y_x^2) \\
 & + C_{23} (2y_y z_z + z_y^2))
 \end{aligned}$$

Die Anzahl der Constanten so ist 6. Die xy Ebene ist zugleich auch eine Symmtriebene, dann P bleibt unverändert wenn man z mit $-z$ vertauscht. Ein solcher Körper gehört dem rhombischen System. Ist der Körper so beschaffen dass die Struktur um eine Axe symmetrisch ist dass die Druckcomponenten sich nicht ändern wenn man z . B. x mit y vertauscht so wird die Anzahl der Constanten noch geringer.

Sei der Körper symmetrisch um z -Achse, so ist dann

$$\begin{aligned}
 a_1 = a_2 \quad a_{16} = a_{11} \\
 \int dm' F(\rho) \xi^4 = \int dm' F(\rho) \eta^4 \quad \int dm' F(\rho) \zeta^2 \eta^2 = \int dm' F(\rho) \xi^2 \zeta^2 \\
 C_{11} = C_{22} \quad C_{13} = C_{23} = C_{44} = C_{55}
 \end{aligned}$$

Mithin ist in diesem Fall

$$\begin{aligned}
 P = \frac{1}{2} & (C_{11}(x_x^2 + y_y^2) + C_{33}z_z^2 + C_{12}(2x_x y_y + y_x^2) \\
 & + C_{13}(2x_x z_z + 2y_y z_z + x_z^2))
 \end{aligned}$$

Was sich in der That nicht ändert, wenn x mit y vertauscht.

Solche Körper sind Krystalle des quadratischen Systems.

Ist der Körper so gebaut, dass er zugleich z . B. um die y -Axe symmetrisch gebaut ist, dass man auch z mit x vertauschen kann so muss

$$\begin{aligned}
 a_1 = a_3 \quad a_{12} = a_{10} \\
 \int dm' F(\rho) \xi^4 = \int dm' F(\rho) \zeta^4
 \end{aligned}$$

$$\int dm' F(\rho) \xi^2 \eta^5 = \int dm' F(\rho) \zeta^2 \eta^2$$

$$C_{11} = C_{33} \quad C_{13} = C_{13}$$

Die Funktion P wird

$$P = C_{11}(x_x^2 + y_y + s_z^2) + C_{12}(x_y^2 + y_z^2 + s_x^2) \\ + 2C_{12}(x_x y_y + s_z y_y + s_z x_x)$$

Solche Körper heissen reguläre Krystalle. Die Druckcomponenten des, regulären Krystalls sind

$$X_x = \mu(C_{11}x_x + C_{12}y_x + C_{12}s_z)$$

$$Y_y = \mu(C_{11}y_y + C_{12}(x_x + s_z))$$

$$Z_z = \mu(C_{11}s_z + C_{12}(y_y + s_z))$$

$$Xx = \mu C_{12}xy$$

$$Ys = \mu C_{12}ys$$

$$Zx = \mu C_{12}sx$$

Wir betrachten jetzt Körper, welche dem hexagonalen System angehören, die Structur des Körpers soll so beschaffen sein, dass sie symmetrisch ist in Bezug auf drei auf einander senkrechten Ebenen, dass eine Drehung um 60° oder einen beliebigen Winkel in einen von der ursprünglichen nicht unterscheidbaren Stellung führt.

Es ist im diesen Fall

$$P = \frac{1}{2}(C_{11}x_x^2 + C_{22}y_y + C_{33}s_z + 13(2x_x s_x x_z^2) + C_{12}(2x_x y_y y_x^2) \\ + C_{23}(2y_y s_z + s_y))$$

die Hauptachse sei s -Achse, und in Holz würde s -Axe die Richtung des Longitudinalen sein. Wir führen ein zweites Coordinatensystem $x'y'$ ein welche gegen das alte $x' y'$ um den Winkel χ geneigt ist

$$x = x'a - y'\beta \quad \alpha = \cos \chi$$

$$y = x'\beta + y'a \quad \beta = \sin \chi$$

$$\alpha^2 + \beta^2 = 1$$

und daher

$$u = u'a - v'\beta$$

$$v = v'a$$

$$v = u'\beta + v'a$$

Es ist

$$\begin{aligned} \frac{du}{dx} = x_x &= \frac{du'}{dx'} a^2 + \frac{dv'}{dy'} \beta^2 - \left(\frac{du'}{dy'} + \frac{dv'}{dx'} \right) a\beta \\ \frac{dv}{dy} = y_y &= \frac{du'}{dx'} \beta^2 + \frac{dv'}{dy'} a^2 + \left(\frac{du'}{dy'} + \frac{dv'}{dx'} \right) a\beta \\ \frac{du}{dy} + \frac{dv}{dx} = x_y &= 2 \left(\frac{du'}{dx'} - \frac{dv'}{dy'} \right) a\beta + \left(\frac{du'}{dy} + \frac{dv'}{dx'} \right) (a^2 - \beta^2) \\ \frac{dv}{dz} + \frac{dw}{dy} = y_z &= \left(\frac{dv'}{dx'} + \frac{dw'}{dy'} \right) a + \left(\frac{dv'}{dx'} + \frac{dw'}{dz'} \right) \beta \\ \left(\frac{du}{dx} + \frac{dw}{dy} \right) = x_z &= \left(\frac{dw'}{dx} + \frac{du'}{dy} \right) a - \left(\frac{dw'}{dy} + \frac{dv'}{dz'} \right) \beta \end{aligned}$$

oder wir die leichtverständlichen Bezeichnungen einführen

$$\begin{aligned} x_x &= x_x' a^2 + y_y' \beta^2 - y_x' a\beta \\ y_x &= x_x' \beta^2 + y_y' a^2 + y_x' a\beta \\ y_x &= (x_x - y_y') a\beta + y_x' (a^2 - \beta^2) \\ z_y &= z_y' a + x_z' \beta \quad x_z = (x_z) a - z_y' \beta \end{aligned}$$

Wir bilden die Ausdrücke

$$X_x = \mu \frac{dP}{dx_x} \quad Y_y = \mu \frac{dP}{dy_y} \quad Z_z = \mu \frac{dP}{dz_z} \quad \text{etc.}$$

und setzen die Werthe der Druckcomponenten in neuen Coordinatensystem

$$\begin{aligned} X_x' & Y_y' & Z_z' & Z_y' & Y_x' & X_y' \\ X_x' &= \mu \frac{dP}{dx_x'} & X_y' &= \mu \frac{dP}{dy_y'} & Z_z' &= \mu \frac{dP}{dz_z'} \\ Y_x' &= \mu \frac{dP}{dy_x'} & Z_x' &= \mu \frac{dP}{dz_x'} & X_y' &= \mu \frac{dP}{dy_y'} \end{aligned}$$

Es ist nun

$$X_x' = \mu \left(\frac{dP}{dx} \frac{dx}{dx_x'} + \frac{dP}{dy} \frac{dy}{dx_x'} + \frac{dP}{dz_z} \frac{dz_z}{dx_x'} + \frac{dP}{dy_z} \frac{dy_z}{dx_x'} + \frac{dP}{dy_y} \frac{dy_y}{dx_x'} \right)$$

$$\frac{dx_x}{dx_x'} = a^2 \frac{dy_z}{dy_x'} = \beta^2 \quad \frac{dy_z}{dy_x'} = 0 = \frac{dy_z}{dx_x'} = \frac{dz_x}{dx_x'} = 0$$

$$\frac{dy_y}{dx_x'} = \frac{dy_x}{dx_x'} = +2a\beta$$

$$X_x' = X_x a^2 + Y_y \beta^2 + 2Y_x a\beta$$

und auf dieselbe Weise

$$\begin{aligned} Y_y' &= X_x \beta^2 + Y_y a^2 - 2Y_x a\beta \\ Z_z' &= Z_z \end{aligned}$$

$$Y_y = Y_x = (Y_y - X_x)\alpha_i\beta + X_y(\alpha^2 - \beta^2) \quad (\text{I})$$

$$Y'_z = Z'_y = -X_z\beta + Y_z\alpha$$

$$Z'_x = X'_z = X_z\alpha_i\beta + Y'_z$$

Es ist nun

$$\begin{aligned} X_x &= \mu(C_{11}x_x + C_{12}y_y + C_{13}z_z) \\ &= \mu(x'_x(\alpha^2 C_{11} + C_{12}\beta^2) + e_x(\alpha_i\beta(C_{11} - C_{12}) + C_{13}\alpha_i z_z)) \end{aligned}$$

$$\begin{aligned} Y_y &= \mu(C_{22}y'_y + C_{12}x_x + C_{13}z_z) + y'_y(C_{11}\beta^2 + C_{12}\alpha^2) \\ &= \mu(x'_x(C_{22}\beta^2 + C_{12}\alpha^2) + y'_y(C_{22}\alpha^2 + C_{12}\beta^2) \\ &\quad + y'_z\alpha_i\beta(C_{22} - C_{12}) + C_{23}z_z) \end{aligned}$$

$$\begin{aligned} Z_z &= \mu(C_{32}z_z + C_{13}x_x + C_{13}y_y) \\ &= \mu(x'_x(C_{13}\alpha^2 + C_{32}\beta^2) + y'_y(C_{22}\alpha^2 + C_{12}\beta^2) + y'_z\alpha_i\beta(C_{23} - C_{13}) \\ &\quad + C_{33}z_z) \end{aligned}$$

$$X_y = Y_x = \mu C_{12}y_x = \mu C_{12}(2(x'_x y'_y)\alpha_i\beta + y'_x(\alpha^2 - \beta^2))$$

$$Y_z = Z_y = \mu C_{12}z_z = \mu C_{23}(z'_y\alpha + x'_z\beta)$$

$$Z_x = X_z = \mu C_{13}x_z = \mu C_{13}(x'_x\alpha - z'_y\beta)$$

Die Einführung dieser Ausdrücke in (I)

$$\begin{aligned} \frac{X'_x}{\mu} &= x'_x(\alpha^4 C_{11} + \beta^4 C_{22} + 2C_{12}\alpha^2\beta^2 + 4\alpha^2\beta^2 C_{12}) + e'_y(\alpha_i^2\beta^2(C_{11} + C_{22}) \\ &\quad + C_{12}(\alpha^4 + \beta^4) - 4C_{12}\alpha^2\beta^2 + z_z^2(C_{13}(\alpha^2 + C_{23}\beta^2) \\ &\quad + y'_x(-\alpha^2(C_{11} - C_{12}) + \beta^2(C_{22} - C_{12}) + 2(\alpha^2 - \beta^2)C_{12})\alpha_i\beta \end{aligned}$$

$$\begin{aligned} \frac{Y'_y}{\mu} &= x'_x(\alpha^2\beta^2(C_{11} + C_{22}) + C_{12}(\alpha^4 + \beta^4) + 4C_{12}\alpha^2\beta^2) + e'_y(\beta^4 C + \alpha^4 C_{23}) \\ &\quad + z_z^2(C_{13}\beta^2 + C_{23}\alpha^2) + e'_x(-\beta^2(C_{11} - C_{12}) \\ &\quad + \alpha^2(C_{22} - C_{12}) - 2(\alpha^2 - \beta^2)C_{12})\alpha_i\beta \end{aligned}$$

$$\frac{Z'_z}{\mu} = (C_{13}\alpha^2 + C_{23}\beta^2) + y'_y(C_{13}\beta^2 + C_{23}\alpha^2) + \alpha_i\beta y'_x(C_{23} - C_{13}) + C_{33}z_z$$

$$\frac{Z'_y}{\mu} = \alpha_i\beta x'_z(C_{23} - C_{13}) + z'_y(C_{23}\alpha^2 + C_{13}\beta^2)$$

$$\frac{X'_z}{\mu} = x'_z\alpha_i\beta(C_{23}\beta^2 + \alpha^2 C_{13} + C_{12}(\alpha^2 - \beta^2) - z'_y\alpha_i\beta(C_{23} - C_{13}))$$

$$\frac{Y'_z}{\mu} = x'_x(\alpha_i\beta)(C_{22}\beta^2 - \alpha^2 C_{11} + C_{12}(\alpha^2 - \beta^2) + 2C_{12}(\alpha^2 - \beta^2))$$

Wenn der Körper so gebaut ist, dass die Constanten von χ unabhängig wird wenn man die Coordinatenachsen um χ dreht, so wird bei der Drehung um χ wieder sein

$$\frac{X'_x}{\mu} = C_{11}x'_x + C_{11}y'_y + C_{13}z'$$

$$\frac{Y'_y}{\mu} = C_{12}y'_y + C_{12}x'_x + C_{33}z'$$

$$\frac{Z'_z}{\mu} = C_{23}z'_z + C_{23}y'_y + C_{13}x'_x$$

$$X'_y = Y'_x = \mu C_{13}y'_x \quad Y'_z = Z'_y = \mu C_{23}Z_y \quad Z'_x = X'_z = \mu C_{13}x'_z$$

So müssen sein

$$\left. \begin{aligned} a^4 C_{11} + \beta^4 C_{22} + 6C_{12}a^2\beta^2 &= C_{11} \\ a^2\beta^2(C_{11} + C_{22}) + C_{12}(a^4 + \beta^4) - 4C_{12}a^2\beta^2 &= C_{12} \\ C_{13}a^2 + C_{33}\beta^2 &= C_{13} \\ \beta^2(C_{22} - C_{12}) &= a^2(C_{11} - C_{12}) + 6(a^2 - \beta^2)C_{12} = 0 \end{aligned} \right\} \text{I}$$

$$\left. \begin{aligned} \beta^4(C_{11} + a^4 C_{22}) + 6C_{12}a^2\beta^2 &= C_{22} \\ C_{13}\beta^2 + C_{23}a^2 &= C_{13} \\ \beta^2(C_{11} + C_{12}) + a^2(C_{22} - C_{12}) - 2(a^2 - \beta^2)C_{12} &= 0 \\ C_{13} - C_{23} &= 0 \end{aligned} \right\} \text{II}$$

$$\left. \begin{aligned} C_{22}\beta^2 - a^2 C_{11} + C_{12}(a^2 - \beta^2) + 2C_{12}(a^2 - \beta^2) &= 0 \\ C_{12}a^2 - \beta^2 C_{11} - C_{12}(a^2 - \beta^2) - 2C_{12}(a^2 - \beta^2) &= 0 \\ a^2\beta^2(C_{22} - C_{12}) + a^2 - \beta^2(C_{11} - C_{12}) + C_{12}(a^2 - \beta^2) &= C_{12} \end{aligned} \right\} \text{III}$$

diese II Gleichungen erfüllt man durch die Annahme

$$C_{13} = C_{23} \quad C_{11} = C_{12} = 3C_{13}$$

und da

$$a^2 + \beta^2 = 1$$

ist. Mithin erhalten wir für einen Körper, der um die z -Achse symmetrisch gebaut ist, wie bei den Hölzern,

$$P = 3C_{13}(x^2 + y^2) + C_{13}(x_x^2 + z_y^2 + 2x_x z_z + 2y_y z_z) + C_{33}z_z^2 \\ + C_{12}(2x_x y_y + y_x^2)$$

diese Gleichung gilt auch für jeden Krystall, der dem hexagonalen System gehört, denn die Werthe χ sind die einzigen durch die Gleichungen gleichzeitig erfüllt werden denn die letzten Gleichungen von I und II lassen sich auch so schreiben

$$\beta^2 C_{12} - a^2 C_{11} + 3(a^2 - \beta^2)C_{12} = 0 \\ a^2 C_{22} - \beta^2 C_{11} + 3(a^2 - \beta^2)C_{12} = 0$$

Addition giebt denn

$$C_{22} = C_{11}$$

Aus den beiden Gleichungen in III folgt

$$C_{11} = C_{22} = 3C_{12}$$

$$\begin{aligned} a_1 &= \int dm' F(\rho) \xi^4 a_2 = \int dm' F(\rho) \eta^4 \\ &= 3a_{12} = 3 \int dm' F(\rho) \xi^2 \eta^2 \end{aligned}$$

Wir fassen jetzt einen Körper ins Auge, welcher so gebaut ist, dass die Druckcomponenten sich nicht ändern, wie man auch die Coordinatenachsen legen mag, einen Körper von dem man sagt er sei isotrop. Die Funktion P behält immer dieselbe Gestalt, wie man auch die Coordinatenachsen drehen mag, als wenn der Körper in jeder Richtung sich verhält, wie ein regulärer Körper. Wir setzen daher

$$\begin{aligned} P &= C_{11}(x_x^2 + y_y^2 + z_z^2) + C_{12}(x_y^2 + y_z^2 + z_x^2) \\ &\quad + 2C_{12}(x_x y_y + z_x z_y + z_x x_x) \end{aligned}$$

und setzen zugleich

$$C_{11} = -K(1 + k' + k)$$

$$C_{12} = -\frac{k}{2}$$

$$2C_{12} = -2k'K.$$

Wo Kkk' gewisse Constanten sind, wengleich nach dieser Definition $k' = \frac{1}{2}$ sein sollte so lassen wir sie einfach bestehen aus Gründen, die wir später noch unten auseinander setzen wollen. Es ist

$$C_{11} + C_{12} = -k'K - K$$

da

$$K = -2C_{12}$$

$$C_{11} + C_{12} = -k'K + 2C_{12}$$

$$C_{11} - C_{12} = -k'K$$

$$-k'K = \int dm' F(\rho) (\xi^2 - \eta^2) \xi^2$$

für einen isotropen Körper ist $k' = 0$. Wenn also k' verschwindet, so ist der Körper isotrop da in jedem um z -Achse symmetrisch gebauten Körper

$$a_{11} = 3a_{12}$$

$$-k'K = 2 \int dx x \xi^2 \eta^2 F(\rho)$$

muss durch $\xi\eta$ zum verschwinden gebracht werden, damit der Körper als isotrop betrachtet werden kann. Die $\xi\eta$ sind intermoleculare Grösse, und der Querschnitt des Holzstäbchen muss darum so klein werden, dass

$$2 \int \mu d + \xi^2 \eta^2 F(\rho)$$

verschwindet. Legt man die z -Achse so in die Länge eines Körpers etwa des Holzes, und x und y -Achse so verringert, dass

$$\int dm' F(\rho) (\xi^2 - \eta^2) \xi^2$$

verschwindend klein betrachtet werden kann. Ein Streifen Holzfaser ein Holzstäbchen von verschwindendem Querschnitt kann daher als ein isotroper Körper betrachtet werden, die Funktion P wird

$$P = -K(x_x^2 + y_y^2 + z_z^2 + \frac{1}{2}(x_y^2 + y_z^2 + z_x^2)) + k(x_x + y_y + z_z) + k'(x_x^2 + y_y^2 + z_z^2)$$

die Grössen

$$x_x + y_y + z_z \\ x_x^2 + y_y^2 + z_z^2 + \frac{1}{2}(x_y^2 + y_z^2 + z_x^2)$$

d. h.

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} \\ \left(\frac{\partial u}{\partial x}\right)^2 + \left(\frac{\partial v}{\partial y}\right)^2 + \left(\frac{\partial w}{\partial z}\right)^2 + \frac{1}{2}\left(\frac{\partial v}{\partial x} + \frac{\partial u}{\partial y}\right)^2 + \frac{1}{2}\left(\frac{\partial u}{\partial z} + \frac{\partial w}{\partial x}\right) \\ + \frac{1}{2}\left(\frac{\partial v}{\partial y} + \frac{\partial w}{\partial z}\right)^2$$

ändern sich nicht, wenn das Coordinatensystem gerändert wird, wohl aber die Grösse

$$x_x^2 + y_y^2 + z_z^2 = \left(\frac{\partial u}{\partial x}\right)^2 + \left(\frac{\partial v}{\partial y}\right)^2 + \left(\frac{\partial w}{\partial z}\right)^2$$

um dies zu beweisen führen wir die Hauptdilatation $\lambda_1 \lambda_2 \lambda_3$ ein und bezeichnen die Cosinuse dieser Richtungen, welche sie mit den Coordinaten schliessen mit

$$a_{1i} \beta_{ij} \gamma_{j3}$$

Es sind uns bekannt

$$\frac{\partial u}{\partial x} = x_x = a_1^2 \lambda_1^2 + a_2^2 \lambda_2^2 + a_3^2 \lambda_3^2$$

$$\begin{aligned} \frac{\partial v}{\partial y} &= j_y = \beta_1^2 \lambda_1 + \beta_2^2 \lambda_2 + \beta_3^2 \lambda_3 \\ \frac{\partial w}{\partial z} &= s_z = a_1 \lambda_1 + a_2 \lambda_2 + a_3 \lambda_3 \\ \frac{1}{2} \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \right) &= \frac{s_y}{2} = \beta_1 \gamma_1 \lambda_1 + \beta_2 \gamma_2 \lambda_2 + \beta_3 \gamma_3 \lambda_3 \\ \frac{1}{2} \left(\frac{\partial w}{\partial x} + \frac{\partial u}{\partial z} \right) &= \frac{x_z}{2} = \gamma_1 a_1 \lambda_1 + \gamma_2 a_2 \lambda_2 + \gamma_3 a_3 \lambda_3 \\ \frac{1}{2} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) + \frac{j_x}{2} &= a_1 \beta_1 \lambda_1 + a_2 \beta_2 \lambda_2 + a_3 \beta_3 \lambda_3 \end{aligned}$$

Wenn wir jetzt die drei ersten Gleichungen addiren, so kommt

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = x_x + j_y + s_z = \lambda_1 + \lambda_2 + \lambda_3$$

was die Volumendilatation bedeutet und von den Coordinationsystem ganz und gar unabhängig ist

Man findet ferner

$$\begin{aligned} x_x^2 + j_y^2 + s_z^2 &= \lambda_1^2 (a_1^4 + \beta_1^4 + \gamma_1^4) \\ &\quad + 2\lambda_1 \lambda_2 (a_1^2 a_2^2 + \beta_1^2 \beta_2^2 + \gamma_1^2 \gamma_2^2) \\ &\quad + \lambda_2^2 (a_2^4 + \beta_2^4 + \gamma_2^4) \\ &\quad + 2\lambda_1 \lambda_3 (a_1^2 a_3^2 + \beta_1^2 \beta_3^2 + \gamma_1^2 \gamma_3^2) \\ &\quad + \lambda_3^2 (a_3^4 + \beta_3^4 + \gamma_3^4) \\ &\quad + 2\lambda_2 \lambda_3 (a_2^2 a_3^2 + \beta_2^2 \beta_3^2 + \gamma_2^2 \gamma_3^2) \end{aligned}$$

$$\begin{aligned} \frac{1}{2} (s_y^2 + x_z^2 + j_x^2) &= 2\lambda_1^2 (\beta_1^2 \gamma_1^2 + \gamma_1^2 a_1^2 + a_1^2 \beta_1^2) \\ &\quad + 2\lambda_2^2 (\beta_2^2 \gamma_2^2 + \gamma_2^2 a_2^2 + a_2^2 \beta_2^2) \\ &\quad + 2\lambda_3^2 (\beta_3^2 \gamma_3^2 + \gamma_3^2 a_3^2 + a_3^2 \beta_3^2) \\ &\quad + 4\lambda_1 \lambda_2 (\beta_1 \gamma_1 \beta_2 \gamma_2 + a_1 \gamma_1 a_2 \gamma_2 + a_1 \beta_1 a_2 \beta_2) \\ &\quad + 4\lambda_1 \lambda_3 (\beta_1 \gamma_1 \beta_3 \gamma_3 + a_1 \gamma_1 a_3 \gamma_3 + a_1 \beta_1 \beta_3 a_3) \\ &\quad + 4\lambda_2 \lambda_3 (\beta_2 \gamma_2 \beta_3 \gamma_3 + a_2 \gamma_2 a_3 \gamma_3 + a_2 \beta_2 a_3 \beta_3) \end{aligned}$$

die letzten drei Glieder lassen sich anderes schreiben. Es ist

$$a_1 a_2 + \beta_1 \beta_2 + \gamma_1 \gamma_2 = 0$$

das Quadrat hiervon ist

$$\begin{aligned} &a_1^2 a_2^2 + \beta_1^2 \beta_2^2 + \gamma_1^2 \gamma_2^2 + 2(a_1 a_2 \beta_1 \beta_2 + a_1 a_2 \gamma_1 \gamma_2 + \beta_1 \beta_2 \gamma_1 \gamma_2) \\ &- (a_1^2 a_2^2 + \beta_1^2 \beta_2^2 + \gamma_1^2 \gamma_2^2) \\ &= 2(a_1 a_2 \beta_1 \beta_2 + a_1 a_2 \gamma_1 \gamma_2 + \beta_1 \beta_2 \gamma_1 \gamma_2) \end{aligned}$$

ganz auf dieselbe Weise

$$\begin{aligned} & -(\alpha_1^2 \alpha_3^2 + \beta_1^2 \beta_3^2 + \gamma_1^2 \gamma_3^2) \\ & = 2(\alpha_1 \alpha_3 \beta_1 \beta_3 + \alpha_1 \alpha_3 \gamma_1 \gamma_3 + \beta_1 \beta_3 \gamma_1 \gamma_3) \\ & -(\alpha_2^2 \alpha_3^2 + \beta_2^2 \beta_3^2 + \gamma_2^2 \gamma_3^2) \\ & = 2(\alpha_2 \alpha_3 \beta_2 \beta_3 + \alpha_2 \alpha_3 \gamma_2 \gamma_3 + \beta_2 \beta_3 \gamma_2 \gamma_3) \end{aligned}$$

Es folgt hieraus

$$\begin{aligned} \frac{1}{2}(y_x^2 + s_y^2 + x_z^2) &= 2\lambda_1(\beta_1^2 \gamma_1^2 + \gamma_1^2 \alpha_1^2 + \alpha_1^2 \beta_1^2) \\ &+ 2\lambda_2(\beta_1^2 \gamma_2^2 + \gamma_2^2 \alpha_2^2 + \alpha_2^2 \beta_2^2) \\ &+ 2\lambda_3(\beta_3^2 \gamma_3^2 + \gamma_3^2 \alpha_3^2 + \alpha_3^2 \beta_3^2) \\ &- 2\lambda_1 \lambda_2 (\alpha_1^2 \alpha_2^2 + \beta_1^2 \beta_2^2 + \gamma_1^2 \gamma_2^2) \\ &- 2\lambda_1 \lambda_3 (\alpha_1^2 \alpha_3^2 + \beta_1^2 \beta_3^2 + \gamma_1^2 \gamma_3^2) \\ &- 2\lambda_2 \lambda_3 (\alpha_2^2 \alpha_3^2 + \beta_2^2 \beta_3^2 + \gamma_2^2 \gamma_3^2) \end{aligned}$$

die Addition dieses zu $x_x^2 + y_y^2 + s_z^2$, so heben sich die mit $\lambda_1 \lambda_2$, $\lambda_1 \lambda_3$ und $\lambda_2 \lambda_3$ multiplicirten Glieder auf. Mithin

$$\begin{aligned} & x_x^2 + y_y^2 + s_z^2 + \frac{1}{2}(s_y^2 + y_x^2 + x_z^2) \\ &= \lambda_1^2(\alpha_1^2 + \beta_1^2 + \gamma_1^2) + \lambda_2^2(\alpha_2^2 + \beta_2^2 + \gamma_2^2) \\ &+ \lambda_3^2(\alpha_3^2 + \beta_3^2 + \gamma_3^2) \\ &= \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \end{aligned}$$

d. h. unabhängig von der Lage der Coordinatenachsen, in dem für P angestellten Ausdruck ist aber nach das Glied

$$k'(x_x^2 + y_y^2 + s_z^2)$$

vorhanden, das im allgemeinen von der Richtung der Coordinatenachsen abhängig ist. Soll aber P in jedem rechtwinkeligen System unverändert bleiben, so muss

$$k'$$

verschwindend sein, d. h. irgend wie

$$\int dm' F(\rho) (\xi^2 - \eta^2) \xi^2 = k' K$$

unendlich klein sein, damit der Körper ein Isotroper sein könnte, welchem System er auch angehören möchte.

Wir erhalten für einen isotropen Körper

$$\begin{aligned} P &= -K(x_x^2 + y_y^2 + s_z^2 + \frac{1}{2}(y_z^2 + s_x^2 + y_x^2)) + k'(x_x + y_y + s_z)^2 \\ C_{11} &= -k(K + k) \\ C_{12} &= -\frac{k}{2} \end{aligned}$$

Die Constant k ist theoretisch in der That

$$= \frac{1}{2}$$

Es war für jeden um die s -Axe symmetrischen Körper

$$C_{11} = 3C_{12}$$

was auch der Fall für den isotropen Körper ist

$$a_{11} = \frac{\alpha_1}{2} = \int \mu d\tau \xi^4 F(\rho)$$

$$a_{12} = \frac{\alpha_{12}}{2} = \frac{1}{2} \int \mu d\tau \xi^2 \eta^2 F(\rho)$$

Wir führen neue Coordinaten $\xi' \eta' \zeta'$ ein welche so defnirt werden

$$\xi = \alpha_1 \xi' + \beta_1 \eta' + \gamma_1 \zeta'$$

$$\eta = \alpha_2 \xi' + \beta_2 \eta' + \gamma_2 \zeta'$$

$$\zeta = \alpha_3 \xi' + \beta_3 \eta' + \gamma_3 \zeta'$$

wo $\alpha \beta \gamma$ wieder die Cosinusse sind. Er ist

$$\xi^2 + \eta^2 + \zeta^2 = \rho = \xi'^2 + \eta'^2 + \zeta'^2$$

$$C_{11} = \frac{C_{11}}{2} = \frac{1}{2} \int \mu d\tau (\alpha_1 \xi' + \beta_1 \eta' + \gamma_1 \zeta') F(\rho)$$

$$\frac{1}{2} (\alpha_1^4 + \beta_1^4 + \gamma_1^4) \int \mu d\tau \xi'^4 F(\rho)$$

$$+ \frac{1}{2} (\alpha_1^2 \beta_1^2 + \beta_1^2 \gamma_1^2 + \gamma_1^2 \alpha_1^2) \int \mu d\tau \xi'^2 \eta'^2 F(\rho)$$

Da in Folge der Gleichmässigkeit der Struktur des Körpers

$$\int \mu d\tau F(\rho) \xi'^1 \eta'^2 = \int \mu d\tau F(\rho) \xi'^2 \eta'^1 = \text{etc} = a_{12}$$

$$\int \mu d\tau F(\rho) \xi'^1 \eta'^3 = \int \mu d\tau F(\rho) \xi'^3 \eta'^1 = \text{etc} = 0$$

$$\int \mu d\tau F(\rho) \xi'^4 = \int \mu d\tau F(\rho) \eta'^4 = \text{etc} = a_2$$

mithin haben wir auch

$$\frac{\alpha_1}{2} = (\alpha_1^4 + \beta_1^4 + \gamma_1^4) a_1 + 3(\alpha_1^2 \beta_1^2 + \beta_1^2 \gamma_1^2 + \gamma_1^2 \alpha_1^2) a_{12}$$

Es ist nun

$$1 = (\alpha_1^2 + \beta_1^2 + \gamma_1^2) = \alpha_1^4 + \beta_1^4 + \gamma_1^4 + 2(\alpha_1^2 \beta_1^2 + \beta_1^2 \gamma_1^2 + \gamma_1^2 \alpha_1^2)$$

folglich

$$a_1 = (\alpha_1^4 + \beta_1^4 + \gamma_1^4) a_1 + 3a_{12} (1 - \alpha_1^4 - \beta_1^4 - \gamma_1^4)$$

$$a_1(1 - a_1^4 - \beta_1^4 - \gamma_1^4) = 3a_{12}(1 - a_1^4 - \beta_1^4 - \gamma_1^4)$$

folglich

$$a_1 = 3a_{12}$$

mithin

$$C_{11} = 3C_{12}$$

$$K(1+k) = \frac{3k}{2}$$

$$k = \frac{1}{2}$$

ob ein solches Verhältniss herrscht ist eben so zweifelhaft wie der Einwurf dagegen, da jede kleine Heterogeität des Materials stark dieses Verhältniss beeinflussen muss, und man bei jedem Versuche nie sicher sein kann dass, das verwendete Material homogen sei.

Caginard de la Tour (Poggendorff Band 12 S. 518) fand in der That für einen Eisendraht

$$k = \frac{1}{2}$$

Wertheim (Poggendorff B. 78 S. 381) dagegen

$$k = \frac{1}{2}$$

F. Naumann fand allerdings für Eisen den Werth $k = \frac{1}{2}$ und für andere Stoffe näherte sich k nach seinen Versuche dem Wertheimschen Werth. Die Untersuchung von Kirchhoff (Poggendorff Band 108 Seite 369.) haben das negative Resultat geliefert, dass zwischen den beiden Constanten für die isotropen Körper kein constantes Verhältniss stattfindet. Cornu (Comptes Rendus T. 69. p. 333. 1899) fand für Glas durch optisches Mittel, das Verhältniss

$$\frac{1}{2} \left(\frac{1}{2k+1} \right)$$

zwischen 0.22 und 0.26, was den theoretischen Werth $\frac{1}{4} = 0.25$ nahekommt. Mallock (Proc. Rog. Loc. V 29. 157, 1879) fand für einen weichen Stahl das Verhältniss 0.259 aber für andere Stoffe grössere Werthe. W. Voigt (Wiedemann 15. p. 497, 1882) fand für ein galvanisch niedergeschlagenes Kupfer genau den Werth $\frac{1}{4}$. jedoch kann man diese Abweichung keinesweges der Theorie zu Grund liegenden Hypothese zur Last legen. Denn die Frage ist eben noch eine offene, in wie weit die beobachteten Abweichungen der Ungleichmässigkeit des Gefüges des Materials zur Last fällt.

Es mag dem k sein, wie es sein wolle. Es handelt sich darum, das

Holz so zu dimensioniren dass man es als isotrop betrachten kann, dass

$$\int \rho d\tau = \int \rho r^2 F(\rho)$$

als unendlich klein ansehen kann. Ich habe viereckige Stäbchen von Holz bilden lassen von ungefähr 1 cm Quadrat und von der Länge 60 cm und dieselben der Biegung unterworfen, und K auf gewöhnliche Weise bestimmt. Unterscheidet dabei eine Biegung in tangentialer Richtung, und eine Biegung dazu senkrechter radialer Richtung, und nahm an, dass die beiden Biegungen dieselbe Grösse K liefern müssen, wenn das Holz als isotrop betrachtet werden kann.

Das Instrument, das ich angewendet habe, war wie folgt, beschaffen (Fig 1). Eine starke eiserne Bank DD trägt zwei scharfe Keile LL, die man längs einer Rinne in der Bank auf und niedergeschoben werden kann. LL dient dem Holzstäbchen HH als Stützen, CC ist ein Metallrahmen mit einem Keil, und hat eine Spalte, so dass man den Keil genau auf die Mitte des Holzstäbchens legen kann. Ein Spiegel von grosser Brennweite dreht sich auf dem Stifte T, und ruht auf dem Stiftchen S, welches das Plättchen M (Fig 2) berührt, das Plättchen M ruht auf einen Bügel CC, der mittelst der Schraube ∂ am dem Metallrahmen AA auf und niedergeschoben werden kann. Die Schneiden LL können mittelst der Schrauben s in jeder Höhe befestigt werden. Der Metallrahmen (AA) steht mit dem Metallrahmen B in Verbindung, und das Holzstäbchen H ruht auf dem Keil k, und wird durch ein Gewicht G in der Schale herabgezogen, und so gebogen, in dem das Holzstäbchen H gebogen wird, wird S herabgezogen und so wird der Spiegel B gedreht, und das Spaltenbild n wird emporgehoben bis m. Der Winkel i um den der Spiegel gedreht wird gemessen

und

$$\text{tag } i = \text{tag } 2\alpha$$

$$\text{tag } 2\alpha = \left(\frac{mn}{an} \right)$$

der Biegungspfeil δ ist dann annähernd

$$\delta = l \cdot \sin \alpha$$

Wo l die Länge des Stiftchens (S) ist. Indem δ auf und nieder schraubt kann m mit n zum Zusammenfall gebracht werden. Die

Empfindlichkeit des Instrumentes ist dann sehr gross, und ein $\frac{1}{1000}$ Milimeter von dem Biegungspfeile kann leicht bestimmt werden dass manche Übelstände sich zeigen, wie der Einfluss der Eindrücke an der Schneide, die den Holzstäben als Stützen dienen, der Einfluss der Luftfeuchtigkeit.

Standort	Altr	Fallzeit	Breite des Herbstrings	K. gebogen tang	K. gebogen radial	Location
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Thuja obtusa. (Hinoki)

Takaosan	130	März	0,140 cm	$14,03 \times 10^7$	$12,93 \times 10^7$	Kern
—	—	—	0,100 cm	$13,73 \times 10^7$	$13,15 \times 10^7$	„
Gifu	160	unbekannt	0,030	$7,13 \times 10^7$	$6,72 \times 10^7$	„
Kishū	130	November	0,150	$11,70 \times 10^7$	$11,79 \times 10^7$	„
„	„	„	0,170	$11,70 \times 10^7$	$11,70 \times 10^7$	„
„	60	August	0,140	$14,48 \times 10^7$	$14,80 \times 10^7$	„
„	„	„	0,150	$14,37 \times 10^7$	$15,27 \times 10^7$	„

Takaosan	130	März	0,150	$14,86 \times 10^7$	$12,88 \times 10^7$	$\frac{2}{3}$ Kern $\frac{1}{3}$ Splint
„	„	„	0,050	$14,89 \times 10^7$	$13,58 \times 10^7$	Splint
Gifu	160	unbekannt	0,060	$14,26 \times 10^7$	$12,89 \times 10^7$	„
„	„	„	0,090	$6,56 \times 10^7$	$6,25 \times 10^7$	„

Thuja pisifera. (Sawara)

Takaosan	90	December	0,090	$11,16 \times 10^7$	$11,41 \times 10^7$	Kern
„	„	„	0,110	$10,70 \times 10^7$	$11,28 \times 10^7$	„
„	„	„	0,130	$10,75 \times 10^7$	$10,99 \times 10^7$	„
„	„	„	0,080	„	„	Splint
„	„	„	0,080	$11,18 \times 10^7$	$10,16 \times 10^7$	„
„	„	„	0,090	$9,69 \times 10^7$	$8,70 \times 10^7$	„

Standort	Alter	Fallzeit	Breite des Herbstrings	K. gebogen tang	K. gebogen radi	Locati on
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Thuopsis dolabrata. (Hiba)

Kiso	150	unbekannt	1,190	$10,38 \times 10^7$	$10,35 \times 10^7$	Kern
"	"	"	0,260	$10,93 \times 10^7$	$11,61 \times 10^7$	"
"	"	"	0,200	$8,68 \times 10^7$	$8,69 \times 10^7$	$\frac{1}{2}$ Kern $\frac{1}{2}$ Splint
"	"	"	0,170	$9,81 \times 10^7$	$9,36 \times 10^7$	Splint

Sciadopitys verticillata. (Kōyamaki)

Kiso	80	November	0,300	$6,55 \times 10^7$	$6,06 \times 10^7$	Kern
unbekannt	unbekannt	unbekannt	0,200	$10,81 \times 10^7$	$10,87 \times 10^7$	"
Kiso	80	November	"	$6,58 \times 10^7$	$6,43 \times 10^7$	Splint
unbekannt	unbekannt	unbekannt	"	$11,16 \times 10^7$	$11,39 \times 10^7$	"

Cryptomeria japonica. (Sugi)

Takaosan	200	November	0,095	$5,15 \times 10^7$	$5,31 \times 10^7$	Kern
"	"	"	0,070	$5,17 \times 10^7$	$4,98 \times 10^7$	"
Kishu	140	unbekannt	0,120	$6,35 \times 10^7$	$6,04 \times 10^7$	$\frac{1}{2}$ Kern $\frac{1}{2}$ Splint
"	"	"	0,130	$5,15 \times 10^7$	$5,61 \times 10^7$	$\frac{1}{2}$ Kern $\frac{1}{2}$ Splint

Tsuga Sieboldii. (Suga)

Kiso	100	November	1,180 cm	$11,35 \times 10^7$	$11,50 \times 10^7$	Kern
"	"	"	0,210	$9,33 \times 10^7$	$5,64 \times 10^7$	Splint

D. Kitao:

Standort	Alter	Fallzeit	Breite des Herbstrings	K. gebogen angent	K. gebogen radial	Location
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Zelkova accuminata. (Keyaki)

Takaosan	unbekannt	December	0,112	$9,41 \times 10^7$	$9,98 \times 10^7$	Kern
"	"	"	0,158	$10,11 \times 10^7$	$10,77 \times 10^7$	"
"	"	"	0,165	$10,09 \times 10^7$	$10,46 \times 10^7$	"
"	"	"	0,120	$9,54 \times 10^7$	$9,95 \times 10^7$	Splint
"	"	"	0,620	$4,69 \times 10^7$	$5,93 \times 10^7$	"
"	"	"	0,650	$4,53 \times 10^7$	$4,57 \times 10^7$	"

Castanea vulgaris var. *Japonica.* (Kuri)

Takaosan	40	unbekannt	0,126	$13,21 \times 10^7$	$13,36 \times 10^7$	Kern
"	"	"	0,290	$12,00 \times 10^7$	$12,38 \times 10^7$	"
"	"	"	0,280	$12,03 \times 10^7$	$13,21 \times 10^7$	Splint
"	"	"	0,300	$13,07 \times 10^7$	$15,25 \times 10^7$	"

Magnolia hypoleuca. (Hō)

Takaosan	unbekannt	December	0,230	$9,94 \times 10^7$	$11,36 \times 10^7$	Kern
"	"	"	0,250	$10,62 \times 10^7$	$11,04 \times 10^7$	"
"	"	"	0,190	$10,22 \times 10^7$	$11,42 \times 10^7$	"
"	"	"	0,300	$10,15 \times 10^7$	$11,01 \times 10^7$	Splint
"	"	"	0,165	$9,85 \times 10^7$	$10,83 \times 10^7$	"

Fagus sylvatica var. *Sieboldi.* (Buna)

Takaosan	130	December	0,130	$12,16 \times 10^7$	$13,52 \times 10^7$	½ Kern ½ Splint
"	"	"	0,160	$11,41 \times 10^7$	$12,22 \times 10^7$	Splint
"	"	"	0,230	$11,31 \times 10^7$	$12,96 \times 10^7$	"

Standort	Alter	Fallzeit	Breite des Herbstrings	K. gebogen tangent	K. gebogen radial	Location
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Quercus glauca. (Shirakashi)

Takaosan	unbekannt	December	0,160	$11,69 \times 10^7$	$11,71 \times 10^7$	Kern
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Quercus acuta. (Akagashi)

unbekannt	40	unbekannt	0,500	$13,18 \times 10^7$	$13,36 \times 10^7$	Kern
„	40	„	0,350	$12,11 \times 10^7$	$12,68 \times 10^7$	Splint

Quercus thalassica. (Matebagashi)

Nishigahara	25	September	0,45	$12,10 \times 10^7$	$12,19 \times 10^7$	Kern
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Fraxinus Sieboldiana. (Shioji)

Chichibu	48	unbekannt	0,276	$15,02 \times 10^7$	$14,73 \times 10^7$	Kern
„	„	„	0,200	$14,13 \times 10^7$	$14,41 \times 10^7$	„

Panlownia tomentosa. (Kiri)

Nishigahara	7	September	0,400	$4,16 \times 10^7$	$4,21 \times 10^7$	Kern
„	„	„	0,450	$4,62 \times 10^7$	$4,12 \times 10^7$	„

Pinus densiflora. (Akamatsu)

Standort	Alter	Fallzeit	Breite des Herbstrings	K. gebogen auf tangential	K. gebogen auf radial	Location
Mimuneya	95	November	0,123	$10,36 \times 10^7$	$9,03 \times 10^7$	Kern
"	"	"	0,136	$9,01 \times 10^7$	$9,68 \times 10^7$	Splint
Takaosan	50	"	0,180	$11,86 \times 10^7$	$11,52 \times 10^7$	Kern
"	"	"	0,236	$12,73 \times 10^7$	$12,21 \times 10^7$	"
"	"	"	0,350	$10,94 \times 10^7$	$11,31 \times 10^7$	"
"	"	"	0,198	$10,94 \times 10^7$	$11,59 \times 10^7$	Splint
"	"	"	0,175	$10,56 \times 10^7$	$10,47 \times 10^7$	"
"	"	"	0,197	$10,73 \times 10^7$	$10,08 \times 10^7$	"

Abies umbellata. (Momi)

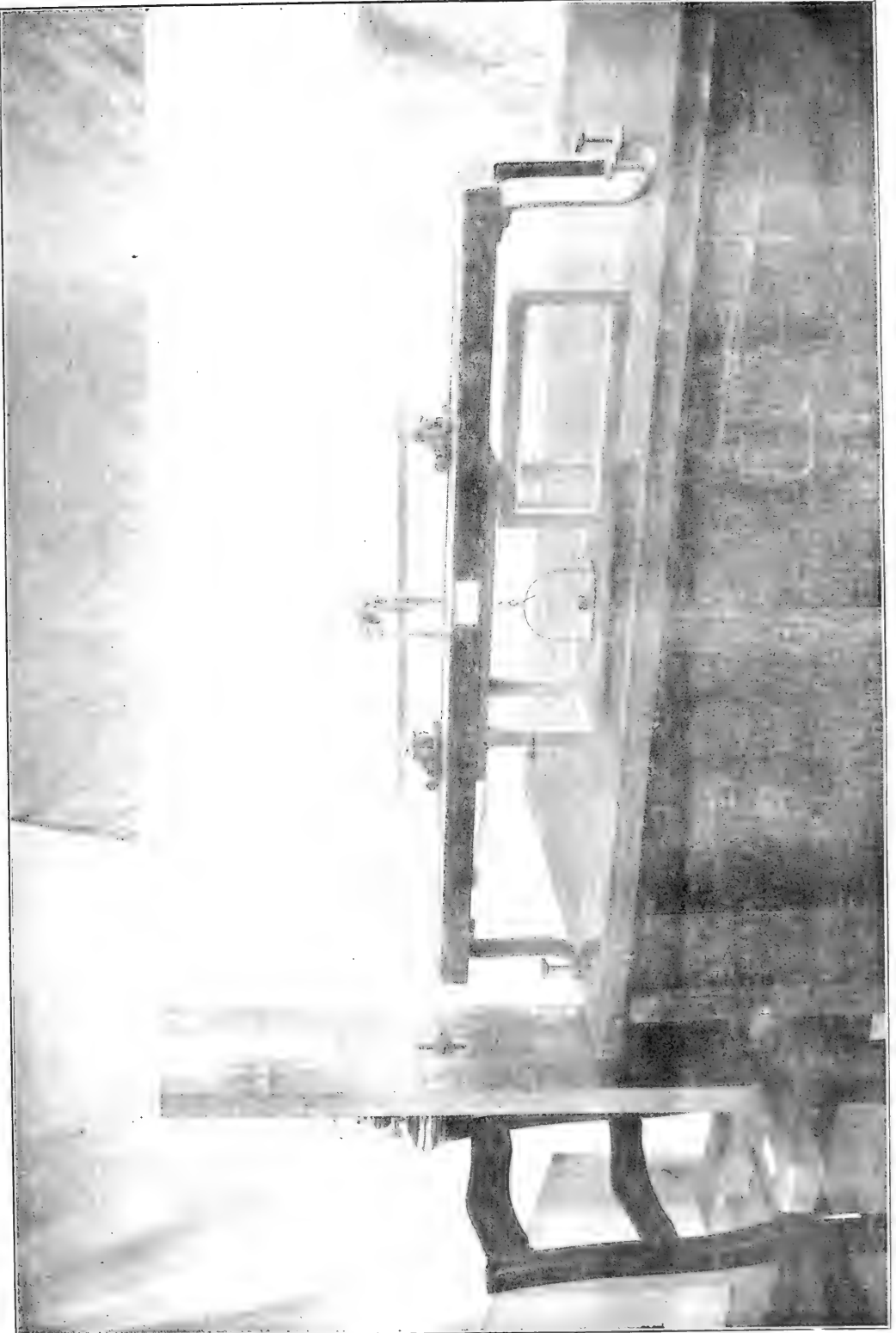
Takaosan	160	December	0,200	$9,34 \times 10^7$	$9,12 \times 10^7$	Kern
"	"	"	0,120	$9,33 \times 10^7$	$9,86 \times 10^7$	"
"	"	"	0,230	$10,48 \times 10^7$	$10,73 \times 10^7$	"
"	"	"	0,170	$10,26 \times 10^7$	$10,14 \times 10^7$	Splint
"	"	"	0,260	$9,67 \times 10^7$	$9,89 \times 10^7$	"
"	"	"	0,360	$10,18 \times 10^7$	$9,76 \times 10^7$	"

Ich habe 6—7 Male Biegungspfeile bestimmt und K für jeden Pfeil des Stäbchens ermittelt und daraus das Mittel genommen. Wenngleich die Stäbchen sich seit zwei Jahren in Zimmer des Laboratoriums befanden, und darum vollkommen lufttrocken sind, so war der Einfluss der Luftfeuchtigkeit auf den Werth von K so gross, dass die erste Decimalstelle sich oft ändert, dass die Luftfeuchtigkeit mehr die Änderung des K verursacht als die Breite des Herbstrings. In sofern die beiden Werthe von K mit der Breite des Herbstrings in keinerlei Zusammenhang stehen, und ihre Differenzen nur durch die Luftfeuchtigkeit veranlasst sein können, können

wir annehmen dass die beiden Werth von K einen und denselben Werth zeigen könnten, wenn der Querschnitt des Stäbchens viel geringer gewesen wäre, als ein Quadrat 1 cm. Ich behalte mir vor, Stäbchen von viel geringeren Querschnitt in Untersuchung zu nehmen.









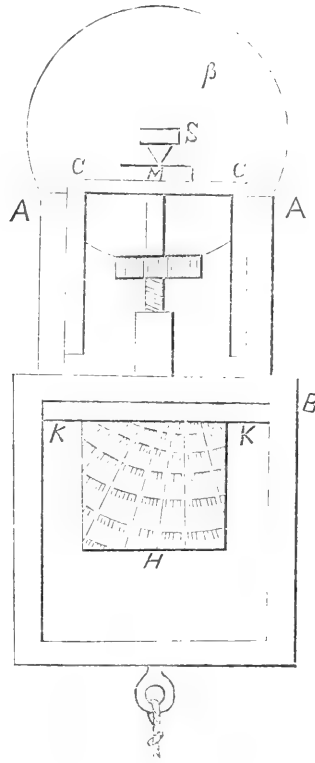


Fig. 2

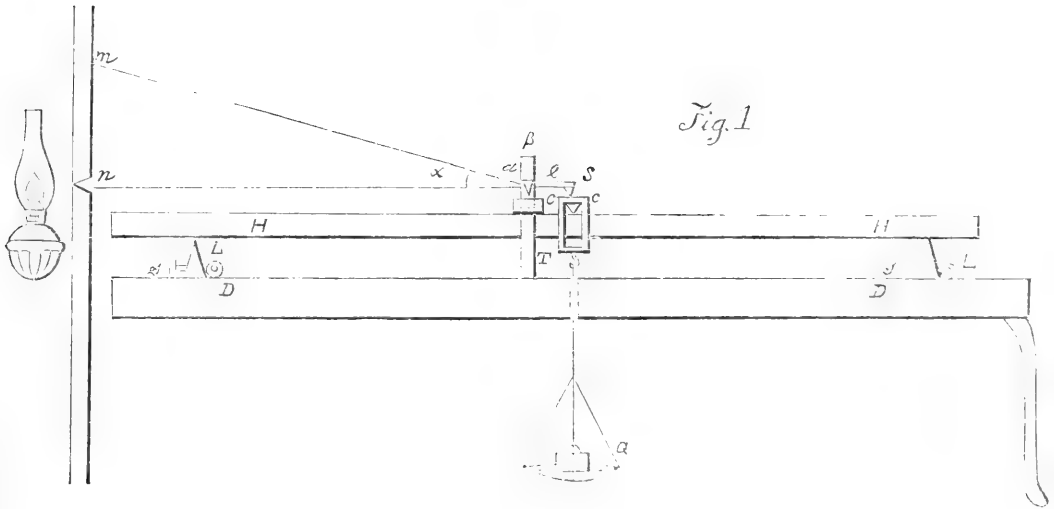


Fig. 1



Studies in the Physiological Functions of Antipodals and the Phenomena of Fertilization in Liliaceae.

I. *Tricyrtis hirta*.

BY

T. Ikeda.

With Plates III—VI.

Introductory.

Recent investigations have brought to light many interesting phenomena relating to the reproduction of Angiosperms. We have an enormous number of papers upon this subject, which are indeed valuable on account of the important morphological data which they contain, but from the physiological point of view, there still remain many problems to be solved; among others, we may cite the physiological functions of the so-called antipodal cells. In fact, the opinions of the authors respecting this problematic organ within the embryo-sac do not agree, and it is easy to see that this divergence of opinion is mainly due to the specific difference of the plants used by the respective investigators. A general conclusion from the phenomena relating to antipodals cannot therefore be drawn until researches on various and widely different types of plants have been made. The investigations of antipodals have till now been restricted to the Ranunculaceae, Leguminosae, Compositae, Gramineae and a few others;¹ and since similar researches on the Liliaceae, which have been so often subjects of investigations in regard to the phenomena of fertilization, were still wanting, except some short remarks by Westermaier² on a few

¹ Westermaier, 1890 and 1896; Osterwalder, 1898; Goldflus, 1898—9.

² Westermaier, 1896.

forms (*Muscari*, *Hyacinthus*, *Allium*), I began to make a study of this group of plants in this respect, not however neglecting to study the phenomena of fertilization. First of all, I took up as the subject of my researches *Tricyrtis hirta* Hook., native in this country. The results of the investigations discussed in this paper still present some gaps, but since, on account of other business, I cannot for a while continue work in this line, I will publish them here as I now have them.

Materials and Methods.

The material was gathered from September till the middle of October 1900, when the plants were in full blossom in our botanical garden. The methods of investigation were as follows:—

1. Free-hand sections of fresh specimens: besides observations on microtome-sections, microchemical reactions were tested on free-hand sections from fresh materials.

2. Microtome-sections: The material was immediately fixed after its collection and serial sections were made according to the ordinary methods.

Flemming's strong and weak solutions, absolute alcohol, and Keiser's sublimate acetic acid mixture were employed as fixing media. Of these, Flemming's weak solution was mostly used, but its action was somewhat inferior to the stronger one, which always afforded excellent results. After dehydration through ascending grades of alcohol, the material was put successively in xylol-alcohol and pure xylol, and then imbedded in paraffine through xylol-paraffine. The sections were cut 5—10 μ thick. For staining, several reagents were used: Flemming's safranin-gentian-violet-orange, Delafield's haematoxylin, haematoxylin-glycerine, Heidenhain's iron-alum haematoxylin, Schaffner's anilin-safranin, his picronigrosin, Baumgarten's acid-fuchsin and methylene-blue, Gram's gentian-violet and fuchsin iodine-green mixture. Besides these, various combinations of stains were sometimes tried. Of these, the first mentioned always proved to be the best, though the others, except Schaffner's reagents, gave pretty good results.

Formation of the Embryo-sac.

The formation of the archesporial cell has nothing extraordinary: it arises as usual at the expense of a sub-epidermal cell, which soon becomes conspicuous by its larger size. In its earliest stage, the archespore has the homogeneous cytoplasm, which is equally distributed therein. Its nucleus, large and spherical, has its single nucleolus suspended in the nuclear reticulum (Fig. 1). No tapetal cell is cut off, so that the archespore develops directly into the embryo-sac-mother-cell. The amount of chromatin, which is rather scanty in the earliest period, soon increases until it forms a thick convoluted spireme thread. Often beautiful mitotic figures are visible in the nuclei of the nucellar cells, but any calculation of the number of the minute and crowded chromosomes was impossible.

The embryo-sac-mother-cell grows into a somewhat funnel-shaped cell, of which the distal end intrudes within the underlying tissue (Fig. 2 and the following). During the growth of the embryo-sac-mother-cell, the chromatin of its nuclei arranges itself as usual in a fine, long, convoluted spireme thread. The nucleolus is still present in the nucleus, which is surrounded by the dense cytoplasm (Fig. 2 and 3). The spireme thread gradually shortens itself, leaving a clear space around it. The cytoplasm, which was homogeneous and dense up to this period, becomes now more or less reticular in its structure, which is probably caused by the rapid growth of the cell (Fig. 4). The convoluted spireme then undergoes a longitudinal splitting (Fig. 4, 5), which is soon followed by its transverse division into chromosomes (Fig. 6), whose number is not exactly known. The microsomes which constitute each chromosome are often clearly visible. Of these, those which are found at both ends of the chromosome gradually enlarge and this expansion of microsome granules is always accompanied by the shortening process of the chromosome, until it becomes condensed into a short dumb-bell-shaped rod and when the condensation proceeds, the final products are nuclear tetrads (Fig. 7); besides, X-, V-, and))- shaped chromosomes are also visible. These chromosomes, which now lie along the nuclear wall or are suspended on

the delicate fibres of the linin-reticulum, arrange themselves on the equatorial plane of the spindle; and then the axis of each chromosome runs parallel to that of the spindle (Fig. 8). Of achromatic figures, the conducting fibres alone are visible, and do not converge towards one point. Of the nuclear tetrads, six were calculated in many cases, and though the number of chromosomes in the nuclei of vegetative cells, for example those in the nucellar cells, could not be counted, yet it is clear that the number of chromosomes in the latter case must be far greater than in the case of the division in the embryo-sac-mother-cell; besides, the mode of division, as before described, is the so-called heterotypic one. From these observations, as well as from a comparison with those of various plants, it is clear that the numerical reduction of chromosomes takes place in the embryo-sac-mother-cell. After the formation of the septum between two daughter cells by the first division (Fig. 10), their respective nuclei undergo a second division, which may take place at the same time or at times differing in the case of each of them. It must be noted that the daughter nuclei produced by the first mitosis never enter the resting stage and soon begin to undergo the next division.

When the second mitosis proceeds, the minute colourless particles as well as the highly stained bodies are found scattered in the cytoplasm (Fig. 11). These bodies might be regarded as fragments of the nucleolus, which do not perform any function in the mitosis—the so-called extranuclear nucleoli.

Of the four sister cells produced by two successive divisions, the upper three obliterate, are gradually driven off in the micropylar direction on account of the active growth of the lowermost one, and at length are visible as cap-shaped pieces directly above the future embryo-sac (Fig. 12). Thus in the formation of the embryo-sac in *Tricyrtis hirta* there takes place a tetrad-division¹ of the first type, as recently stated by Schniewind-Thies.² Guignard,³ in his researches on the embryo-sac formation, studied also *Tricyrtis hirta*; according to him, only one, the lower of the

¹ In the sense of Jucl (cf. Jucl, 1900).

² Schniewind-Thies, 1901.

³ Guignard, 1882.

two daughter cells formed by the first mitosis, undergoes the second, so that only three cells are formed and this mode of development corresponds to the second type of Schniewind-Thies. The divergence between Guignard's results and my own may be explained on the ground that he perhaps overlooked the second division in the upper daughter cell, which might easily have occurred at the time of his investigation (1882), when only free-hand sections could be examined.

Maturation of the Embryo-sac.

The cell destined for the embryo-sac now begins to grow vigorously by the absorption of food materials through the underlying tissue. It enlarges immensely; its growth is not, however, accompanied by an increase of cytoplasm. Its chalazal end, which as before stated, had been prolonged into a funnel-shaped body, gradually penetrates into the underlying tissue in the direction of the median longitudinal axis of the ovule, and serves probably as the haustorium for collecting the nutriment for the embryo-sac. In this case the single nucleus, which is more or less flattened, lies near the neck of this haustorium; as it seems to me probable that the nucleus tends to occupy the best position for the elaboration of food for further development, the position of the nucleus in this stage is the natural consequence of the constructive metabolism in this haustorial part (Fig. 12). The cytoplasm of the embryo-sac in this early stage is small in amount and consists only of a few delicate strands along the wall. Besides there are present a few granular particles, especially plentifully deposited around the nucleus (Pl. VI, fig. 43); they have been proved to be some kinds of dextrine about whose distribution in the embryo-sac and elsewhere the later chapter is to be consulted.

The embryo-sac nucleus in this early stage is relatively deficient in chromatin, which is suspended on the linin-net-work, together with two or three spherical nucleoli (Fig. 12). After a certain period it undergoes the first division and the two resulting daughter nuclei gradually depart toward opposite poles, until at last they reach respectively the two extremities of the embryo-sac (Fig. 13). They are small in size, with a round nucleolus in the centre. At this stage the cytoplasm still remains

unincreased and attached simply to the periphery of the embryo-sac, except around these nuclei, in which dextrine granules are deposited. I have had no opportunity of observing the following two successive divisions of the embryo-sac nucleus, by which eight nuclei are formed and the bipolar grouping of the cells is completed.

The eight nuclei produced by these processes soon become bipolarly grouped at the two opposite ends of the embryo-sac, and both groups of cells show at first no difference whatever from each other (Fig. 13). At the beginning of the bipolar grouping all these nuclei agree in their structures: their chromatin granules are small in amount and are suspended on the achromatic fibres traversing the intranuclear space; they have each a single nucleolus, which sometimes contains a few vacuoles. The cytoplasm of the embryo-sac is finely granular and plentifully provided with fine colourless particles, which are proved by microchemical methods to be some kind of dextrine. The embryo-sac, which is still in rapid growth, possesses large vacuoles in its centre, so that the communication of the egg-apparatus with the antipodals is by means of cytoplasmic strands through the axial portion of the embryo-sac, in which the polar nuclei are suspended. The antipodal cells now fill up the chalazal protuberance of the embryo-sac (Fig. 14). Their cytoplasm becomes soon afterwards very granular and compact having no vacuoles, while on the other hand the egg-apparatus is somewhat deficient in cytoplasmic contents and highly vacuolated. The nuclei of the antipodals are always larger than those of the egg-apparatus, because after the completion of the embryo-sac the former undergoes much more vigorous growth than the latter, until at length, before the time of pollination, the antipodal cells become several times larger than the egg-apparatus.

The union of the polar nuclei also takes place in this stage (Fig. 15). These nuclei as well as their nucleoli are spherical and of immense size, and besides are furnished with some refractive vacuoles. When the two polar nuclei fuse together, the nucleoli of both usually unite at the same time; but sometimes they remain separate long after the union of the nuclei, for I have sometimes met with the embryo-sac nucleus with two large nucleoli, even shortly before fertilization.

The fusion-product of these polar nuclei, i.e. the primary endosperm nucleus, is characterized by its large size and spherical shape, as well as by possessing a large, highly stainable nucleolus (see for example fig. 16, *p.*); the amount of chromatin is much greater than that of either of its two components. The union of the two polar nuclei takes place in the upper half or near the centre of the embryo-sac, but the fusion-nucleus sinks towards the top of the antipodal cells and again takes its route upward when the egg-cell is ready for fertilization. The primary endosperm nucleus, which is at first spherical, gradually becomes elongated and irregular in shape. The amount of chromatin does not show any increment corresponding to its growth, but its single nucleolus (or often two nucleoli) becomes extremely large, and shortly before the entrance of the pollen-tube refractive vacuoles of various sizes are always found therein. Shortly before fertilization, the outline of the nucleus becomes very irregular, often taking the lenticular shape in optical section.

Of the egg-apparatus, the largest is the ovum, which locates itself somewhat eccentrically along the lateral wall of the embryo-sac (Fig. 16). It is always vacuolated in its basal portion and furnished abundantly with certain reserve-materials in the cyto-reticulum. The two synergidae are somewhat smaller than the ovum and also are vacuolated; they have no reserve food material and often persist to the time of the early endosperm formation, but sooner or later they undergo degeneration, usually at the time of fertilization, but sometimes afterwards.

The increase of the cytoplasm of the embryo-sac goes on parallel with its vigorous growth in size, so that the whole space of the fully grown embryo-sac is filled with the homogeneous and compact cytoplasm. This increase of cytoplasmic contents seems to begin after the union of the polar nuclei.

Antipodals, Integuments, Nucellus, Chalaza, etc.

a. Antipodal Cells.

The antipodal cells of *Tricyrtis hirta* never change their original orientation in relation to the embryo-sac, as is the case with *Nigella* as described by Westermaier. He wrote about the protrusion of the chalazal

end of the embryo-sac of *Nigella* towards the underlying tissue as follows¹:—"Es vertieft sich nämlich der Embryosack auf einer Seite an der Basis der „Antipoden," indem Zellen des Knospenkernes resorbiert oder irgendwie verdrängt werden;....." So far the process accords perfectly with ours. In *Tricyrtis hirta*, however, the plane of insertion of the antipodal cells remains unchanged throughout the whole period of development, that is, they are perpendicular to the longer axis of the ovule and are always placed in the funnel-shaped portion of the embryo-sac at its chalazal end. The prolongation of the antipodal cells downwards begins very early, until they attain their maximum length at the time of fertilization or a little later.

Before going further I will describe here an important cytological feature bearing on the nutritive function of the antipodals. In the youngest ones, the cytoplasm is finely granular and compact in appearance. The small nucleus is furnished with a single, highly stainable nucleolus and possesses the scanty chromatin, which remains for a long time in the reticular state (Fig. 14). During development these features undergo a considerable change: when the chalazal end of the antipodal cells becomes more and more elongated downwards their nuclei become highly enlarged and now begins gradually an extraordinary increase of chromatin. The chromatin substance becomes variously aggregated within the nucleus, especially along the inner periphery of the membrane: it forms a number of big extraordinarily dense and consequently highly stainable, usually angular chromatin-masses (Fig. 18). The single nucleolus gradually becomes very much smaller, so that often it is hardly to be distinguished from these chromatin-masses.

The phenomenon of chromatin-aggregation of the nuclei constitutes the most remarkable fact during the development of the antipodals. What may be the significance of this peculiar process? Let us go at first to the literature.

In animal as well as plant cells it has been observed by various authors that gland cells show the phenomena of chromatin-aggregation

¹ Westermaier, 1890, p. 5.

during their activity. Of secretory cells in the animal kingdom we have various interesting examples described in Rosenberg's work on *Drosera*,¹ which will not be repeated here. Turning to vegetable secretory-cells, we have an extensive work on septal glands by Schniewind-Thies. Her conclusion is as follows:²—"The nuclei of secretion-tissue of nectar are always distinguished from those of parenchyma by their containing a large quantity of chromatin..... The abundance of cytoplasm and probably sometimes also the extraordinary size of nuclei in nectar cells stand in relation to their great activity, for they must absorb raw materials necessary for the nectar formation, elaborate the latter by themselves, and transport this nectar outwards....." Huie³, and later Rosenberg⁴, observed the chromatin-aggregation in tentacle-cells of *Drosera* leaves when they are nourished with various substances. Also recently W. Magnus⁵ discovered similar phenomena in digestive cells of the endophytic mycorrhiza of various Orchideae. In his study on *Aconitum Napellus*, Osterwalder observed the same phenomenon in the nucleus of antipodals and brought this into relation with their nutritive activity.⁶ All these observations of various authors as to the significance of chromatin-aggregation, together with other concomitant circumstances, which are to be described later, led me to conclude as probable that *the chromatin-aggregation in the nuclei of antipodals of Tricyrtis is also the expression of their metabolic activity,—that therefore these organs play a most essential rôle in the nutrition of the embryo-sac,—that they are indeed the metabolic centre for the absorption, elaboration, and transportation of nutritive materials of the latter.*

This nutritive function of antipodal cells seems to continue from the time of the full maturation of the embryo-sac till that of the endosperm formation. The antipodal cells change their structure again after fertilization, when assimilation and secretory activities are gradually weakened and approach their end: the big chromatin masses produced by the aggregation now begin to dissolve gradually, so that they become smaller and smaller and the single nucleolus, which has been found

¹ Rosenberg, 1899.

² Schniewind-Thies, 1897.

³ Huie, 1897-1899.

⁴ Rosenberg, l.c.

⁵ Magnus, 1900.

⁶ Osterwalder, l.c.

throughout the former stages, degenerates. The antipodals sometimes elongate upwards along the wall of the embryo-sac and in such later stages their cytoplasm takes a very characteristic feature: it becomes fibrillar and imitates the structure of pancreas-cells in Amphibia, as described by Mathews;¹ the arrangement of these fibrillae is very varied and often they are more or less coiled up, somewhat similar to the case of Amphibia, studied by the same author (Fig. 19,20). According to him,² the pancreas-cell is filled before secretion with metaplasmic zymogen-granules, which disappear during secretion, the cell meanwhile becoming filled with protoplasmic fibrils. The fact that at the end of their activity the antipodal cells become filled with fibrillae, is an interesting cytological feature imitating the behaviour of the pancreas-cells above described. The antipodal cells devoted to the nutrition of the embryo-sac throughout all the developmental stages are finally gradually reduced in size and driven off downwards by the growth of the endosperm; or sometimes, they are seen attached to the lateral wall of the embryo-sac, entirely surrounded by the mature endosperm. In the later period of endosperm-formation, they are more and more carried away downwards, till at last they dwindle away to small flattened pieces (Fig. 48) and finally disappear entirely.

b. Integuments, Funiculus, and Raphe.

The outer and the inner integuments of the ovule are each composed of two layers of cells. The limiting membrane between the outer and the inner integument, as well as that between the latter and the nucellus, which is only one layer thick, are already cuticularized in the earliest stage, except in the micropylar region of the inner integument and the nucellar cap (Fig. 44 and the following). I have tested microtome-sections, made from materials fixed with sublimate-acetic acid mixture and absolute alcohol with chloriodide of zinc, and observed that these membranes are never coloured blue or violet, but always yellowish brown. They are insoluble both in concentrated sulphuric acid and in copper-oxide-ammonia. Through the whole stage of development, their reactions towards such reagents remain unaltered. These cuticularized membranes

¹ cf. Wilson, 1900, p. 44.

² Wilson, l.c., p. 350.

gradually increase in thickness. Therefore, from the earliest period there exists no direct communication between the integuments and the nucellus or the embryo-sac through the limiting membranes. But it is an interesting fact that throughout all stages the micropylar region of the inner integument never undergoes cuticularization; the possible significance of this phenomenon will be discussed later.

The funiculus and the raphe consist each of four structural elements, namely the epidermis, cortical parenchyma, phloem and xylem. In the early period of the embryo-sac development the procambium string composed of cells with much elongated nuclei is designed for the conducting of tissue of nutriment towards the embryo-sac. Subsequently its function is taken up by the vascular bundles, which are themselves developed from this procambium. In the cross-section of the ovary, which at the same time passes longitudinally through the median plane of the ovules (Fig. 21), we can clearly trace the course of the vascular system through the funiculus and raphe. This vascular bundle in the ovule is the branch of the main-trunk running through the ovary in its axial direction.

Before entering into the funiculus the vascular system passes through a special group of cells, which is placed near the placenta (Fig. 21, *pl.*, Fig. 22 *a* and *b*). These cells are characterized by their small size, relatively large nuclei and abundant cytoplasmic contents, and persist unchanged throughout the whole period. The xylem of this vascular system consists only of a bundle of spiral tracheides, while the phloem is formed by a number of long columnar cells with delicate membranes, forming two or three layers around the xylem. The presence of sieve-tubes is doubtful. The cortical parenchyma consists of elongated cells with large rod-like nuclei. These vascular bundles run through the funiculus and their termination is found among a special group of cells in the chalaza. This group of cells, (Fig. 21, *ch.*) similar to that near the placenta, is characterized by their constituents, which are small in size, rich in cytoplasmic contents and furnished with a round and relatively large nucleus rich in chromatin.

Afterwards the nucleus of these cells becomes gradually small and

irregular in shape until the intranuclear space becomes apparently empty; and then the cytoplasmic contents of the cells disappear and are replaced by a hyaline fluid. These cell-groups, in the chalaza as well as near the placenta, are probably the place of the enzyme formation, as will be shown later; the special character of the cells of these groups speaks also for their nature.

c) Nucellus and Chalaza.

When we examine the nucellus in its young stage, we find that its portion underlying the embryo-sac is composed of polygonal or cubical cells, which are arranged in the regular manner, forming only one layer around one axial cell-group. (See Fig. 23, which shows the cross-section of that region of the nucellus). At the beginning there is no visible difference among these cells: they are all characterized by the presence of a large amount of cytoplasm as well as of a huge, spherical nucleus rich in chromatin. Through the rapid growth of the embryo-sac in the direction of the longitudinal axis of the ovule, that portion of the nucellus becomes more and more elongated and narrow.

While the cells of the outer layer retain their original cubical shape, those of the axial group become gradually elongated, until each cell takes a long columnar shape (Fig. 17) and its nucleus becomes correspondingly long. This is due not only to the longitudinal growth of cells, but probably also to the pressure exerted upon them by the surrounding ones. This axial row of nucellar parenchyma has already reached its maximum elongation at the stage of the full maturation of the embryo-sac. It has various names given by many authors, for example "Conducting passage" („Zuleitungsbahn"), "Starch route" („Stärkestrasse") by Westermaier;¹ "Pseudo-chalaza" by Goldflus,² etc. It persists even to the later period of the endosperm-formation, though in a more or less degenerated condition.

Cells of the outer layers, which are characterized by their large nuclei, never become elongated like those of the axial row but undergo gradual decomposition at the time of the maturation of the embryo-sac. This degeneration begins with the decay of delicate, parenchyma cells directly

¹ Westermaier, l.c.

² Goldflus, l.c.

beneath the embryo-sac: at first their nuclei disorganize, then the cytoplasm and and lastly the cell membranes. In this process, when the nucleus begins to disorganize, it becomes highly stainable; its chromatin granules become scattered within it; but no linin-network is visible and no nucleolus is met with. When the nuclear membrane fades away, the nuclear contents are scattered throughout the cytoplasm.

It is very probable that this process of degeneration contributes in no small degree to the endosperm-formation. The products of degeneration may be directly absorbed by the antipodals or conveyed through the conducting passage to the antipodals, which elaborate these materials for the purpose of the endosperm-formation. When the embryo-sac is already fully matured, it has been often observed in preparations made from materials fixed in Flemming's strong solution that an immense aggregation of granular substance is found in the cells of the conducting passage (Fig. 17). It is probable that these granules are the protein matter derived from the degeneration of the nucellar cells above described, precipitated by Flemming's solution during the course of their transit through the conducting passage. It is also evident that certain soluble ferments must intervene for this degeneration of nucellar cells; where these enzymes are formed is not clear, but it is not improbable that they are formed in the antipodals themselves, for the destruction of nucellar cells begins close to the antipodals and proceeds gradually downwards.

The portion of the nucellus, which forms the lateral side of the embryo-sac, is only one cell-layered. It begins to disorganize generally at the time of fertilization, but remains till a pretty advanced stage of the endosperm-formation, though in a degenerated condition; finally it becomes absorbed evidently into the embryo-sac.

For a long time the antipodals have been considered to be merely rudimentary prothallial cells. It is to Westermaier that is due the honour of having proved for the first time their important nutritive function on the basis of anatomical structures and microchemical reactions of various well selected examples.¹

¹ Westermaier, l.c.

Osterwalder, in his study of *Aconitum Napellus*, confirmed Westermaier's view on the ground of the particular situation of antipodals, their cytological feature, as well as the structure of the basal portion of the nucellus.¹ The opinion of Miss. Goldflus on the antipodals of the Compositae is also in perfect accordance with Westermaier's view:² her conclusion is based chiefly on the anatomical structure of the neighbouring tissue of the antipodals. Miss. Balicka-Iwanowska, on the contrary, in studying the embryo-sac of certain Gamopetalae, attributes little importance to the nutritive function of antipodals; for she found that "the antipodals, when they exist at all, seem to have a transitory function, possess mostly poor contents, and disappear very quickly."³ Campbell,⁴ in his study of the embryo-sac of *Lysichiton* and *Sparganium*, observed an extraordinary multiplication of antipodals, whereupon he came to the probable conclusion that they must play an important rôle in the nutrition.

The divergence of opinions about the function of antipodals is, as already stated in the Introduction, no doubt due to the specific difference of the plants used by the various authors.⁵ For while in some plants they represent the most important nutritive organs, in others they may be mere functionless prothallial cells. Whether they are important in the nutrition is therefore to be decided only upon the basis of detailed studies of each particular case, so that to infer their importance solely on the ground of their extraordinary multiplication, as Campbell does, would not be justified, as Miss. Sargent⁶ states quite rightly in her recent interesting paper.

The nutritive function of the antipodals of *Tricyrtis hirta* will, I think, be concluded on the basis of various facts described till now, though necessarily more or less hypothetically. These facts are:—

¹ Osterwalder, l.c.

² Goldflus, l.c.

³ Balicka-Iwanowska, 1899, p. 68.

⁴ Campbell, 1899.

⁵ Guignard says for example as to the Leguminosae: "Les antipodes disparaissent souvent avant la fécondation, par suite de la résorption du tissu nucellaire sous-jacent; d'ailleurs leur rôle, encore assez problématique, paraît terminé peu de temps après leur formation; dans d'autres plantes, au contraire, on les voit s'accroître d'une façon notable, même après la fécondation." (1881, p. 200.)

⁶ Sargent, 1900.

1.) The phenomena of chromatin-aggregation in the nuclei of the antipodals.

2.) The formation of a bundle of long columnar cells in the portion of the nucellus below the antipodals, the so-called "Conducting passage," which extends to them on one side and to the special cell-group in the chalaza on the other. On account of the long columnar shape of the cells, as well as their situation in relation to antipodals, etc., they are evidently to be considered as the means of transmission of food material, both carbohydrates and protein matter.

3.) The termination of the vascular bundles of the funiculus within the cell-group in the chalaza, indicating the transportation of raw material from the exterior to that place.

4.) The cuticularization of the limiting membrane between the inner integument and the nucellus at an early period, so that food material from the exterior enters the embryo-sac chiefly through the vascular bundles of the funiculus, the cell-group in the chalaza, the conducting passage, and the antipodals.¹

It is to be noted that not only do the antipodals elaborate food material for the endosperm-formation, but also for the growth of the egg-apparatus. For in the young embryo-sac, no sooner are the ovum and synergidae differentiated, than the antipodals begin to show their characteristic cytological feature, the conducting passage begins to be formed, and at the same time the egg-apparatus is active in increasing its cytoplasmic contents, so that the material for this growth evidently comes through the antipodals and the conducting passage.

When we take into account all these facts, we are led to the conclusion already stated that the antipodals play a most essential rôle in the nutrition of the embryo-sac, for not only do they absorb raw material and transmit it, but also they elaborate it into a proper form.

¹ In various plants investigated till now by many authors for example, in Compositae, according to Goldflus, the membrane of the embryo-sac is cuticularized, so that all food going to the latter must pass the antipodals. In *Tricyrtis hirta*, as just stated, the limiting membrane between the nucellus and the inner integument is cuticularized but *the cell-wall of the embryo-sac itself is not*, so that though food materials from the exterior must pass generally through the antipodals, it is not impossible that the nucellar cells forming the lateral side of the embryo-sac, when degenerated, are absorbed directly by the latter.

This conclusion drawn only from morphological facts, is confirmed by microchemical reactions, which are treated of in the next chapter.

Microchemical Observations.

The microchemical reactions were made on fresh ovules, collected between 1-2 p.m.

a.) Soluble Carbohydrates.

Of soluble carbohydrates the microchemical identification of cane-sugar and glucose in the ovule was tried. According to Molisch,¹ α -naphthol and thymol were employed for this purpose.

After the treatment of free hand sections of ovules, chiefly longitudinal ones, with an alcoholic solution (20%) of α -naphthol under the cover-glass, a few drops of strong sulphuric acid are added.

Within at most two minutes, the nucellus, the chalaza and the micropylar region of the inner integument, are coloured deep violet. The vascular bundles in the funiculus as well as in the raphe are coloured at first greenish blue, but become deep violet only after half an hour; this delay of the reaction is probably due to the fact that the penetration of the reagent to the vascular bundles is made possible only after the death of the surrounding tissue. The foregoing experiment is not however sufficient to demonstrate the presence of soluble carbohydrates, because concentrated sulphuric acid may split sugars from glucosides, change starch and cellulose into sugar and thus may give rise indirectly to the same colour reaction.² Hence for the purpose of control, living specimens were heated at first with boiling water for a short time under the cover-glass and then treated with α -naphthol and strong sulphuric acid. The reaction in question occurs in the same manner, but markedly later than before, often after half an hour or more, so that it is highly probable that in the living materials of the ovule, there exists at least some kind of soluble carbohydrates.

The reaction towards Fehling's solution was very different from what was expected. When the sections, after the treatment with this reagent,

¹ Zimmermann, 1892.

² Zimmermann, l.c. p. 73.

are carefully and not too strongly heated, until little bubbles begin to be formed, the nucellus, the chalaza, and the inner integument, are coloured slightly violet ; but no red precipitates of copper suboxide are observed, as might be expected.

This reaction is no doubt due to the so-called Biuret reaction, caused by the presence of albuminous matter within the cell contents.

From the foregoing experiments, as well as from the widely different opinions of various authors, it is impossible to determine what kind of soluble carbohydrates is present in the ovule, but it seems very probable to me that some soluble carbohydrates, such as sugars are there present, which have been transformed from insoluble ones, such as starch ; especially the intense violet reaction of the nucellus, the chalaza, and the micropylar region of the inner integument towards Molisch's test, points to the probable occurrence of the saccharification-process in these particular portions of the ovule. The accounts of this process must be given later.

b.) Starch and Dextrines.

In order to ascertain the distribution of starch and dextrines through all the developmental stages of the ovule, the microtome-sections made from materials fixed with absolute alcohol and Keiser's sublimate and acetic acid mixture, were treated with the chloriodide of zinc, instead of potassium iodide solution, in order to obtain a rapid reaction.

Some idea of the distribution of starch and dextrines throughout all stages may be obtained by means of the several diagrams in Pl. VI.

In the period of the archespore (Fig. 40) no starch reaction is obtained within the ovule. Starch grains are found in the wall of the ovary, except in its epidermis ; they are probably the products of carbon-assimilation in these places themselves, where chlorophyll grains are abundantly present. These starch grains become gradually smaller both in amount and in size towards the ovule, which is entirely free from starch and so is coloured only yellow by chloriodide of zinc.

As the development proceeds (Fig. 41), starch grains appear in the epidermis of the ovule, in the funiculus and the raphe, as well as in the inner layer of the outer integument, so that the conclusion is highly

probable that starch in the ovarian wall penetrates into these parts in some dissolved state, and is here again transformed into starch.

Through the period of the development of the ovule, starch penetrates more and more into various parts of the ovule evidently by the same process, so that the outermost layer of the ovule, the inner layer of the outer integument, the funiculus, the outer layer of the inner integument and the nucellar cap come to possess starch grains, though in the two latter parts the quantity of starch is scanty (Fig. 42). In the earliest stage of the development of the embryo-sac (Fig. 43) the distribution of starch is almost identical with that in the preceding stage, except for the presence of a few grains in the inner layer of the inner integument; but the relative amount of starch is widely different. For, as is shown in Fig. 43, the inner layer of the outer integument and the nucellar cap become richer in starch.

At the same time fine grains which are coloured red (somewhat tinged with brown) by the chloriodide of zinc, are found aggregated around the nucleus; they are evidently some kind of dextrine and are derived from starch, which has been absorbed into the embryo-sac in some soluble form.

In the following stage (Fig. 44), the distribution of starch in the ovule is nearly the same as in the foregoing. In the embryo-sac, the dextrine granules are visible in both the egg-apparatus and the antipodal cells. Starch in the inner integument disappears and minute dextrine granules appear in the micropylar region of the inner integument.

After the union of the polar nuclei (Fig. 45) the distribution of starch is almost the same as that in the foregoing stage, but the number of dextrine grains deposited in the tissue of the inner integument surrounding the micropyle, gradually increases. These granules are found in the parietal cytoplasm of the embryo-sac as well as in the ovum, but they are never observed in synergidae.

Later, when the embryo-sac is ready for fertilization (Fig. 46), the relative distribution of starch grains in various parts of the ovule still remains unaltered, but their amount in the funiculus and the raphe has been largely augmented, while at the same time their great decrease in the outermost layer of the ovule and their certain decrease in the inner layer of the outer integument, is to be noticed. The amount of dextrine granules

in the micropylar region of the inner integument attains its climax in this stage ; besides the amount of them within the egg-cytoplasm has gradually increased. As to the dextrine granules aggregated in the micropylar region of the inner integument, it is very probable that they serve for the nutrition of the pollen-tube, which makes its way into the micropyle along the ovarian wall. Various reasons speak for this probability : they attain their maximum amount just before fertilization (Fig. 46) and disappear soon after it ; also the fact of the non-cuticularization of the micropylar region of the inner integument (cf. the foregoing chapter) is in favour of this hypothesis.

When fertilization is over and the endosperm nucleus begins to divide (Fig. 47) the distribution of starch grains becomes gradually modified. In general, their amount decreases, especially in the outermost layer of the ovule and the inner layer of the outer integument. The dextrine granules deposited [in the egg-cytoplasm increase in amount concurrently with development of the embryo ; they are probably used for the purpose of egg-nutrition and kept in the cytoplasm as reserve materials.

When the formation of the endosperm proceeds, the decrease of the reserve starch grains in various parts of the ovule is immense. Except the small quantity in the outer integument and the pretty large amount in the funiculus and the raphe, no starch grains are found. The amount of reserve dextrine in the embryo increases gradually. The fertilized ovum remains undivided, until an immense quantity of the endosperm is produced. At this stage of the endosperm-formation (Fig. 48), the antipodal cells already show signs of disintegration and remain attached to the endosperm as flattened discoidal pieces. The supply of nutriment for the purpose of endosperm-formation is furnished by the conducting passage, already in the process of degeneration. Even in this stage, no starch grains are visible in the endosperm.

When ripening is near (Fig. 49), the relative distribution of starch becomes distinctly altered. No starch or at most only a trace of it is found in various parts of the ovule, except in the inner integument and the endosperm tissue where it is abundantly present. On account of the growth of the endosperm, the funicular portion and consequently its vascular

bundles and cortical parenchyma now become so extremely compressed as to obliterate their lumen. The passage of nutriment from outside would not therefore take place through such elements; the only possible way is probably the epidermis.

The only noticeable fact, which has not taken place in all the foregoing stages, is the immense aggregation of starch in the inner integument. This phenomenon, which is seen only in the latest period of the endosperm-formation, has possibly some connection with the subsequent development of the endosperm after the supply of food from outside has been stopped.

The aggregation of starch grains in the endosperm begins at first in the micropylar end of the embryo-sac and then proceeds toward the chalazal end.

From what has been described up to this point, it will be seen that starch has never been met with in the chalaza, the antipodals, or the whole nucellus (including the conducting passage) except the nucellar cap. As already stated, starch formed in cells of the ovarian wall is transported into the ovules, where it is again transformed into the original form and deposited in various tissues of the ovule. This reserve starch afterwards undergoes again the chemical change into soluble form and is transported into the embryo-sac. The place where the diastatic enzyme, which intervenes during this transformation, is formed, is the cell-group in the chalaza, which, as above stated, is characterized by the smallness and scanty cytoplasmic contents of its constituents; and water necessary for this hydrolysis may be supplied by the vascular bundle and especially by the spiral tracheïdes, which terminate amid the chalaza in somewhat knot-like enlargements. For the same reason, starch in the ovarian wall may change into soluble form by the action of diastase, which probably is secreted by a special cell-group near the placenta, characterized also by the smallness and rich cytoplasmic contents of its constituents;¹ water necessary for its hydrolysis may be supplied also by spiral tracheïdes, which run through the centre of this group of cells.

The chalaza and the conducting passage, which are always free from

¹ Billings (1901) has recently applied to this cell-group the name of "nutritive tissue" („Nährgewebe“).

starch, show the intense violet reaction in the living state, so that they possess soluble carbohydrate instead of starch.

This result is in contradiction to the statement of Westermaier, who always found starch in the conducting passage, so that in our case the name "starch route" („Stärkestrasse") given by him to this tissue must be changed into "sugar route."

Fertilization.

The so-called "double fertilization" takes place in *Tricyrtis hirta*.

No generative nuclei with coiled or vermiform shapes as discovered by many authors were observed in my preparations, but as the male nuclei in their free state in the embryo-sac were not found, it is not yet decided whether they are not vermiform from the beginning, as is the case with *Endimyon* investigated by Guignard.¹ Besides the accurate observation of both sperm nuclei in the pollen-tube was not possible on account of the too deep staining of the contents of the latter.

The two generative nuclei discharged from the pollen-tube take their course to unite with the egg-nucleus and the primitive endosperm nucleus respectively. Though direct observation is wanting, it is almost undoubted that the considerable changes in the structure, size, and shape of these generative nuclei must have taken place during this transition through the embryo-sac. The egg-nucleus, ready for fertilization, is always flattened or lenticular in its optical section; it has a single nucleolus in its centre, and is scanty in chromatin. The ovum is poorer in cytoplasm than the synergidae, but is always richly loaded with dextrine granules (Fig. 50); even after the fusion with the male nucleus, no change in the physical consistency of cytoplasm and nucleus in the ovum takes place. The nucleus of the synergidae, which is greatly reduced in size just before fertilization, lies in the dense granular cytoplasm.

The union of both germ nuclei is at first a mere apposition; the apposed nuclei become flattened against each other and compressed to a certain degree, so that the amount of chromatin seems to show a

¹ Guignard, 1899.

relative increment. The size and shape of the male and female nuclei in the state of apposition are almost identical. No structural difference was observed between them, except the absence of the nucleolus in the former (Fig. 24, *e.n.* and *g.n. 1*). The septum of the apposed nuclei then gradually fades away and the fusion of both nuclei is accomplished. The resulting nucleus with a single nucleolus as before becomes more and more spherical (Fig. 25). The egg-cytoplasm possesses the alveolar structure as before fertilization and then the ovum grows in size. It remains entirely or almost without growth long after the fertilization, even till after the formation of many nuclei from the endosperm nucleus (Fig. 33). During this development, the nucleolus of the egg-nucleus becomes divided into two or three pieces and prepares for the subsequent division. The synergidae, one of which was observed to be still alive at the time of fertilization, after it, undergo, sooner or later the process of destruction.

The second generative nucleus discharged into the embryo-sac makes its course towards the primitive endosperm nucleus, and during this time an enormous change in its size and shape, as well as in its structure, seems to take place. The second generative nucleus is much larger than the other, being sometimes equal to that of the primitive endosperm nucleus (Fig. 24, *p.* and *g.n. 2*). Its shape is also similar to that of the latter, being sometimes ellipsoidal or cone-shaped. The paternal and maternal chromatin elements of the resulting nucleus are distinguishable long after the fusion.

The absence of the true nucleolus in the generative nucleus is here also to be noted. Vacuoles are sometimes present. The primitive as well as the definite endosperm nucleus is extraordinarily large and the single nucleolus is of huge size. The fusion nucleus is always irregular in contour; and towards the side of fusion there seems to be a denser aggregation of chromatin.

Formation of the Endosperm.

The most remarkable facts brought out in the study of the endosperm-formation are the manner of its formation and the behaviour of the endosperm nuclei.

After a certain period of rest the definitive endosperm nucleus begins to divide according to the ordinary mode of mitosis. During these changes, the giant nucleolus becomes gradually reduced in size (Fig. 27 *a* and *b*) and finally disappears. The outline of the two resulting daughter nuclei is very irregular, always taking more or less long rod-like shapes, and we find here at the beginning several, usually spherical nucleoli (Fig. 28). Then they divide both at the same time (Fig. 29) and thus give rise to four daughter nuclei (Fig. 30).

In the latter nuclei as well as in those derived by later divisions, we find in the resting stage the nucleoli of various sizes. They are at first spherical but later they develop pseudopodia-like processes (Fig. 30) and begin to break up into several pieces (Fig. 31, 33, 34), so that there are often seen some nucleolar fragments scattered within the nuclear cavity. As chromatin begins to increase the nucleoli begin to draw back their pseudopodia-like processes. Even in the advanced stage of nuclear division nucleolar fragments are found scattered near the nuclear spindle, (Fig. 36 *a*). The whole behaviour of the nucleolus above described then corresponds no doubt to what was observed by Zimmermann¹ during the nuclear division in various plants and led him to the erroneous conclusion „Omnis nucleolus e nucleolo.”² For example, our figure 36 *a* corresponds exactly to his figure 32 (cell from the stem-apex of *Psilotum triquetrum*), and also our figure 32 somewhat resembles his figure 27 (cell from the root-apex of *Vicia Faba*) or 38 (spore-mother-cell of *Equisetum palustre*).

During these periods of development, the nuclei themselves become elongated and assume various remarkable forms, resembling those concerned in amitotic division (Fig. 34).

Their mitotic division was often met with, where the arrangement of chromosomes in the equatorial plane was pretty irregular (Fig. 36 *a* and *b*).

The peculiar forms assumed by the endosperm nuclei, as above described, might perhaps give rise to the erroneous conclusion that they were concerned in amitosis, but as real mitosis was observed repeatedly, it is highly probable that this phenomenon is to be considered as that of

¹ Zimmermann, 1883.

² Zimmermann, 1896. p. 64.

surface extension for the purpose of metabolic interchanges between the nucleus and the cytoplasm. Phenomena which might be included in the same category have been observed in both animal and vegetable cells. Of the former, we may cite the well-known discovery of Korschelt in the water-beetle *Dytiscus*: the egg contains at a certain period the dense granular nutritive masses, which are believed to have come from outside; the germinal vesicle becomes amoeboid, sending out long pseudopodia, which are always directed towards the principal mass of granular substances.¹ Of the vegetable cells, Kohl² observed in the living nuclei of marginal cells in the leaves of *Elodea canadensis*, as well as in those of the hair-cells in leaves of *Tradescantia virginica*, that by the action of an asparagine solution these nuclei are incited to make amoeboid movements, and he attributes this phenomenon to an energetic interchange between the nuclei and the cytoplasm.

The endosperm nuclei, thus formed by the mitosis are uniformly distributed throughout the granular, compact cytoplasm, and after a long time when the embryo has become nearly ripe the membranes are formed between these nuclei, and thus the formation of the endosperm is completed.

Even when seeds are near ripening, these nuclei are characterized by their curious shape (Fig. 37); they are scanty in chromatin and furnished with several round nucleoli, but finally they take the usual shape and undergo the ordinary mode of karyokinesis. Each endosperm cell possesses scanty cytoplasm, but is filled with an immense amount of starch grains (Fig. 39 *a, b*). The formation of cell-membranes between these endosperm nuclei begins at the micropylar region of the endosperm and proceeds towards the chalazal portion. This always happens after the full aggregation of cells with starch.

The embryo remains very small and shows no differentiation, even when the seeds are almost ripe (Fig. 38).

From what has been described before, we see that the endosperm formation of *Tricyrtis* differs widely from the ordinary course. For it is a

¹ See Wilson, 1900, p. 349-350.

² Kohl, 1897.

well-known fact that usually during this process the cytoplasm of the embryo-sac forms at first a thin parietal layer, where successive nuclear divisions take place, and then afterwards the hollow inner space is gradually filled up. But here the embryo-sac is from the very beginning filled up with dense cytoplasm and the nuclei formed by successive divisions are scattered in it uniformly. Such a mode of development seems, according to Lloyd,¹ to take place also in *Vaillantia hispida* belonging to the Rubiaceae.

Summary.

1. The archespore arises as usual from a subepidermal cell and develops directly into an embryo-sac-mother-cell. Then two successive divisions occur and thus four cells are formed, of which the lowest one develops into the embryo-sac, while the three above become obliterated.

2. Soon after the maturation of the embryo-sac, the union of two polar nuclei takes place.

3. The antipodals are prolonged downwards towards the funnel-shaped haustorial part of the embryo-sac at its chalazal end. The nucleus of the antipodals is at first scanty in chromatin, but soon it begins to show the phenomena of chromatin-aggregation. This is due to metabolic activity, so that when their activity approaches its end, the chromatin-masses begin gradually to dissolve away.

4. Of the nucellar cells, which are placed at the basal portion of the embryo-sac, those of the axial row soon take a long columnar shape and form the so-called "conducting passage." According to the microchemical test, the latter is always free from starch and seems to contain soluble carbohydrates. The conducting passage continues to a special cell-group in the chalaza, in which the vascular bundles of the funiculus terminate. These vascular bundles pass through another cell-group near the placenta and unite with the main trunk of the vascular bundles of the ovary. When we take these anatomical structures as well the microchemical reactions into account, we can imagine the mode of transmission of starch. It is transformed into soluble carbohydrates by diastase secreted probably by these

¹ Lloyd, 1899.

cell-groups near the placenta and the chalaza, then the soluble carbohydrates pass through the funicular vascular bundles and the conducting passage respectively and are absorbed into the antipodals, which either elaborate them there into their proper form or transmit them to the proper place.

5. The nucellar cells surrounding the conducting passage degenerate probably on account of enzymes secreted by the antipodals. These products of degeneration may be absorbed directly by the antipodals or indirectly through the conducting passage and are used for the nutrition of the embryo-sac. The immense number of granular masses met with in the conducting passage is derived probably from these degeneration-products.

6. All these facts—the cytological features of the antipodals, the anatomical structure of the neighbouring tissue, especially the formation of the conducting passage, as well as the results of microchemical tests—all justify the conclusion that the antipodals in *Tricyrtis hirta* are the centre of the absorption of raw materials, their elaboration into the proper form and the means of the transmission of food to the proper place.

7. During development, dextrine granules are deposited in the antipodals, the ovum, the parietal cytoplasm of the embryo-sac, and in the micropylar region of the inner integument. They are evidently the reserve material. As to the dextrine granules in the micropylar region, there are various reasons for the hypothesis that they serve for the nutrition of the pollen-tube, which passes through this region.

8. The so-called "double fertilization" takes place. Whether the generative nuclei are vermiform or not, is not yet decided.

9. During the endosperm-formation, the nuclei take various curious shapes. This is probably for the purpose of surface extension, due to the metabolic activity between cytoplasm and nuclei.

10. The mode of endosperm-formation differs greatly from the ordinary one in this that the embryo-sac is from the beginning filled with the compact cytoplasm and successive nuclear divisions occur within this cytoplasm.

This work was carried on in the Botanical Laboratory of the College of Agriculture of the Imperial University of Tokio, under the guidance of Professor Ikeno, to whom I have therefore in the first place to express my sincere gratitude. I am also indebted to Prof. Ishikawa for his valuable counsel throughout the progress of the work.



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EXPLANATION OF FIGURES.

PLATE I.

- Fig. 1—10. Various stages of the first nuclear division of the embryo-sac-mother-cell. Fig. 9 and 10, Zeiss, homogeneous immersion $\frac{1}{2}$ and ocular 3, all others the same obj. and oc. 4.
- Fig. 11. Second nuclear division of the embryo-sac-mother-cell. $3 \times \frac{1}{2}$.
- Fig. 12. Beginning of the embryo-sac with three degenerating sister-cells. $3 \times \frac{1}{2}$.
- Fig. 13. Embryo-sac with two nuclei. $2 \times \frac{1}{2}$.
- Fig. 14. Embryo-sac with two polar nuclei. $3 \times \frac{1}{2}$.
- Fig. 15. Two polar nuclei concerned in copulation. $2 \times \frac{1}{2}$.
- Fig. 16. Mature embryo-sac. *o*, ovum; *syn.*, synergidae; *ant.*, antipodals. Nucleolus of primary endosperm nucleus extremely large! $4 \times \frac{1}{2}$.
- Fig. 17. Cells of the conducting passage with protein granules. *ant.*, antipodals. $3 \times \frac{1}{2}$.
- Fig. 18. One antipodal short after fertilization. Chromatin-aggregation in the nucleus! $4 \times \frac{1}{2}$.
- Fig. 19. Two antipodals. Later stage than that of Fig. 18. Chromatin-aggregation less remarkable. Fibrillar structure in cytoplasm! $4 \times \frac{1}{2}$.
- Fig. 20. One antipodal later than in Fig. 19. Fibres in cytoplasm now becoming very distinctive. Leitz. $4 \times \frac{1}{2}$.

PLATE II.

- Fig. 21. Cross-section of an ovary with two ovules longitudinally cut. *ch.*, chalaza; *f.*, funiculus; *o. w.*, ovarial wall; *pl.*, cell-group in the placenta. $2 \times A$.
- Fig. 22. a. The same in cross-section, showing the cell-group in the placenta. $2 \times D$. b. The cell-group in the placenta much magnified.
- Fig. 23. Cross-section of an ovule. *ax. c.*, axial cell-group; *n. p.*, parietal layer of the nucellus; *i. i.*, inner integument; *o. i.*, outer integument; *ep.*, epidermis. Leitz 4×4 .
- Fig. 24. Double fertilization. *e. n.*, nucleus of the ovum; *g. n. 1*, first generative nucleus; *g. n. 2* second; *p.*, primary endosperm nucleus. $3 \times \frac{1}{2}$.
- Fig. 25. Second generative nucleus and primary endosperm nucleus in fusion. The fertilization of the ovum already accomplished. $3 \times \frac{1}{2}$.
- Fig. 26. Secondary endosperm nucleus in spireme stage. $2 \times \frac{1}{2}$.
- Fig. 27. a. b. The same in anaphase. Two consecutive sections. $3 \times \frac{1}{2}$.
- Fig. 28. Two daughter-nuclei derived from the first endosperm division. $3 \times \frac{1}{2}$.
- Fig. 29. Two above daughter nuclei dividing. *p. t.*, pollen-tube; *o*, ovum. $2 \times \frac{1}{2}$.
- Fig. 30. Endosperm formation. Nucleoli with pseudopodia-like processes. $4 \times \frac{1}{2}$.
- Fig. 31. The same. Advanced stage. Nucleoli fragmenting.
- Fig. 32. The same. Advanced stage. Nucleus having peculiar shape. $4 \times \frac{1}{2}$.
- Fig. 33. The same. Nucleoli fragmenting. *o*, fertilized ovum.

PLATE III.

- Fig. 34. Endosperm-formation. Nuclei of various remarkable shapes. $4 \times \frac{1}{2}$.
- Fig. 35. The same. Nuclei in spireme stage. $4 \times \frac{1}{2}$.

Studies in the Physiological Functions of Antipodals, etc.

- Fig. 36. The same. Nuclear division. a. side-view ; b. polar view. $3 \times \frac{1}{1\frac{1}{2}}$.
Fig. 37. The same. Cell-walls already formed. Nuclei of remarkable forms. $4 \times \frac{1}{1\frac{1}{2}}$.
Fig. 38. Embryo in almost ripe seed ; no differentiation ! $4 \frac{1}{1\frac{1}{2}}$.
Fig. 39. Nuclei in later stage of endosperm formation. a. Resting nuclei. b. Upper cell with the nucleus in dispireme stage ; in the lower one the longitudinal division of chromosomes figured. Cells filled with starch grains. $4 \times \frac{1}{1\frac{1}{2}}$.

PLATE IV.

Figs. 40—49, schematic representations of various stages of development of the ovule.

Abbreviations :

o. w., ovarial wall ; *e. i.*, external integument ; *ar.*, archespore ; *i. i. m.*, micropylar region of the inner integument ; *p. n.*, polar nucleus ; *p. e. n.*, primary endosperm nucleus ; *nu. c.*, nucellar cap ; *i. i.*, inner integument ; *nu.*, nucellus ; *o. e.*, epidermis of the ovule ; *cu.*, cuticularized membrane ; *ch.*, chalaza ; *f.*, funiculus ; *ov.*, ovum ; *syn.*, synergidae ; *e. n.*, endosperm nucleus ; *ant. des.*, disorganized antipodals ; *des. e.*, disorganized sister cells of the embryo-sac ; *rh.*, raphe ; *p. t.*, pollen-tube.

- Fig. 40. Archespore formation.
Fig. 41—42. Stages of the embryo-sac-mother-cell.
Fig. 43. Embryo-sac formation.
Fig. 44. Polar nuclei not yet united.
Fig. 45. Polar nuclei already united. Dextrine granules in the ovum and around the synergidae.
Fig. 46. Fully matured embryo-sac.
Fig. 47. Short after fertilization.
Fig. 48. Endosperm formation.
Fig. 49. Later stage of endosperm formation.
Fig. 50. Ovum with dextrine granules.



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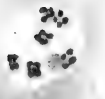




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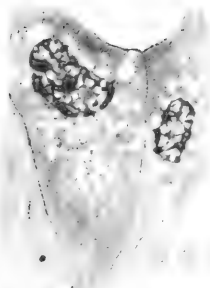


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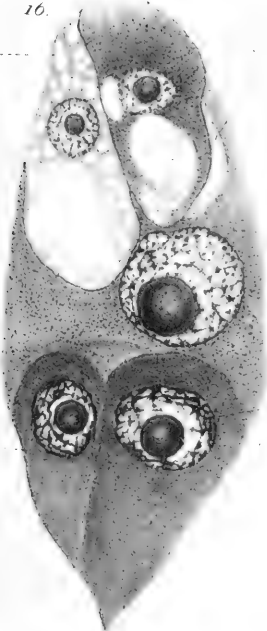


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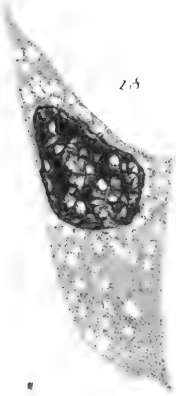
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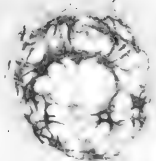
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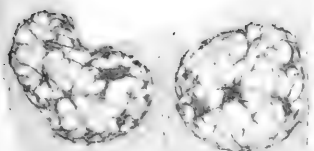


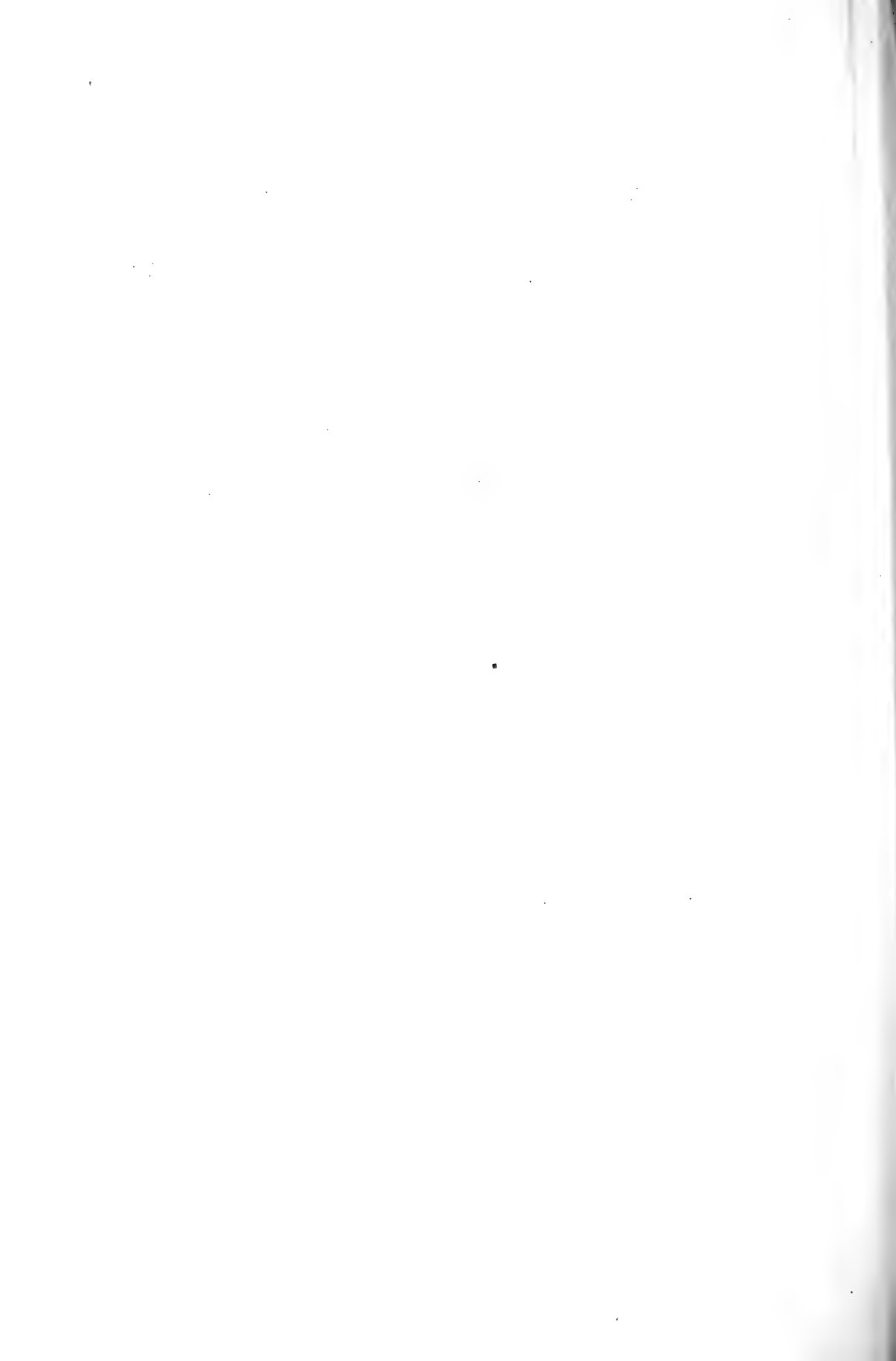
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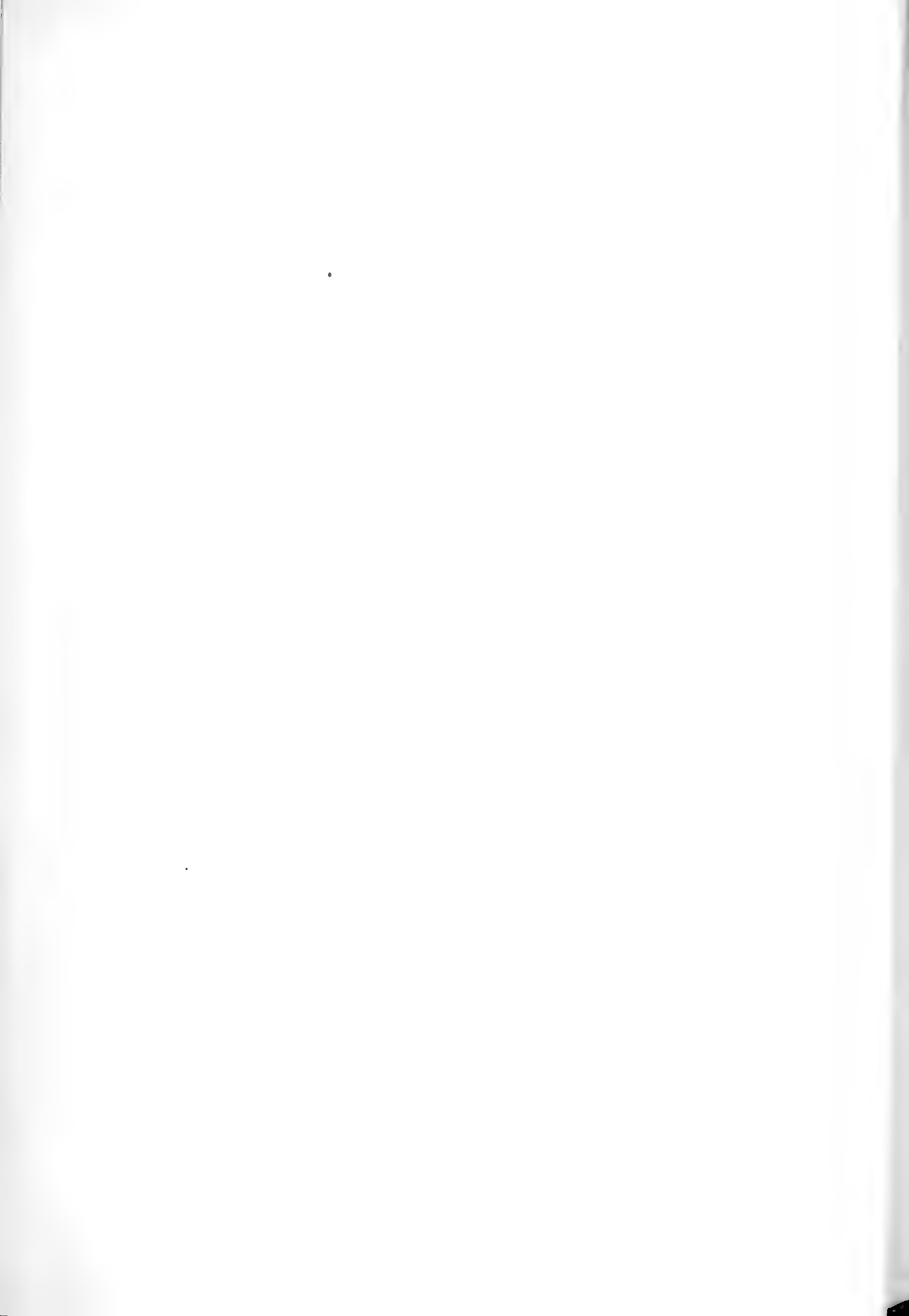
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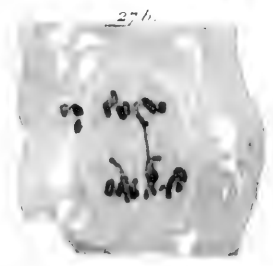
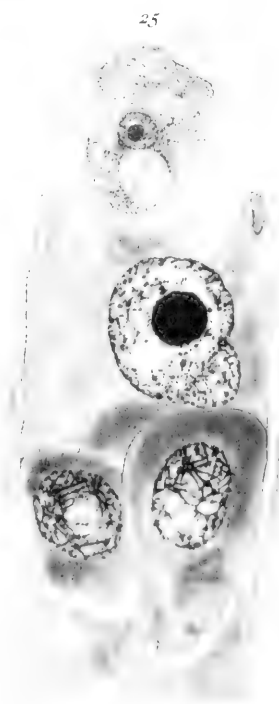
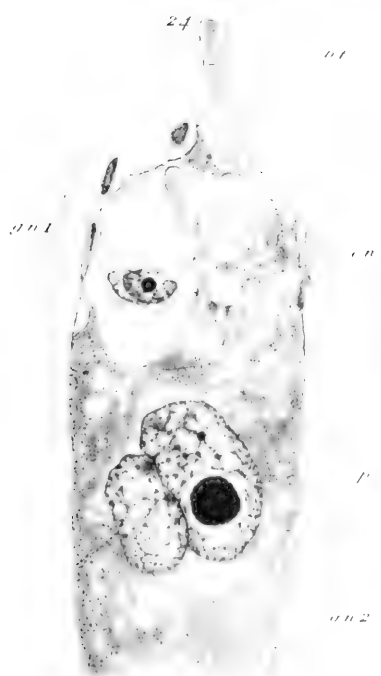
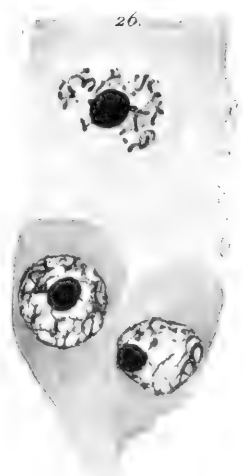
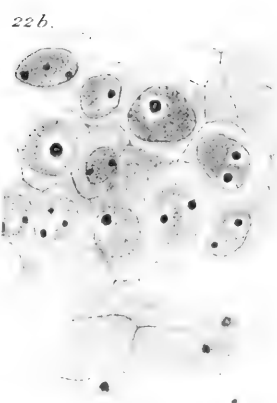
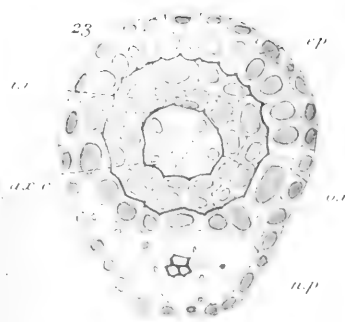


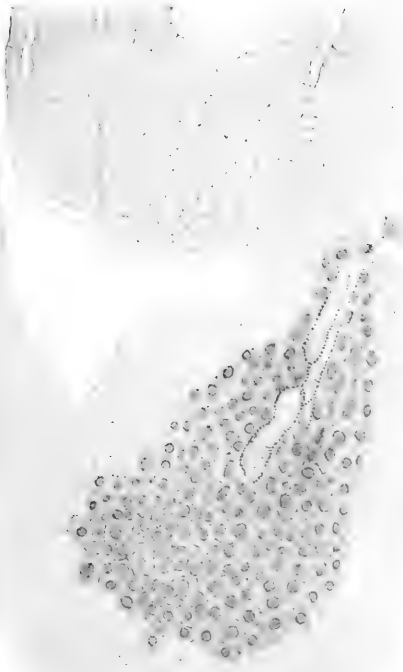
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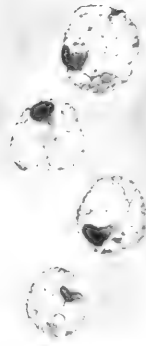


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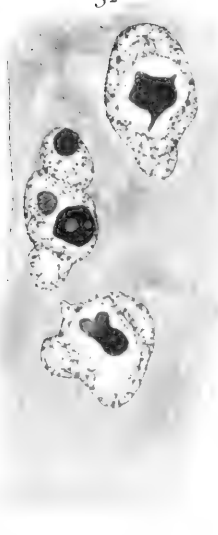
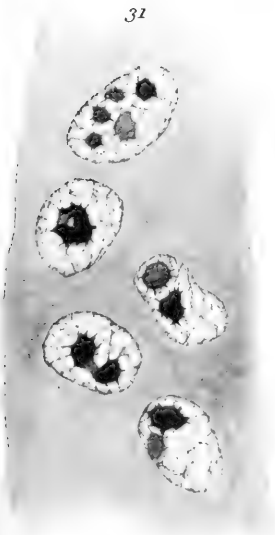
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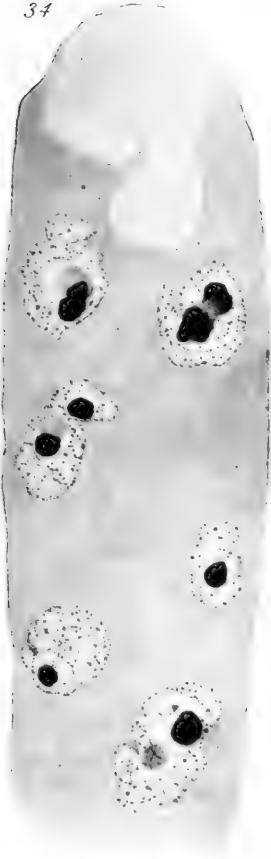
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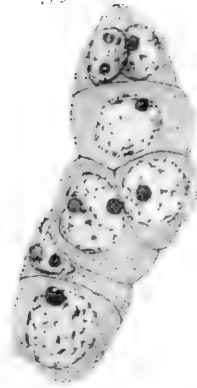
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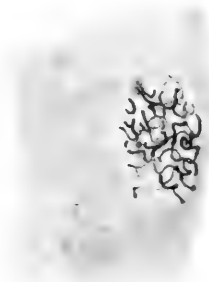
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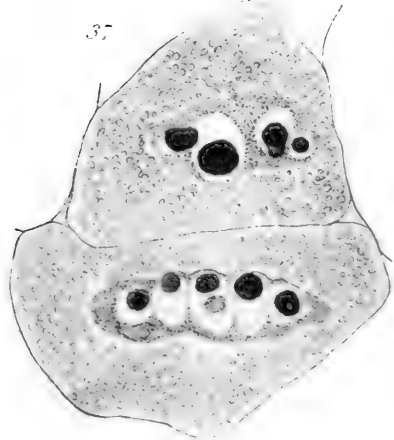
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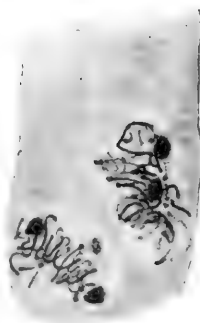
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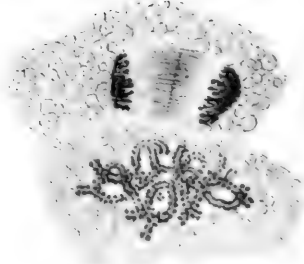
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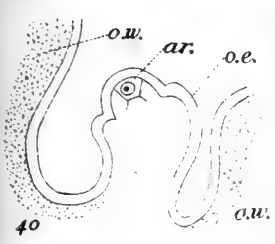
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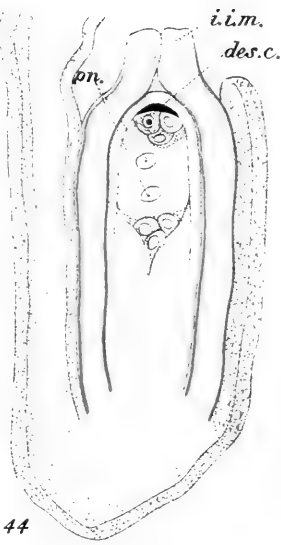
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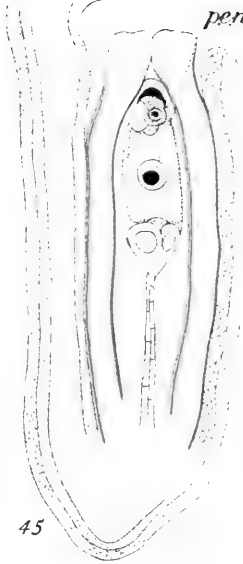
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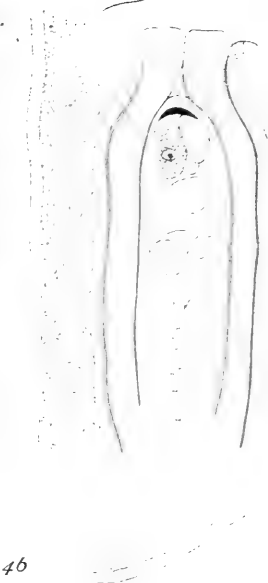
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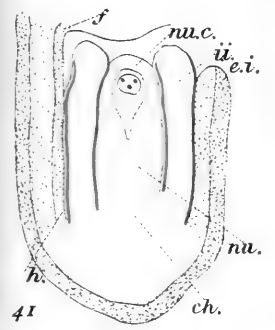
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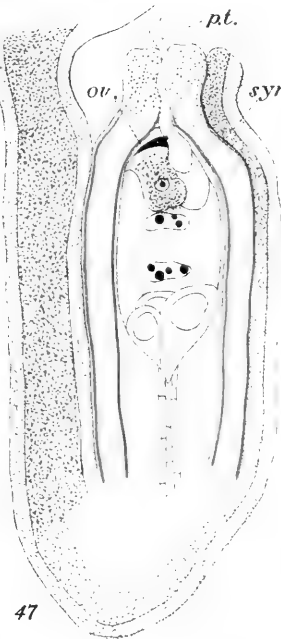
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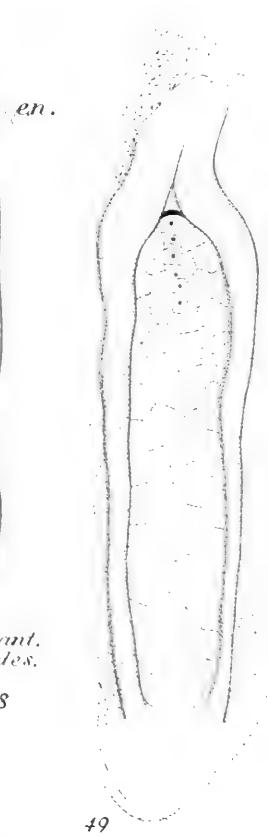
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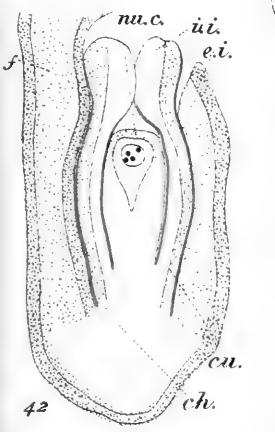
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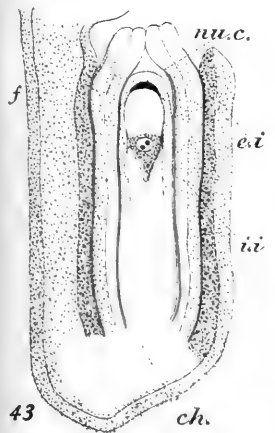
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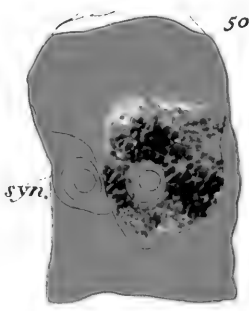
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-  Starch (abundant).
-  Do. (scanty).
-  Dextrine.
-  Cuticularized membranes.
-  Cytoplasmic contents.



Contributions to the Study of Silk-Worms.

I. ON THE EMBRYOLOGY OF THE SILK-WORM.

BY

K. Toyama.

(With Plates VII—XI.)

Since the publication of Tichomiroff's beautiful work on the "Développement du ver a soie du murier dans l'oeuf," about ten years ago, no important facts have been added, so far, as I am aware, by any renewed researches, except a short description by Graber ('90).

The observations which are described in the following pages, were carried on in the Zoological Institution of our College during last year, and though they may not bring to light anything new, yet they are here published with the hope that they may be found to be of some value.

Before going further, I wish to express my heartiest thanks to Prof. Ishikawa for affording me much assistance and advice in the present investigation, and to Prof. Sasaki for kindly placing at my disposal various publications out of his own library.

Methods.

Eggs deposited on a karton are killed by the solution (1—2%) of chromic acid or by the Flemming's weak triacid solution, both heated to 80—90°C. The latter reagent is fitted for young stages, while the former is used only for advanced ones. A great number of eggs hardened by hot corrosive sublimate, picro-sulphuric or picro-acetic acids, proved to be useless. The eggs treated by the chromic or Flemming's acids for two or more hours, are thoroughly washed with water and transferred for hardening to 70% alcohol in which they remain for several weeks or even months.

By this process the content of the egg which adheres closely to the shell is shrunk a little and a narrow space is left between the surface of the egg and the shell, so that we can easily remove the shell with dissecting needles. It may be noted here that the eggs treated by the chromic acid solution are preserved in the most satisfactory manner, both the nuclei in resting and in karyokinetic states being beautifully brought out and the yolk being made capable of being cut without difficulty, so that a complete series of sections can be obtained.

Staining is done on the slide with alum-carmin, glycerine or alum haematoxylin, and gives beautiful results in all stages as will be seen from the figures, which are all drawn from these preparations.

A. My own observations.

I. The formation of mesoderm and entoderm.

In our country, univoltine silk-worms generally deposit their eggs in June or July. Five or six hours after deposition, the first division of the segmentation nucleus will be observed in the middle of the anterior portion of the egg. Some of the increased nuclei migrate towards the surface where they form the blastoderm at the end of about one day, while the others which remain in the interior of the yolk become vitellophags.

If we cut an egg at the end of the second day after its deposition, the ventral plate will be seen completely detached from the other portion of the blastoderm. In this stage, the lower layer (Unteresblatt) is not yet completely formed and the inward growth of cells will be seen to take place from the primitive furrow at the anterior portion of the ventral plate as has been already described and figured by Tichomiroff.*

The closure of the blastopore takes place very slowly, as will be seen in an embryo represented in Fig. 1, Pl. VII., where it appears as a wide furrow tapering at both ends (Fig. I, bl. a). This embryo was killed at the end

* Tichomiroff l. c. Fig. 16.

of August or September. A circular depression will be observed at the anterior end of the furrow (Fig. 1, o).

The internal developmental processes of this stage are shown in Figs. 2, 2^a, 2^b, 3, 4, 5 and 5^a. Fig. 2 is a transverse section passing through the anterior end of the blastopore. The ectoderm invaginates here in the form of a deep sac. A similar invagination at the primary head segment was also observed in a younger stage than this by Tichomiroff ('82) who represents it in his Figure 14, but has failed to give its fate. Further development brings the lips of the invaginated pocket close together in the median line (Fig. 2^a) thus forming a closed sac, with an irregularly shaped lumen which is compressed dorso-ventrally. This corresponds to the "primordial Spalte" of Heider. Fig. 2^b shows the median longitudinal section of this stage, in which the lumen is distinctly visible. The cells composing the wall of the sac gradually become more and more irregular and wandering out into its lumen completely fill it up, and form a loose cell-mass. These changes are noticeable when we compare Fig. 2 with Figs. 2^b and 3. On closer examination, we see that the mass is composed of large round cells of soft and succulent appearance and with a clear outline (Fig. 3). Some of these cells will frequently be seen to detach from the mass, as is shown in Figs. 7, 7^a ms².

The invagination of the ectoderm just described is only observed in the primary head segment where the circular depression above mentioned is situated (Figs. 1 and 6, o, o¹), while in the mandibular or maxillary region the inner layer is formed by an inward growth of ectodermal cells from the base of the shallow blastoporous furrow, as will be seen in Fig. 4. In a more posterior portion of the embryo where the blastoporous opening is wide (Fig. 1, a.), the inner layer is formed by a lateral overgrowth of the ectoderm as is seen in Fig. 5. And in the anal segment we observe a wide and shallow ectodermal furrow from the bottom of which the migration of cells takes place to form the inner layer (Fig. 5¹, pf). This may be regarded as a modified form of the deep invagination that is seen at the anterior end of the blastopore.

Not infrequently, in this stage, we meet with cells which detach themselves from the lateral portions of the ectoderm and enter the yolk-

mass (Fig. 5^a, par). Tichomiroff ('82) has also observed these immigrating cells and has represented them in his Figs. 14 and 15, m¹. He considers them to be the secondary entoderm and comes to the following conclusion :—

“Pendant les premiers stades de développement, cet entoderme secondaire, au moment même de sa formation, se convertit immédiatement en mésoderme.”

According to my observations, however, they correspond to the “Paracyten” of Heymons ('95) and like them gradually disintegrate into small granules having no share in the formation of mesoderm. Migratory cells of similar nature were also observed in many insects by Graber.

Although the ectoderm does not as yet show any sign of segments in this stage (Fig. 1), certain alterations are already found in the inner layer. The most important of these is its metameric arrangement. This process begins at the middle portion of the germ-streak and proceeds both forwards and backwards, as has already been observed by Tichomiroff. In the present stage, there are 17 or more segments faintly marked off from one another, and among these the first which is derived from the deep invagination of the blastopore, as above mentioned, is the largest.

At the end of November, when the embryo attains the stage given in Fig. 6, the blastopore is nearly closed, being represented only by a faint line, except at the posterior half (Fig. 6 a), where it remains somewhat open. The cells on both sides of the central cell-mass at the anterior end of the blastopore (Fig. 7 ms¹), are now separated from it and form a mass of small-cells (ms) closely attached to the ectoderm. These are the mesoderm cells and are now easily to be distinguished from the cells of the central mass by their smaller size and by their nuclei being deeply stained. The cells of the central mass are, on the contrary, larger and of a spherical form, and their cytoplasm is very much vacuolated staining faintly with haematoxylin or carmine (Fig. 7 a), thus making them easily distinguishable from the others even under a low power. Cells are seen to detach from the central mass in this as in the preceding stage, and to migrate into the yolk-mass (Figs. 7 & 7a ms²). They differ,

however, entirely from the vitellophags or "Paracyten" in the structure of the nucleus and also in form and size.

The central cell-mass thus far considered may be compared with the endoderm-anlage of *Hydrophilus* as is described by Heider, or with that of *Doryphora* as given by Wheeler, but it is of quite a different nature as will be shown later on, and for this reason we will call it the oral cell-mass. An interesting question on this point is: Whether the oral cell-mass will remain as a definite tissue? To this we shall come later on.

After the closure of the blastopore, there remains a round ectodermal depression in the middle of the primary head segment where the oral cell-mass is situated (Figs. 6, 7^o). This is a structure of a transitory nature, disappearing in a more advanced stage where it is only represented by a shallow median furrow (Figs. 12 and 13^o).

In the maxillary or thoracic region, the inner layer has already separated off from the ectoderm as a distinct layer (Fig. 8 ms), while the median ectodermal furrow still exists (Fig. 8, pf) which closely resembles the neural furrow.

As we go towards the posterior, however, the boundary of the ectoderm and the inner layer gradually obliterates (Fig. 9) until finally the inner layer becomes exposed to the surface (Fig. 10). In the anal segment, the wide furrow above mentioned (Fig. 5^a, pf) becomes narrow and the two distinct layers, the ectoderm and the inner layer, are again formed (Fig. 11). Moreover, in this stage, the inner layer is heaped up in the median portion, its lateral arrangement becoming visible only when the segmentation of the ectoderm begins to appear.

Soon afterwards, the narrow groove extending from the oral depression to the anal segment fades away with the closure of the posterior opening of the blastopore, but no trunk segment is as yet to be seen (Fig. 12). It is in this stage that the embryo passes the winter, namely from December to the end of January, or the first part of February. Among the hundreds of embryos we studied, we did not find one that had passed beyond this stage. Other varieties of the silk-worm, such as the bivoltine.

multivoltine etc. also pass the winter in this stage, so that we may call it a resting stage.

Figs. 13—16 represent transverse sections through an embryo of this stage, i.e. the resting stage. The first section (Fig. 13) passes through the primary head segment; the oral cell-mass (Fig. 13, ms^1) elongates considerably into the yolk, and at its distal portion some immigrating cells (ms^2) are to be seen. Besides these, we first meet with other cells migrating in the yolk-mass such for example as degenerating cells (d.c) which will be considered later in a separate section. The next sections (Figs. 14 and 15) pass through the anterior portion of the thoracic region, the former representing the segmental portion, while the latter the intersegmental. The segmental arrangement of the mesoderm is now distinctly visible. These segments are 18 in number, the first (the oral cell-mass) and the last (the anal segment) being the largest (compare Figs. 13 and 16, with Figs. 14 and 15). This reminds us of Wheeler's Fig. 72, Pl. XX., which represents a longitudinal section of an embryo of *Doryphora*, of which he says: "These two masses of cells are the independent sources of the entoderm, which grows backwards as two strings from the anterior mass and forwards as two strings from the posterior mass." These cell-masses in *Bombyx mori*, however, are not the entoderm-anlage as we shall see further on.

The warm days of spring awake the embryo from its winter sleep. It now increases greatly in length, and with this the procephalic lobe extends more laterally. A number of outer segments also make their appearance developing in number posteriorly. With these changes of the external parts, the internal portions also change. The median mesodermal cell-mass flattens and spreads out below the entire ectoderm and finally becomes divided into two lateral streaks (mesodermal streaks) by the withdrawal of its cells from the median line (Fig. 26, Pl. VIII). The cells constituting the lateral streak are clearly of two layers, the upper consisting of cylindrical cells arranged regularly, while the lower layer consists of flattened cells arranged irregularly. These evidently correspond to the "paradermalen" and the "paralecithalen Schicht" of Heider.

One of the most interesting changes in this stage is the disintegration of the oral cell-mass to form migratory cells. This is seen in Fig. 17, which is a longitudinal section through the primary head segment. Here the cells are clearly seen migrating from the periphery of the oral cell-mass (Fig. 17, ms²). Tichomirowff who first observed this cell-mass in this stage (see his Fig. 17) considers it to be the mesoderm-anlage saying that " nous voyons que le premier des dix-huit segments anterieurs du mesoderme diffère par sa forme de tous les autres. Ce segment tire son origine de la partie la plus profonde du sillon primitiv. Les cellules different également du reste du mésoderme : elles sont un peu plus grosses que les cellules mesodermiques ordinaires et leur plasma est plus clair." Moreover, he homologizes it with the structure which Hatschek ('77) for the first time found in *Bombyx* and considered to be the entoderm-anlage (a structure which in reality is not the entoderm-anlage but the suboesophageal body) and concludes with the following words: " Nous allons voir qu'en réalité il n'en est rien ; cet epithelium (Middarmepithelium) a une origine differente." The further changes concerning the cell-mass are not, however, given.

Fig. 18 is a surface view of a more advanced embryo in which the neural furrow has made its first appearance as a faint median line. In the primary head segment, we again meet with a wide depression. In this stage, we observe not infrequently the bifurcation of the neural furrow at the anal segment, resembling closely the bifurcation of the blastopore at the caudal end of the *Xiphidium* embryo as is observed by Wheeler ('93), or of the *Lina* embryo observed by Graber ('90). Fig. 73 which represents a cross section at this portion of the embryo shows the two ectodermal furrows at the bottom of which cell-proliferation is to be observed, but the true nature of these furrows is as yet quite obscure and requires further study.

Let us now consider the oral cell-mass. Its fate will be best understood when we examine Figs. 19—26, which are a series of transverse sections passing through the primary head segment of an embryo in the same stage as that represented by Fig. 18. In the anterior portion (Fig. 19), the mesoderm flattens out on both sides of the median line

forming two lateral masses. These gradually approach each other as we go posteriorly until they join together in the median line and with the oral cell-mass (Figs. 20—21). Figs. 22-26 are consecutive sections of the oral cell-mass in which the disintegration of its cells into migratory cells will be clearly seen. The immigration of cells begins at the anterior periphery of the oral cell-mass (compare Figs. 17, 22, 23, and 25), and proceeds gradually to its distal end which elongates considerably into the yolk (Fig. 27) and forms the two arms of an inverted Y (in Fig. 28 only the left arm of the Y is represented). The cells usually detach from the distal end of the arms one at a time but in some cases also in groups (Fig. 22 ms²).

Some important changes are to be observed in the embryo shortly after the preceding stage (Fig. 20): cephalic and thoracic appendages now become distinctly formed as lateral outgrowths of their respective segments. The antennae (at) originate as lobular outgrowths from the posterior edges of the procephalic lobes. The stomodial depression (st.) now distinctly appears and from its anterior edge the labrum (lb) will be seen as two separated processes. The three thoracic segments are very slightly or not at all broader than the two maxillary segments. The appendages of these six segments are also nearly alike in shape, size, and position except that those on the mandibular segment are larger than the others (Fig. 30 md). The mandibular appendages also differ from the others in this that they are directed horizontally, while the other appendages are placed latero-posteriorly. The space between the primary head segment and the mandibular segment is occupied by ganglion cells (Fig. 30 vk) and represents the "Vorkiefersegment" of German authors. Proctodium now appears also in the form of a faint and shallow depression at the posterior end of the anal segment (Fig. 70 a).

Returning now again to the consideration of the oral cell-mass, we find that the disintegration of its cells becomes more vigorous with the ingrowth of the stomodium until at last the entire cell-mass completely disappears. This is clearly to be seen in Figs. 29, 31 and 32. Fig. 29 is a median longitudinal section of the primary head segment where

a shallow stomodial depression (st) makes its first appearance just in front of the oral cell-mass. In Fig. 31 the stomodial depression is more marked and with this the curvature of the ectoderm is increased, which evidently accelerates the detachment of the oral cell-mass from the ectoderm. And when the stomodial tube becomes more elongated, and its terminal portion becomes broader as in Fig. 32, not even a remnant of the cell-mass is to be seen.

From what has been above described, we may safely conclude that *the invaginated cell-mass at the anterior end of the blastopore, although it greatly resembles the entoderm-anlage of other authors such as Heider, Wheeler etc., is certainly not to be regarded as such in the present case.*

Then the questions naturally arise : (1) Whence arises the entoderm? ; (2) What is the oral cell-mass and the cells migrating from it? The first question will be considered of in the next paragraph ; while the second will be discussed later on under the heading, "The vitellophags and cellular elements found in the yolk-mass."

The formation of the mid-gut (Mitteldarmanlage.)

As before said the stomodium makes its first appearance as a shallow ectodermal depression at the anterior portion of the oral cell-mass which represents the anterior end of the blastopore. As the development of the embryo advances the depression gradually deepens and proceeds backwards along the ventral wall of the embryo, as is shown in Figs, 38 and 39. At the two lateral corners of its free ventral end the elongation of the stomodial ectoderm takes place (Figs. 37, 38 a). These ectoderm-elongations correspond to the "vordere Epithellamelle" of Voelatzkow and Heymons, who first observed them in Coleoptera, Orthoptera etc., and they give rise to the formation of the epithelium of the mid-gut.

The epithelial cells of the mid-gut originate, as has just been said, from the ectoderm-elongations at the posterior end of the stomodium, and have nothing to do with the cells of the blastopore, which latter do not, as has been said before, now exist as a definite tissue. Nor do the mesoderm cells of the "Vorkiefersegment" have any relation to

the formation of the mid-gut epithelium. These are beautifully visible in Figs. 33—40, which represent a series of sagittal sections of the embryo given in Fig. 30.

In the embryo given in Fig. 30, the stomodial tube takes an oblique course along the ventral wall as already referred to, and the ventral apex (*a*) of the tube is more elongated than its dorsal corner (*c*) (Figs. 37, 38, 39). It will also be seen that the anterior portion of the ectoderm of the "Vorkiefersegment" is taken into the formation of the ventral wall of the stomodium and helps the curving of the ectoderm at this place. The mesoderm attached to this portion gradually detaches itself from the ectoderm and proceeds posteriorly along the ventral wall of the stomodium, forming a structure which was first discovered by Hatschek ('77) in *Bombyx*, and erroneously considered to be the entoderm-anlage. Wheeler ('93), who also found this same structure in *Xiphidium*, has given it the name of the subœsophageal body. Tichomiroff ('82) also observed this body in a more advanced silk-worm embryo, calling it by the name of the "corps adipeux du seconde ordre," and derived it from the central mass of yolk-cells.

When fully formed, its cells are large and it stains more faintly by carmine or haematoxylin than any other structures found in the body of the embryo. The cytoplasm is very granular and has a distinctly yellow tint even in the unstained sections. With the elongation of the stomodial tube the subœsophageal body proceeds backwards until it becomes situated in the ventral side of the fore-gut within the methothorax.

Figs. 41—51 represent transverse sections through the primary head segment of an embryo taken out from the same deposit as the one just described, but a little more advanced than the former. Fig. 41 passes through the anterior portion of the primary head segment; Fig. 42 six sections behind it, and in front of the stomodium. Here the ectodermal depression is more developed and its median portion is elevated into a ridge (*a*). The depression becomes deeper as we go backward (Fig. 43), and its lateral lips come close together in the median line until a tube is formed (Figs. 44—47) which is compressed dorso-ventrally. Between the ectoderm and the stomodial tube are seen some free cells (Figs. 46,

47, *b.c*) which represent blood cells. It may be here remarked that the compressed lateral edge of the stomodium (Figs. 45—47, *a*) elongates somewhat laterally as a distinct tissue. This portion corresponds to the elongation of the ventral wall of the stomodium already referred to (Figs. 37—39), and becomes more and more developed concurrently with the development of the embryo, and the epithelium of the mid-gut is formed by the proliferation of this tissue as will be seen in the following pages.

In the vicinity of the distal end of the stomodium we observe many free cells in the yolk (Figs. 49—51). We can not accurately determine the origin of these cells. They may arise from the oral cell-mass or some of them may come from the mesoderm. We believe, however, that they arise from both the oral cell-mass and the mesoderm. Whatever their origin may be, we are certain that they take no part in the formation of the entoderm. We specially directed our attention to this point, but we were not able to meet with even a single case in which the formation of the entoderm by these wandering cells could have occurred.

Like all other insects that have a stage during which the body is greatly elongated (Fig. 18), the silk-worm passes into a series of stages during which the germ-band is gradually shortened (Fig. 52). The shortening is accompanied by a broadening of all the segments, a growth of the appendages, and very important internal changes; the cephalic and thoracic appendages have meanwhile assumed a more definite character. The first and second maxillae and thoracic appendages have each become three jointed. The abdominal appendages now also make their appearance on the first ten segments with the exception of the anal, as Kowalevsky ('71) and Tichomiroff ('82) long ago observed in Lepidopterous insects. Graber ('88), however, doubts the observations of Kowalevsky and erroneously states that Tichomiroff did not discover them. But, as is stated above, we are not only able to say that abdominal appendages do really exist in silk-worms, but we can also confirm the statement made by Packard on this point, that "these structures appear in the embryos of certain Lepidoptera and Hymenoptera, though they are much less distinct and more evanescent than in the lower orders of insects."

It is interesting to note here, moreover, that the stigma appears in each segment from the first thoracic to the eleventh. In the meso- and metathoracic segments we are able to observe faint depressions of the ectoderm, which may represent the rudiments of stigma. Of these the one on the mesothorax together with the stigmata on the last two abdominal segments disappears entirely, while the remaining ten pairs of stigmata persist in the larval stage. The rudimentary stigmata on the metathorax does not disappear, but remains as a small opening without external chitinous ring and internal closing apparatus, such as the closing lever, closing band, or closing bow. Tichomirow (92) has given a quite correct description of these metathoracic stigmata in saying that "le stigmaté du metathorax ne disparaît pas, il demeure sous la forme d'un stigmaté rudimentaire avec son faisceau correspondant trachéal, même chez la larve adulte; ce stigmaté rudimentaire se trouve fortement avancé vers le mesothorax." We are now able to add that these rudimentary stigmata do not only persist in the larval, but also in the imaginal stage, in which they are clearly recognizable. Their internal structures are, however, different from those on the prothorax or abdomen, where a stigma is provided with a closing bow, a closing band, and a closing lever, as is described by Krancher (81) in *Smerinthus*, whereas the metathoracic stigmata have only the closing bow with its well developed muscles. Recently Boas (99) has observed the presence of stigmata in each thoracic segment in the larva of *Cossus ligniperda*, which, however ends blindly. We can not detect any trace of closed stigmata in silk-worms.

Fig. 54 is a median longitudinal section of the primary head segment of the embryo above described. The stomodium has now become longer than in the preceding stage and its distal end widens out. The wall of the stomodium consists of thick epithelium, except at the bottom where it becomes quite thin (gl.), constituting the "Grenzlamelle" of Heymons. The "vordere Epithellamelle" has developed more than before and close to its ventral side the subœsophageal body (sb.) appears as a distinct mass of cells.

A cross section through the antennal region of an embryo in the same stage, is shown in Fig. 53. The stomodium, as already described,

is found to be a compressed tube ; its lateral edges (ent) are thicker than its dorsal and ventral walls, and project into the yolk as distinct structures. This is the anlage of the entoderm already referred to. Attached to the ventral wall of the stomodium we again meet with the subœsophageal body. But the development of the entoderm-anlage will become clear when we come to examine the serial sections mentioned below.

Fig. 55 is a longitudinal section through the primary head segment of a slightly more advanced stage, showing the elongation of the "vordere Epithellamelle." Series of transverse sections of the head segments of this stage are given in Figs. 56—63, showing more clearly the relations between the lateral projections of the stomodial wall and the epithelium of the mid-gut. The first section (Fig. 56) represents the section through the bottom of the stomodial invagination whose ventral wall presents here three thick folds, on each side of which will be seen lateral projections of cell-mass (ent) very well developed while the dorsal wall, which represents the cross section of the "Grenzlamelle," is very thin. The stomodium becomes more and more compressed as we proceed posteriorly (Fig. 57), until in the section represented by Fig. 58 the lumen of the stomodium has completely disappeared and only a flat cell-mass resting on the subœsophageal body is to be seen. In this flat cell-mass we again observe the thick lateral portions (ent) which have been already referred to. In the section passing through the anterior portion of the mandibular segment (Fig. 59), we observe only these lateral cell-masses (ent) and a portion of the subœsophageal body (sb) attached to the ventral sides, while the median portion has entirely disappeared. In the next section here represented (Fig. 60), which passes through the posterior portion of the same segment, the subœsophageal body is no longer observed, and the lateral cell-mass or the entoderm-anlage only are left attached directly to the lateral mesoderm (Fig. 69). In these mesoderm masses we can not make any distinction between the splanchnic and the somatic portions, all the lateral masses consisting of irregularly shaped cells (Fig. 60 ms). In the maxillary segments (Figs. 61, 62) the dorsal end of the lateral mesoderm forms a curved compact tissue consisting of one layer of cells, somewhat resembling the head of the figure

3. The curved inner end of this portion of the mesoderm (Figs. 61, 62, 63, sp. ms) represents the splanchnic layer, and the outer portion (Figs. 61, 62 and 63, sm. ms) the somatic layer, but a closed coelomic cavity is not formed. On the dorsal and the inner portions of the splanchnic part of the curved end of the mesoderm will be seen the entoderm-anlage or the prolongations of the lateral cell-masses of the stomodium, which in the anterior part consists of irregularly shaped cell-masses (Figs. 61, ent). The cells of these masses are large and contain vacuoles in the cytoplasm. In the posterior part, however, they consist of high columnar cells arranged in a single layer and firmly attached to the inner dorsal portion of the splanchnic mesoderm (Fig. 62, ent). The entoderm-anlage become smaller as we go posteriorly until in the second thoracic segment (Fig. 63) they are represented by only a few cells, attached to the splanchnic mesoderm.

In the yolk-mass in the vicinity of the distal end of the entoderm-anlage we meet with, not infrequently, small cells containing small but deeply coloured granules. These are to be seen in Fig. 64, which is a cross section of the sixth abdominal segment. On closer examination, they are found to be nothing else than degenerating cells, the function of which is probably to give nutrition to the growing entoderm. These cells together with blood cells are also found in other places in the yolk, but mostly in the neighborhood of the entoderm (Figs. 62, 63, and 64).

In his Fig. 45 (which corresponds to the stage shown in my Fig. 52) Tichomirowff distinguishes "trois trabécules de cellules vitellines"; namely a middle and two laterals, and is of the opinion that the neurilemma and the fat bodies are derived from the former, while the epithelium of the mid-gut comes from the latter. On this point he says; "ils donnent naissance à l'épithelium de l'intestine moyen, épithelium que se constitue des cellules de l'entoderme secondaire, émanant de ces trabécules des cellules." We have also frequently observed such cells corresponding in all particulars to those given in his Fig. 54 *Ep.* (see our Figs. 59, 64, and 91 *bc*), but we have not been able to find any such cells in any stage of transition into the entoderm. On the contrary, we frequently

meet with these cells disintegrating into small granules and showing the phenomena of degeneration. From all that has been thus far said, we may conclude that they are degenerating cells and have nothing to do with the formation of the epithelium of the mid-gut.

Fig. 65 is a frontal section of the posterior portion of the stomodium of a more advanced embryo. We observe in it the karyokinetic division of the nuclei of the epithelium of the mid-gut, by which the cells of this region are multiplied thus causing its closure.

From the foregoing accounts, we may safely conclude that *the anterior entoderm-anlage is formed by the proliferation of the epithelial cells of the stomodium, and is consequently ectodermal in its nature. Neither the mesodermal cells formed by the cells of the blastoporous invagination, nor the yolk cells, have anything to do with the formation of this structure.*

The proctodium and the Malpighian vessels.

The first appearance of the proctodium is always later than that of the stomodium, as has already been observed by many other authors. Even when the stomodial depression becomes as long as is represented by Fig. 30, or 31, the proctodium is seen only as a faint depression at the posterior end of the neural furrow (Fig. 70^a). Its median longitudinal section is represented in Fig. 71. Different from the stomodial depression, it is directed somewhat towards the dorsal side and forms a round tube, the blind end of which consists of thick epithelium (Fig. 72), where an active proliferation of cells will be observed (ent). These cells give origin to the posterior entoderm. The lateral wall of the blind end of the proctodium will now be seen to give rise to short evaginations (Fig. 72, uv), which ultimately become the Malpighian vessels. As regards the formation of these structures, Tichomiroff states that "ces derniers (Malpighian vessels) ne se présentent en effet que comme de simples excroissances tubulaires de l'intestin postérieur. Comme on sait une larve adulte possède six vaisseaux malpighians, qui débouchent dans l'intestin postérieur par deux conduits communs. Ce sont ces conduits

qui apparaissent des le commencement comme de simples excroissances de l'intestin posterieur, se partagent ensuite chacum en trois tubes comme les troncs trachéaux principaux émanant des stigmates, se divisent en rameaux secondaires." Hatschek ('77) also observed three tubules on each side of the proctodium or hind-gut of *Bombyx*, and says that "die drei malpighi'schen Drüsen jeder Seite münden durch ein gemeinschaftliches Anfangsstück in das blinde Ende des Hinterdarmes." According to our observation, these arise as three separate pairs of hollow outgrowths from the beginning as will be seen in Fig. 76, which is taken from an embryo a little more advanced than that represented in Fig. 72. As the entoderm and the Malpighian vessels appear just at the same time, the position of the entoderm-anlage is disturbed and the study of it by cross sections becomes very difficult. Fig. 74 is a sagittal section of the proctodium of a more advanced stage, which corresponds to that shown in Fig. 52. Here we observe that the proliferation of the cell-mass from the ventral wall of the proctodium assumes the form of a short lamella and is directed forwards forming a "hintere Epithellamelle" (eplh). When the revolution of the embryo is about to set in, this becomes more elongated and a dorsal process is formed (Fig. 75).

From the entoderm-anlage, two lateral stripes grow out and assume the form of a **U**, the arms of which are directed forward and become attached to the splanchnic mesoblast in just the same way as the anterior entoderm-anlage. This is shown in Fig. 54, which shows the foremost portion of the posterior entoderm. In this section, we observe some genital cells (g) placed in the somatic mesoderm, from which they are differentiated. This is what has been shown by Wheeler in *Xiphidium*, and by Heymons in *Phyllodromia*. Although the clusters of germ cells are normally seen to occur in the third and the sixth abdominal segments, we often observe them in all other abdominal segments with the exception of the anal; and in one case, we observed them even in the mesothoracic segment. We are thus in position to say that the genital cells originally arise in each body segment. Tichomiroff also found genital cells in a far more advanced stage than this, and first maintained the opinion that they were derived from the entodermal cells, "mais apres m'être familiarisé plus tard

avec le rôle important que remplit l'entoderme secondaire dans la formation de different organes, j'incline à admettre que la partie essentielle des organes sexuels se constitue aussi à ses depens." But they originate from the mesoderm as the former facts show.

Summary: 1. *The posterior entoderm-anlage is derived first from the epithelial wall of the proctodium in the same way as the anterior entoderm-anlage is derived from that of the stomodium.* 2. *Malpighian vessels arise as three separate pairs of outgrowths from the blind end of the stomodium.* 3. *Genital cells differentiate from the cells of the somatic mesoblast.*

II. The vitellophags and other cellular elements found in the yolk.

a. Vitellophags.

Just as in the eggs of many other insects thus far studied, we here also find in the yolk various cellular elements among which the most conspicuous are the vitellophags, or cells left in the yolk at the time when the other cleavage cells are traveling towards the surface of the egg to form the blastoderm. In certain cases, however, all the segmentation-nuclei come to the surface of the egg, and in these cases the vitellophagous cells are formed by the migration of the cells of the blastoderm, as was observed by Patten ('84) in Phryganids, by Uzel ('97) in Campodia, and in many other cases.

In the silk-worm, the vitellophags are formed from the segmentation-nuclei which are left in the yolk. When the segmentation of the yolk-mass is completed, we find a single vitellophag in the centre of the yolk. This multiplies by direct division (Fig. 68) when the embryo attains the stage given in Fig. 1, so that in more advanced stages we see many nuclei in the centre of each of the yolk-segments (Fig. 9, *y.b*).

The cytological nature of these vitellophags is as follows:—The body of the cell is large as compared with other cellular elements found in the yolk. It has a large round nucleus with fine granular chromatin

scattered in it. There is no nucleolus to be seen. The cytoplasm is stained faintly in the younger stages of embryo, while in later stages it produces numerous pseudopodial processes (Fig. 69 vit) and stains well either with haematoxylin or carmine; the fine granular chromosome becomes thicker. Thus they are readily distinguishable from other cellular elements in the yolk and are easily seen (Figs. 2, 7, 9, 22, 24, 25, 32, 33 etc.).

The vitellophags are very often seen collected in the vicinity of the entoderm-anlage (Fig. 61) or other organs, but they have never been observed to transform into other tissues. We frequently observe, however, that their nuclei become irregular in shape and about to dissolve (Fig. 67 vit), and although we have traced their metamorphosis up to the stage when the embryo hatches, yet we have failed to find any direct evidence of their forming other organs, and we can definitely say that they take no part in the formation of the mid-gut-anlage or any other organs, but degenerate in situ and finally undergo dissolution.

b. Migratory cells from the ectoderm.

In younger stages we frequently observe cells which are about to detach themselves from the ectoderm (Figs. 2^a, 5¹, par). These are small ellipsoidal cells with a point like nucleus (Fig. 19, par). These cells probably correspond to the "Paracyten" of Heymons, who described and figured them in his beautiful work "On Dermaptera and Orthoptera" ('95). Tichomiroff ('82, '92) has also observed these cells but considers that they become the mesoderm. It is, however, certain that these as Heymons ('95) rightly says, have no direct share in the formation of any embryonal tissue, but finally dissolve away.

c. The cells migrating from the oral cell-mass.

As already stated, the oral cell-mass disintegrates and forms migratory cells. In the early stages of the embryo, we observe them in the vicinity of the oral cell-mass (Figs. 7, 13, and 17 ms²). In later stages

the oral cell-mass sends out two branches from its free end, as already stated (Figs. 27, 28 *ms*²). These elongate into both sides of the dorsal portion of the embryo where some liquid content is to be seen (Fig. 27 c). Fig. 28 is a magnified drawing of a portion of the left side of the embryo as shown in Fig. 27, and shows the separation and metamorphosis of the cell-mass into free cells. These cells are round, ovoid, or spindle-shaped, having a large nucleus which contains dense chromatin-granules and one or two nucleolei (Fig. 28 *ms*²). The cytoplasm contains numerous vacuoles in its periphery, while it is dense near the nucleus, staining deeply (Fig. 28 *ms*², Figs. 67, 69, *ms*²). Sometimes we see that these cells become irregular in outline and stain faintly by haematoxylin or carmine, and finally dissolve as shown in Fig. 67.

As to the function of these free cells, Schwartz (1909) is of the opinion that these give origin to the blood corpuscles. Although they resemble blood cells in their general appearance, yet we have no direct proof that they are such, and we are inclined to think that they are nutritive cells which have the function of liquefying the yolk and conveying it to other portions of the egg, and that they finally dissolve. This appears the more probable when we learn that some of these free cells pass out from the body of the embryo and wander about the extra-embryonal yolk-mass; and also that with the increase of these free cells, degenerating cells containing small granules increase suddenly in number in the yolk near the oral cell-mass.

d. The blood cells.

As already stated, we observe a different structure in the anterior end of the mesoderm which afterward becomes the subœsophageal body (Figs. 38, 39, 49 a). The mesoderm at this stage of development consists of irregularly shaped cells having homogenous cytoplasm which stains uniformly, except at the anterior end where the cells are seen to detach from the rest (Fig. 40 a). These cells are vacuolated and stain very faintly.

In a more advanced stage in which the subœsophageal body is well

formed, we observe at the same place some free cells of a circular form, containing vacuoles in the cytoplasm and staining faintly except at the nucleus (Figs. 47, 54, 55, b.c). Karyokinetic divisions (Fig. 54 a) are sometimes to be seen among them. These cells are blood corpuscles, but as the number of them produced from this portion of the mesoderm is small, it is probable that they are formed from other portions of the mesoderm in a similar way; this latter point however we are not able to state definitely at present.

In Figs. 20, 21, 22 b.c' we see a number of cells separating off from the mesoderm at other portions of the body, which we had considered to be the blood corpuscles. More careful examinations, however, convinced us that they were in reality not such, but rather degenerating cells which will be described under the next heading.

e. Degenerating cells.

In a younger stage of the embryo, there are only vitellophags and a few ectodermal migratory cells in the yolk-mass. In the spring, when the ventral plate begins to elongate and the disintegration of the oral cell-mass becomes vigorous wandering cells in the yolk-mass gradually increase in number, especially in the vicinity of the primary head and anal segments, as was observed by Wheeler in *Doryphora* (Figs. 17, 22—25).

With the increase of wandering cells, we also observe various cells containing small granules which are stained well by haematoxylin or anilin colors. They may be observed everywhere in the yolk-mass near the germinal streak, but like the other wandering cells they are especially abundant in the vicinity of the primary head and anal segments, as shown in Figs. 43—45, and 74, 76, d.c. At the time when the stomodium, the proctodium etc, begin to be formed, these cells suddenly multiply in such numbers, that they are easily distinguishable even under a low magnifying power.

In the beginning, however, they closely resemble other migratory cells, and contain stainable round granules in the cytoplasm

(Figs. 13 a, 66 d.c). These granules gradually increase in number and with this the nucleus dissolves, till at last the cells are entirely filled up with the granules (Fig. 69 d,c). The size of the cells is not uniform, some being large and others small. Finally the cells disintegrate and the granules alone are left freely suspended in the yolk-mass.

Where do these degenerating cells come from? They may either arise 1. from the migratory cells of the ectoderm; 2. from those of the mesoderm; or 3. from the free cells of the oral cell-mass.

1. That they arise out of the migratory cells of the ectoderm is to be seen when we compare Figs. 2,^a 5¹ with Figs. 13, 19, 22 par. In Figs. 2,^a 5^a we see cells (par) about to detach themselves from the ectoderm and these cells have an appearance similar to certain small cells in the yolk (Figs. 19, 22 par) which are certainly to be recognized as the degenerating cells referred to above, both by the presence of granules in their cytoplasm as well as by their general form and size. 2. their origin from the mesodermal cells can be clearly seen in Fig. 96 where cells of an exactly similar appearance are met with. 3. Lastly, that they arise out of the oral cell-mass is very plainly to be seen from Fig. 69 where these degenerating cells are seen in the neighborhood of the free cells of the oral cell-mass and among them cells of the intermediate condition are often to be distinguished. Moreover, in the spring when the disintegration of the oral cell-mass most vigorously takes place, a sudden increase of the degenerating cells is to be observed as already described.

The function of these cells is difficult to state. But it will not be far fetched if we consider that they give nutrition to the growing portions of the embryo, such as the proctodium, stomodium etc.

From the foregoing statement, we are able to say that *there are four sorts of migrating cells in the yolk. The first are the vitellophags derived from the remainder of the segmentation nuclei. The second are cells separated from the ectoderm and undergoing degeneration. The third are cells migrating from the mesoderm. Some of these become blood corpuscles while others degenerate like the second. Lastly, the fourth are the cells*

which are produced by the disintegration of the oral cell-mass. These also undergo degeneration.

III. The endoskeleton of the head, with reference to the salivary gland and a new gland.

The first trace of the endoskeletons of the head appears in the embryo shown in Fig. 52. In this stage, we observe many ectodermal invaginations in the lateral part of the mandibular and the maxillary segments. Figs. 77—82 are serial sections through the head segments showing these invaginations. The first section (Fig. 77) passes through the anterior portion of the mandibular segment in a somewhat oblique direction. In the right side of the section, we see the antenna and the mandible, from the outer base of which an invagination of the ectoderm (Fig. 77 tent') takes place, while in the left side where the knife passed through the middle of the mandible we observe the tubular structure of the above invagination.

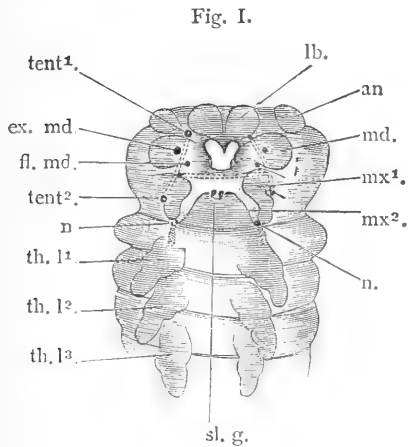
Posteriorly we observe a new tubular invagination on the inner side of the basal portion of the mandible at its posterior portion (Fig. 78 fl. md).

As we proceed more posteriorly we again meet with such an invagination on the lateral sides of the first maxillary segment (Fig. 79 tent²).

A similar invagination also takes place in the next segment at the outer base of the second maxillae (Fig. 80 n). Another pair of invaginations is also to be seen at the inner base of the second maxillae (Fig. 80 slg). The latter are the origin of the silk glands. Figs. 81—82 are the consecutive series of sections next to the section shown in Fig. 80. Here we see posterior portions of the two invaginations at the second maxillary segment.

The fate of these four pairs of tubular invaginations excepting the silk gland, will be seen in Figs. 83—90, which represent a series of sections through the head of a more advanced embryo, in the stage shortly before the revolution. In this stage the head segments are about to

coalesce with one another and the limit of each segment is no more visible (woodcut Fig. I).



tent¹⁻², first and second tentorium; ex. md, attachment of extensor mandibulae; fl. md, attachment of flexor mandibulae. lb, labrum; an, antennae; mx¹⁻², first and second maxillae; sl. g, silk gland; n, new gland or hypostigmatic gland; th. 1¹⁻¹¹¹, first-third thoracic legs; H-shaped dotted line, tentorium anlage within head.

Figs. 83 and 84 represent two consecutive sections through the anterior portion of the head. The invagination between the labrum and the mandible (tent¹), exactly corresponding to the invagination figured in a younger embryo (Fig. 77 tent¹) can be clearly observed. The invaginated tube becomes flattened and proceeds posteriorly along both sides of the stomodium and unites with the invaginated tube at the first maxillary segment (Fig. 89 tent², right side and wood cut, dotted line) which proceeds forwards along both sides of the stomodium. So we see on both sides of the stomodium two parallel tubes opening at both ends, one at the base of the mandible and the other at the base of the first maxilla. These tubes afterwards unite with each other by producing transverse processes from both tubes and together form a H-shaped tube (woodcut Fig. I, dotted line).

These tubes are the anlage of the tentorium of the head. The walls of the head, consequently, are supported or braced within by beams resembling an H which correspond exactly to the tentorium described by Tichomiroff in the head of a silk-worm (Tichomiroff Fig. 35).

Returning again to the mandibular region, we see an invagination at the outer base of the segment (Fig. 85 ex. md; woodcut Fig. I. mx. md). This becomes the seat of the muscle extensor mandibulae. It is short and small, and is the last invagination which takes place in the head. At

the posterior inner end of the mandible we again meet with a large invagination (Fig. 86 fl. md, and woodcut Fig. I. fl. md). It is a flattened tube curved at its anterior portion, so that in a cross section it presents a form like a crescent (Figs. 87, 88, 89 fl. md), while at its posterior portion the tube is circular and slender (Fig. 90 s.g). This anterior portion chitinizes afterwards and becomes the seat of the flexor mandibulae, while the posterior portion gives origin to the salivary glands. These relations will be seen more clearly if we compare the accompanying sagittal section (Fig. 92). Here it will be seen that the invaginated tube sends off a large branch which is directed anteriorly, and the mesodermal cells are largely accumulated around it to form the muscles. Posterior to this branch it proceeds as a round tube (s.g) which becomes the salivary gland. Thus the seat of the muscles, flexor mandibulae, and the salivary gland arise from the same invagination at the posterior base of the mandible.

The invagination at the lateral part of the second maxillary segment above described (Fig. 80) will now be considered. In the shortening of the head-segments to form the head, the appendages on the second maxillary segment become fused together and form a triangular process. This proceeds more forwards and enters between the appendages on the first maxillary segment, as is shown in the woodcut Fig. I. mx². The two openings of the silk glands come close together and become a single opening, while the lateral invagination forms a long cell-mass suspended from the ectoderm into the body-cavity. In consequence of the shortening of the second maxillary segment, the greater portion of it is now situated in the prothorax (see woodcut Fig. I). In the transverse section we observe these cell-masses on both sides of the suboesophageal body in the prothorax (Fig. 90 n), while in the sagittal section the first portion of them appears as an invaginated tube (Fig. 92 n). Fig. 94 represents a frontal section through the head and the thoracic segments in an embryo a little more advanced than the above. It will be seen that the cell-body and also the nuclei of these cells are larger than those of the surrounding tissue and stain somewhat more deeply. This cell-mass persists as a definite body in the larval stage. It is flat and trilobed, resembling fat-tissues in

appearance, and is situated on the ventral side of the first stigma to which it is firmly attached, while its front end is attached to the hind edge of the head. Fig. 95 represents the cell-mass of a larva at the end of the third stage. In the full grown larva, it also exists but it becomes more elongate and produces more branches, as is shown in Fig. 96 which is taken from a larva of the fifth stage. Its length is now about 16 mm. and its breadth 0.09 mm.

This body resembles the fat tissue in general appearance, but it can very easily be distinguished from it by its cells, which are large and contain a dendritic nucleus (Fig. 97), while the cells of the fat tissue are small, their nuclei circular and the cytoplasm mostly with fat granules.

The function of this body is quite obscure, the structure of the nucleus, however, assures us that it is a glandular organ representing perhaps a sort of dermal gland such as the œnocytes or dorsal glands which are not in the prothorax, the other segments of the body are provided with one or two such. But as we have thus far been unable to find any gland of this description mentioned in the literature on insect-anatomy, we will call it "the hypostigmatic gland."

If we now give a short summary of the above statements it will be as follows :

1. *In the mandibular segment, three pairs of invaginations take place; the most anterior (between the labrum and the mandible) becomes the first tentorium, the second pair gives rise to the seat of the extensor mandibulæ, while the last becomes the flexor mandibulæ and salivary gland.*

2. *In the first maxillary segments, there is a pair of invaginations which become the second tentorium.*

3. *In the second maxillary segment, we again meet with two pairs of invaginations, the inner of which forms the silk gland, while the lateral ones grow into a gland which is situated on the inner side of the first stigma in the larva, and which we will call the hypostigmatic gland.*

B. General considerations.

Let us now consider the results obtained by other investigators as compared with those given above.

The mesoderm.

As regards the development of Lepidoptera, Kowalevsky ('71) was the first naturalist who clearly described the formation of the mesoderm in *Sphinx populi*. He noticed that the mesoderm was not formed by the typical groove-shaped invagination of the ectoderm, but by a pair of lateral overgrowths. A comparison of our Figs. 5 and 10 with his Figs. 5 and 6, Taf. XII., will show that the mode of the formation of the mesoderm is the same in both cases.

Next to him comes Bobretzky ('78) whose observations principally concern the formation of the blastoderm, but also give some description of the formation of the germinal layers. According to this author the formation of the mesoderm takes place in Lepidoptera later than in other insects, namely after the formation of the embryonal envelopes, the amnion and serous membranes and "tritt in Form einer seichten, länglichen Rinne auf, deren Bodenzellen, sich vermehrend, sich vom Keimstreifen abbilden." From this we may say that in this species of Lepidoptera studied by Bobretzky, the mesoderm is formed by an inward growth of cells from the bottom of the blastoporous groove.

Tichomirow ('82, '91) in his interesting article above referred to, "Development du ver a soie du murier dans l'oeuf" describes and figures the formation of the germ layers. Concerning the mesoderm formation, he maintains the opinion that "elles procèdent, avant tout, de l'ectoderme et 2) de l'entoderme."

A true invagination tube at the anterior end of the blastopore and an inward growth of cells from the median primitive furrows takes place, which forms the mesoderm (see his Figs. 14 and 15).

Bruce's ('87) observation on *Thyridopteryx* also shows the inward growth of cells from the primitive furrow to form the mesoderm, and

his Fig. VII., Pl. I., corresponds exactly with our Fig. 2 on this point. He says: "The inner layer is not strictly invaginated, for it is cut off from the rest of the embryo before the opposite sides of the median groove have met," and "the median groove deepens, beginning to push dorsally the median portion of the embryo. In subsequent stages, the groove deepens, and the pushed-in-portion of the embryo becomes folded off and forms the inner layer."

Graber ('90) also observed the inward growth of the cells of the median ectoderm to form the mesoderm in *Pieris* and in other *Lepidoptera*, while at the intersegmental region it invaginates as a deep furrow. But the cell-mass which he calls the "Ptychoblast" in his Fig. 129 Pt, seems to me to be the oral cell-mass found in other *Lepidoptera* as above mentioned, and not the inner layer.

Lastly Schwartze ('99) has recently published a valuable paper on this point in *Lepidoptera*; the following is quoted from his description of mesoderm formation:—

"Die Bildung des mesoderms ist bei *Lepidopteren* nicht in ein bestimmtes Schema gebunden, sondern erfolgt bald durch Einsenkung eines Rohres, bald durch Zellwucherung von Boden einer Rinne aus, bald durch seitliche Überschiebung; es kommen sogar in den verschiedenen Körperregionen desselben Embryo verschiedene Form der Mesoderm-bildungen vor."

The results obtained by my investigation on silk-worms as above given quite confirm the opinion of the last named author. The deep invagination in the anterior end of the blastopore closely corresponds to a similar groove on the cephalic lobe described by Bruce and figured in his Fig. VIII. Pl. I., while the inward growth of cells from the bottom of the median furrow in the mandibular or in the maxillary segment greatly resembles that described in the observations made by Bobretzky, Tichomirow, Bruce, Graber et al. Lastly the formation of the mesoderm by the lateral overgrowth of the ectoderm at the median portion in the abdominal segments confirms again the observation made by Kowalevsky.

The entoderm.

In the interpretation of the insect-gastrula the entoderm has always played an important rôle. The origin of the mesoderm has long been known in a general way, but the true origin of the lining membrane of the mid-gut has not yet been completely ascertained; some authors maintain the opinion that this originates from the yolk cells, others think that it comes from the ento-mesoderm, while still others derive it from the ectoderm.

My first intention was to give a comparative description of the entoderm formation in the different orders of insects, but as this has been treated in a masterly manner by Heymons ('95) and Schwartze ('98) we shall confine our remarks mainly to the Lepidoptera.

In the Lepidoptera, Tichomiroff was the first who minutely described the entoderm formation. In his first paper ('79), he considers that it arises from the ento-mesoderm, but in later papers ('81, '91) he maintains the opinion that it is derived from the yolk-cells, that is, the secondary yolk-cells derived from vitellogophags by division, as referred to above. Bruce ('87) was certainly at fault when he considered the formation of the entoderm in *Thrydopteryx* as being formed from the inner layers, the formation being described by him in the following words: "a portion of the inner layer on each side of the embryo becomes separated from the other parts of the inner layer. These portions of the inner layer which may be called entoderm grow together and unite first on what is the ventral surface of the alimentary tract." Ritter ('90) is also similarly mistaken in his study of *Chironomus*.

Grabner ('90) made many valuable observations on the bipolar origin of the entoderm in *Bombyx*, *Pieris*, *Gastropacha* etc. Although he has not given any direct proof on this point, yet from the fact that in many Lepidopterous insects, the division of the yolk-cells does not occur, or occurs only after the formation of the entoderm, he comes to the conclusion that the entoderm is not formed out of the yolk-cells. Comparing other insects, he says; "dabei nehmen wir vorläufig an, dass der vordere und der hintere Drüsenblattkeim aus dem Ptychoblast und nicht aus dem

Ectoderm des Stomo-und Proctodiums hervorgeht." But according to his Fig. III. Taf. X., which closely corresponds to our Fig. 54 or 55, we might say that the entoderm arises out of the epithelium of the stomodium.

Schwartz (98) made a very valuable observation on this point and comes to the following conclusion: "Vorder-und Enddarm entstehen als Ektodermeinstülpungen, dass Mitteldarmepithel aus seitlichen Zelllamellen, die von den blinden Enden Vorder-und Enddarmes aus auf einander zuwachsen, bis sie sich jederseits in der Mitte treffen, und sich dann in Folge starken Breitenwachsthums erst ventral, dann dorsal in der Mediane vereinigen. Der Mitteldarm ist also, abgesehen von der mesodermalen Muscularis, wie Vorder-und Enddarm rein ektodermaler Natur."

From the accounts above given, we may come to the conclusion that the view maintained by Heymons and Schwartz as to the formation of the entoderm from the ectoderm is to be accepted, and that the opinions of Tichomirow and others are untenable, at least in respect to the order of the Lepidoptera.

Among the Coleoptera, Kowalevsky (71) was the first author who worked on *Hydrophilus*, which was investigated in a more detailed manner by Heider (85, '89). Both these observers saw a rhomboidal area at the anterior end of the blastopore, which remained open after the closure of the other portion of the blastopore, thus corresponding exactly with what we saw in the silk-worm. In the sections passing through this portion, Heider distinguishes two cell-layers in the gastrula tube, namely "1. ein Paar von seitlichen Divertikeln, deren Hohlraum durch seitliches Auswachsen des Urdarmlumens hervorgegangen ist und 2. eine mediane an die paarigen Divertikeln sich dicht ausschliessende Zellmasse. Aus den Divertikeln werben die Mesodermmassen des Kopfsegments und des späteren Mandibularsegments, während die unpaare Zellmasse zur Ectodermanlage (Entodermanlage?) wird." A precisely similar result was obtained by Wheeler (89) in *Doryphora*, and it was also confirmed by Graber (90) who says "die Ptychoblastmetameren sind bei *Lina* alle mit Ausnahme der zwei polaren oder Endsegmente, das ist des procephalen und analen

Abschnittes rein mesodermatische Bildungen, beziehungsweise Anlagen, während die genannten zwei Segmente gemischter Natur sind, dass heisst ausser dem auch ihnen zukommenden und sogar sehr stark entwickelten Mesodermantheil zugleich die Entodermanlage enthalten."

Voeltzkow ('89), on the other hand, observed quite correctly the formation of the entoderm from the cells of the stomodium and the proctodium. He was followed by Lecaillon ('98) who worked on the formation of this layer in a leaf beetle.

The subject has been quite recently taken up by Deegener ('00) who made a study on *Hydrophilus* and came to the following conclusion: "Auch ich kann mich mit Kowalevsky und Heider's Darstellung nicht einverstanden erklären und stimme vielmehr mit Voeltzkow und Lecaillon überein. Beide Forscher finden, dass bei *Melolontha* bzw. einer Anzahl untersuchter Chrysomeliden der Ursprung des Mitteldarmepithels in zwei vorderen und zwei hinteren, vom Vorder- bzw. vom Enddarm auswachsenden ventrolateralen ektodermalen Lamellen zu suchen ist, die durch ihre Vereinigung in der Mitte, sowie in der ventralen und später in der dorsalen Medianlinie das Mitteldarmrohr entstehen lassen. Das Wachsthum der ektodermalen Lamellen geschieht überall ohne Beziehung der Zellen des unteren Blattes, so dass an dem ektodermalen Charakter das gesammten Mitteldarmepithels kein Zweifel herrschen kann."

Comparing now the various accounts above referred to with the results above given respecting the formation of the entoderm in the silk-worm, we are strongly inclined to believe that in Coleoptera the entoderm arises much in the same way as in Lepidoptera and that the opinion advanced by Voeltzkow, Lecaillon and Deegener is to be maintained.

The question naturally arises: What is the structure which was observed by Heider and Wheeler?

Now if we compare the Figs. 61, 62, 71, 76, and 77 given by Heider with our Figs. 2 and 2", we shall at once be struck with the similarity of his entoderm to the oral cell-mass of the silk-worm. This similarity becomes more pronounced if we recollect that in *Doryphora* Wheeler saw the migration of the cells out of the structure which he calls the entoderm-centre and which corresponds to the entoderm of Heider in much the

same way as the migration of the cells takes place out of the oral cell-mass in silk-worm. Wheeler says: "Be this as it may, in later stages I believe it can be shown that cells do migrate into the yolk from the embryo and especially from the entoderm-centres. This was shown by me to be the case in *Doryphora*, where many cells pass into the yolk from either entoderm pole. I have since observed an exactly similar phenomena in *Telea polyphemus*¹ in a corresponding stage of development." A similar phenomenon was also observed by Graber ('89), in *Melolontha*. Heymons considers these cells to be the "Paracyten," but we think that they are nothing else than the oral cell-mass.

Tichomirow (92) still maintains the opinion: "Je puis confirmer ice que la participation des cellules vitellines dans la formation du Mésoderme et de l'épithélium de l'intestin moyen est très clairement vue sur les préparations de *M^{mé}*. O. Tichomirow (90) chez la *chrysopa* et la *pulex* et sur mes propres préparations, de la *Calandra granatia* (92)."

Although he (92) has stated that there exists close relationship between the yolk-cells and the entoderm, and shows his Fig. 6 as a proof of it, yet there remains some doubt as to whether yolk-cells are changed into the entoderm, or indeed as to whether there exists any such relationship between them.

In other orders of insects, the formation of the entoderm has been mostly studied in Orthoptera. Ayers ('84, in *Oecanthus*) advanced the opinion that it is derived from the yolk-cells, while a considerable number of observers derive it from the ento-mesoderm (Korotneff, '85, in *Gryllotalpa*; Nussbaum, '88, in *Blatta*; Cholodkowsky, '88, '91, in *Blatta*; Wheeler, '89, '93,* in *Blatta* etc). Heymons (95) as before said, in his beautiful monograph asserts that the entoderm is derived from the stomodial or proctodial wall by cell-proliferation and concludes that "Bei den von mir untersuchten (comparing six genera such as *Forficula*, *Gryllus*, *Gryllotalpa*, *Periplanata*, *Phyllodromia*, *Eclobia*) ist somit der ganze

¹ The cells here are I think undoubtedly the oral cell-mass.

* If we look at his Figs. 32, 33, and 34, and the description that "median portion thus proliferated beyond limits of the ectoderm is the anterior or oral entoderm-centre," it is clear that in *Xyphidium* the entoderm arises from the ectoderm by cell-proliferation.

Darmtraktus ausschliesslich ektodermaler Natur." Rabito ('98) also came to the same conclusion in Mantis.

Concerning the oral cell-mass in Orthoptera as far as we are aware there exists no literature.

Next to Orthoptera Muscidae have been much studied. It was in studies on this insect that Kowalevsky ('86) first advanced the theory of the ento-mesoderm, which in the hands of Heider, Wheeler, Graber et al. inaugurated a revolution in the opinions respecting entoderm-formation in insects.

As early as 1889 Voelotzkow, however, came to the conclusion that in *Musca* the entoderm is formed by the proliferation of the cells of the stomodial and proctodial epithelium. Graber ('90) criticized this and came to the following opinion: "Zudem habe ich in meiner Muscidenarbeit höchst eigenthümliche, bei anderen Insekten bisher völlig unbekannte Verhältnisse nachgewiesen, nach denen es mindestens möglich ist, dass hier die Drüsenblattkeime zum Theile aus ganz selbständigen Einstülpungen des Keimstreifenepithels aus den sogenannten lateralen Gastral-falten entstehen." But in his later paper ('91) he also maintains the ectodermal origin. Ritter ('90) on the other hand, shows that in *Chironomus*, the entoderm is separated off from the "segmentweise Wülste" of the mesoderm (see his Fig. 30).

After considering these results, Heymons ('95) expressed his view in the following words: "Nach den bisherigen Untersuchungen zu urtheilen, ist es daher in hohem Grade unwahrscheinlich, dass das Mitteldarmepithel der Musciden aus dem unteren Blatt (Entomesoderm) entsteht."

Quite recently Escherich ('00, '01) has found three folds in the anterior portion of the blastopore of *Musca*, of which he writes "sie stellen einen Theil der Mesodermanlage dar; die mediane Falte dagegen ist, wie wir gleich sehen werden, die vordere Anlage des Entoderms. Wir haben also in diesem Stadium bei den Musciden in der That ganz ähnliche Verhältnisse, wie bei *Sagitta*, worauf ja bekanntlich schon Bütschli und Kowalevsky hingewiesen haben." And he finally came to the conclusion, "dass 1. bei den Musciden sich sehr frühzeitig ein Entoderm anlegt, dass 2. dieses

Entoderm ein Abkömmling des Blastoderms ist und dass 3. die Differenzierung der beiden primären Keimblätter durch eine typische Invagination eingeleitet wird."

Further investigations are necessary before any decisive opinion on this point can be reached.

Observations on the formation of the entoderm in other groups of insects hitherto considered are scanty, and the opinions formed by various authors vary greatly. Thus Patten ('84) claims that in Phryganids the entoderm arises from the yolk-cells, with which Will ('88) also agrees in his observation of viviparous *Aphis*. Witzlaczil ('84), however, holds the view that in oviparous *Aphis* the entoderm is derived from the ectoderm. Carrière ('90) in his study on the carpenter-bee (*Chalicodoma*) has found a cell-thickening at the fore and hind portions of the midplate, and the cell-mass proliferated from the thickenings is converted into the entoderm-anlage, a fact which stands well with the view that the entoderm is formed out of the ectoderm.

Kulagin's observation ('98) on *Platygaster* differs entirely from the results obtained by all other authors hitherto mentioned. According to his observations the inner layer is formed by the immigration of the blastoderm cells of which he says as follows: "folglich entsteht das Entoderm und Mesoderm gleichzeitig auf dem Wege der Theilung der Zellen des Blastoderms und ihrer Einwanderung." This seems to be the most rudimentary form of the entoderm-formation hitherto discovered.

Now considering the facts and arguments thus brought out by various authors on different kinds of insects, the most usual mode of the formation of the entoderm in the class *Insecta* is that it arises from two separate centres—the oral and the anal. But since the entoderm of many other animals arises from a single centre it is tacitly assumed that such must originally have been the case also with insects, and that the present bipolar condition must be due to a secondary modification. Starting with this postulate, Kowalevsky has formulated his hypothesis that "bei der so in die Länge gezogenen Gastrula der Insekten der mittlere, das Entoderm liefernde sack so ausgezogen ist, das er in der Mitte ganz verschwindet und nur an seinem vorderen und hinteren Ende bestehen

bleibt." But if as we assume that the entoderm of Kowalevsky corresponds to our oral cell-mass which disintegrates into free cells, and that the definite entoderm is formed from the cell proliferation of the stomodium and the proctodium, then it will not be far from the truth, if we say that the original entoderm-anlage are entirely lost in insects, at least in the higher forms, and to supply this want ectoderm cells separate off at their original places and form the entoderm.

Blood cells.

The blood cells of *Bombyx mori* are said by Dohrn ('96) to have some relation to the yolk cells. Ayers ('84) also maintains the same view as Dohrn in his studies on *Oecanthus*. Will ('88) comes to the same opinion in regard to *Aphis* saying that the cell-elements of the blood arise from entodermal yolk-cells, "sowhol innerhalb des Herzens als auch frei in der Leibeshöhle." Cholodkowsky ('91) also holds the same opinion respecting *Phyllodromia*.

Korotneff ('83, '85) on the other hand states that in *Gryllotalpa* at an early period blood cells are found almost everywhere between the yolk and the mesoderm; they are derived, as he states, from the cells of the somatic mesoderm layer which lost their connection with the other parts of the mesoderm, and have fallen into the body cavity. Patten ('84) assumes a similar view in his researches on Phryganids. Wheeler ('89) again confirms the view that the endoderm is formed out of the mesoderm in *Doryphora*, though he differs somewhat from the above two observers as to the place of its origin. Heymon's statement ('95) much resembles that of Korotneff. He says that "bei Forficula, sowie bei den "hier betrachteten Blattiden und Grylliden sind die Blutkörperchen mesodermaler Abkunft. Sie entstehen aus Mesodermzellen, welche nicht bei der Bildung der Ursegmente sich beteiligt hatten, sondern zwischen diesen in der Medianlinie des Körpers ihren Platz beibehalten." A similar view is held by Lécaillon ('98) in the case of *Chrysomelidae*, and by Schwartze in the case of *Lasiocampa*. But in the case of *Lasiocampa*, the last author states that "diese Einwanderung

beschränkt ist auf diejenige Stelle des Embryos, an die sich die Ektodermrinne von zuletzt schliesst, d.h. auf einen sehr geringen Theil der ganzen Länge des Keimstreifs," and further on, in other portions of the mesoderm, he says, that these "zeigen niemals eine Lockerung ihrer Zellen." It seems to us that he has considered that portion of the embryo which corresponds to our oral cell-mass as the only structure which forms the blood cells.

From all the opinions above considered those of Korotneff and Heymons are in best accord with the formation of the blood corpuscles as we have observed it in the silk-worm and as it is described in previous pages, and we must believe that the various views held by the other authors are to be looked upon as being due to their having confused the various cell elements found in the yolk.

First of July, 1901.

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* The asterisk marks the cases in which I have not been able to gain access to the original paper.

Explanation of Plate I.

- Fig. 1. Surface view of embryo with blastopore not yet closed (taken from an egg, one month old: 28th, August.) Zeiss A $\times 4$. o, anterior widening of blastopore; pcl, procephalic lobe; bl, blastopore; a, posterior widening of blastopore; cd, anal segment.
- Figs. 2—5. Cross sections taken from embryo as in preceding figure. Zeiss D $\times 4$.
- Fig. 2. Section through primary head segment; knife passing through anterior portion of blastoporous widening (Fig. I. o).
ect, ectoderm; inv, cavity of invaginated gastrula; vit, vitellophags; yl, yolk-granules.
- Fig. 3. Section through posterior portion of primary head segment.
ect, ectoderm; ms', cell-mass formed by coalescence of invaginated gastrula; other letters as in preceding figures.
- Fig. 4. Section through maxillary segment.
p.f, primitive furrow; ms, mesoderm.
- Fig. 5. Section through posterior blastoporous widening, knife passing through at (a) Fig. I, showing lateral overgrowth of ectoderm to form mesoderm. am, amnion; other letterings as in preceding figures.
- Fig. 5.¹ Section of anal segment.
Letters as in preceding figures.
- Fig. 2.^a Transverse section through anterior widening of blastopore already closed (taken from an embryo somewhat older than Fig. I).
am, amnion; ect, ectoderm; o, ectodermal depression, par, "paracyten," other letters as in preceding figures.
- Fig. 2.^b Longitudinal section of embryo at same stage as in above figure.
Lettering as in the preceding.
- Fig. 6. Surface view of embryo with blastopore about to close. At (a) it remains open (taken from the egg on November 30th.) Zeiss A $\times 4$.
o¹, ectodermal depression formed after closure of anterior widening (Fig. I. o) of blastopore.
- Figs. 7—11. Cross sections taken from embryo as above figured. Zeiss D $\times 4$.
- Fig. 7. Section through ectodermal depression (Fig. 6. o') at primary head segment. am, amnion; ect, ectoderm; o¹, ectodermal depression; ms, mesoderm; ms¹, cell-mass sprung from invaginated gastrula or oral cell-mass; ms², detached cell from oral cell-mass, remaining letters as in the preceding figures.
- Fig. 8. Section through middle portion of embryo.
Lettering as in preceding figures.
- Fig. 9. Section through a portion, little posterior to the preceding figure.
Lettering as in preceding figures.
- Fig. 10. Section through blastopore at Fig. 6. a., showing lateral overgrowth of ectoderm to form mesoderm or inner layer.

Fig. 11. Section of anal segment.

Lettering as in preceding figures.

*Fig. 7.^a Portion of same section, highly magnified, showing two migratory cells from oral cell-mass.

(Zeiss Ap. o. 2 mm × Comp. oc. 6.)

Lettering as in the preceding figures.

Fig. 12. Surface view of embryo after closure of blastopore, (taken from egg on February 2nd.)

(Zeiss A × 4.)

p.cl, procephalic lobe ; cd, anal segment.

Figs. 13—16, cross sections of embryo of same stage as that represented in preceding figures. (Zeiss D × 4.)

Fig. 13. Section of procephalic lobe at region of oral cell-mass. am, amnion ; ect, ectoderm ; o¹, ectodermal depression at primary head segment ; ms¹, oral cell-mass ; ms², detached cells from the oral cell-mass ; d.c, degenerating cells ; vit, vitellophags.

Fig. 13.^a Portion of same section more highly magnified, showing degenerating cells and vitellophags.

(Zeiss Ap. o. 2 mm × Comp. oc. 4.)

Lettering as in preceding figures.

Fig. 14. Section through thoracic region.

Lettering as in preceding figures.

Fig. 15. Section through intersegmental region at the thoracic portion. Lettering as in the preceding.

Fig. 16. Section through caudal plate, showing large accumulation of mesoderm. Lettering as in preceding.

Fig. 17. Median longitudinal section of embryo, taken out on March 18th. (Zeiss D × 4.)

Lettering as in the preceding.

Fig. 18. Surface view of an elongated embryo, taken out on March 27th.

Lettering as in the preceding figures.

Figs. 12—27, consecutive series of sections at anterior portion of embryo, as shown in preceding figure. (Zeiss D × 4.)

Figs. 19—25. Consecutive sections through primary head segment, showing relation of oral cell-mass to ectoderm.

ect, ectoderm ; am, amnion ; ms, mesoderm ; ms¹, oral cell-mass ; ms², detached oral cell-mass ; b.c¹, migrating mesodermal cells ; par., "paracyten" or degenerating cells ; other letters as in preceding figures.

Explanation of Plate VIII.

Figs. 23—25. Sections through primary head segment, explanation as given above.

Fig. 26. Section passing through first abdominal segment. (Zeiss D × 5.)

am, amnion ; n.f, neural furrow ; ect, ectoderm ; ms^a, "paradermalen Schicht" ; ms^b, "paralecithalen Schicht" ; vit, vitellophags.

Fig. 27. Transverse section through procephalic lobe, showing migratory cells from oral cell-mass.

am, amnion; ect, ectoderm; ms, mesoderm; ms¹, oral cell-mass; ms², detached cells from oral cell-mass.

Fig. 28. Portion of same section on left side, more highly magnified, showing migratory cells from oral cell-mass. (Zeiss Ap. o. 2 mm × Comp. oc. 6.)

ms¹, oral cell-mass; ms², detached cells from oral cell-mass; yl, yolk granules.

Fig. 29. Median longitudinal section of embryo at same stage as that represented in Fig. 18, which shows faint depression of stomodium at st.

A, anterior; P, posterior direction; st, stomodium; ms, mesoderm; ms¹, oral cell-mass; ms², detached cells from oral cell-mass; dc, degenerating cells; vit, vitellophags.

Fig. 30. Surface view of embryo taken out on March 27th.

lb, labrum; at, antenna; md, mandible; mx¹⁻², first and second maxillae; th. 1¹⁻³, first, second and third thoracic legs; n.f, neural furrow; cd, anal segment. (Zeiss A × 4.)

Fig. 31. Sagittal section of same embryo as that represented in the above, showing relation between stomodium and oral cell-mass.

Lettering as in preceding figures.

Fig. 32. Sagittal section of head segments of embryo more advanced than above. Here oral cell-mass is entirely lost.

Lettering as in the preceding figures.

Figs. 33—40. Series of sagittal sections of embryo at same stages as shown in Fig. 30, showing stomodial depression. Among these series, Fig. 40 is a section through the median longitudinal line. (Zeiss D × 4.)

st, stomodium; ms, mesoderm; ms², detached cells from oral cell-mass; a, elongation of lateral portion of stomodium, or "vordere Epithellamelle," ms^b, anlage of suboesophageal body, from the anterior portion of which (a) blood cells are formed.

A, anterior; P, posterior direction.

Figs. 41—50. See explanation of Plate III.

Arabic numerals placed between every two sections indicate the number of sections that intervene between the two (exclusive.)

Explanation of Plate IX.

Figs. 41—50. Series of transverse sections through primary head segment of embryo as in Fig. 30, showing deepening of stomodium. (Zeiss D × 4.)

ect, ectoderm; ms, mesoderm; d.c, degenerating cells; st, stomodium; a, lateral prolongation of stomodial wall to form mid-gut; b.c, blood cells. Other letters as in preceding.

Fig. 51. Surface view of shortened embryo, taken from egg in April (Zeiss A × 4.)

lb, labrum; at, antenna; md, mandible; mx.⁽¹⁻²⁾, first and second maxilla; th. 1.⁽¹⁻³⁾, first, second and third thoracic legs; sl.g, opening of silk gland; ab. 1.⁽¹⁻¹⁰⁾, abdominal legs; stg, stigma; n.f, neural furrow. (Zeiss A × 4.)

- Fig. 52. Transverse section through antennal region of same embryo as that represented in Fig. 51 showing entoderm-anlage.
ent, entoderm anlage or lateral prolongation of stomodial wall; n, nerve. Other letters as in preceding figures.
- Fig. 53. Sagittal section through primary head segment of same embryo as in preceding figure, showing stomodial tube and entoderm-anlage.
epl. v, "vordere Epithellamelle"; st, stomodium; ms, mesoderm; g.l, "Grenzlamelle"; s.b, suboesophageal body; b.c, blood cells.
- Fig. 54. Portion of same series of above section more highly magnified, showing blood cells. (Zeiss, Ap. 2. mm x Comp. oc. 6).
ect, ectoderm of "Vorkiefersegment;" b.c, blood cells; a, blood cells taken from dorsal vessel of full grown embryo about to hatch.
- Fig. 55. Sagittal section of embryo, somewhat more advanced than that represented in Fig. 52 (taken from egg on April, 4th. Zeiss D x 4).
b.c, blood cells; ms, mesoderm; st, stomodium; g.l, "Grenzlamelle." epl. v, "vordere Epithellamelle"; s.b, suboesophageal body; g.m, mandibular ganglion; g.mx(1-2), first and second maxillary ganglion; sl.g, silk gland.
- Figs. 56-63. Series of transverse sections of embryo corresponding to preceding figure. (Zeiss, D x 4).
Figs. 56-57, sections through antennal region; Figs. 58-60, sections through mandibular segment; Figs. 61-62, sections through first maxillary segment; Fig. 63, section through mesothoracic segment. at, antenna; g.l, "Grenzlamelle"; st, stomodium; ent, lateral entoderm-anlage; s.b, suboesophageal body; b.c, blood cells; n.c, nerve cord; md, mandible; ms¹, first maxilla; n.f, neural furrow; ms, mesoderm; sp. ms, splanchnic mesoderm; sm. ms, somatic mesoderm; coc, coelomic cavity; tent², second tentorium; d.c, degenerating cells; th. 1², second thoracic leg; æ, ænocytes.
- Fig. 64. Transverse section through sixth abdominal segment, same embryo as above. (Zeiss D x 4).
ent, entoderm; d.c, degenerating cells; g, genital cells; sp. ms, splanchnic mesoderm; stg, stigma.
- Fig. 65. Portion of frontal section through anterior portion of mid-gut showing karyokinetic division of its epithelial cells.
epl. v, "vordere Epithellamelle"; g.l, "Grenzlamelle"; sp. ms, splanchnic mesoderm; st, stomodium. (Zeiss, Ap. 2. mm x oc. II).
- Fig. 66. Portion of transverse section of primary head segment of embryo, corresponding to that represented in Fig. 52. (Zeiss, Ap. 2. mm x Comp. oc. 4).
ect, ectoderm; par, degenerating cells.
- Figs. 67-69. Free cells in yolk, taken from same embryo as in preceding figure (magnification as above).
d.c, degenerating cells; ms³, immigrating cells from oral-cell-mass; vit, vitellophags; yl, yolk granules.

- Fig. 68. Yolk ball, taken from embryo as represented in Fig. I, showing direct division of nucleus.
- Fig. 70. Anal segment of embryo as represented in Fig. 30. (Zeiss, B \times 2).
n.f, neural furrow; a, proctodial depression.
- Fig. 73. Transverse section through anal segment of embryo as represented in Fig. 30, showing bifurcation of neural furrow. (Zeiss D \times 4).
ms, mesoderm; n.f, neural furrow.
- Fig. 76. Transverse section through anterior portion of proctodium of embryo corresponding to Fig. 74. (Zeiss, D \times 4).
pt, proctodium; m.v, malpighian vessel; ms, mesoderm; n.c, nerve cord.

Explanation of Plate X.

- Fig. 71. Longitudinal section of same embryo as Fig. 70. (Zeiss D \times 4).
d.c, degenerating cells; ms, mesoderm; pt, proctodium.
- Fig. 72. Sagittal section through anal segment of an embryo slightly more advanced than the above. (Zeiss, D \times 4).
ent, entoderm-anlage; m.v, malpighian vessels; pt, proctodium; other letters as in preceding figures.
- Fig. 73. Sagittal section through posterior portion of embryo represented in Fig. 51. (from embryo taken on April 1st). (Zeiss, D \times 4).
am, amnion cells; d.c, degenerating cells; epl. h, "hintere Epithellamelle"; pt, proctodium.
- Fig. 75. Sagittal section through abdominal portion of more advanced embryo, taken from egg on April 9th. (Zeiss D \times 4).
Lettering as in preceding figures.
- Fig. 77-82. Series of transverse section through head segment of embryo as represented in Fig. 52, showing various ectodermal invaginations. (Zeiss, D \times 4).
Figs. 77, section through anterior portion of mandibular segment in somewhat oblique direction; Fig. 78, posterior portion of same segment; Fig. 79, first maxillary segment; Fig. 80-82, series of sections through second maxillary segment, at, antenna; br, supraoesophageal ganglion; ent, entoderm; f.n.c, frontal nerve chord; fl. md, flexor mandibulae; s.b, suboesophageal body; sp. ms, splanchnic mesoderm; sl.g, silk gland; md, mandible; mx (1-2), first and second maxilla; n, new gland or hypostigmatic gland; st, stomodium.

Explanation of Plate XI.

- Figs. 83-91. Consecutive series of transverse sections through head of embryo more advanced than that represented in Fig. 52, (taken from egg on April 6th). (Zeiss D \times 4).
Figs. 83 and 84, sections through anterior portion of mandibular region; Fig. 85 through posterior portion of same region; Figs. 86 and 87, through portion between mandible and first maxilla; Figs. 88 and 89, through second maxilla; Fig. 90, through posterior

portion of head; Fig. 91, through first thoracic segment, br, supraoesophageal ganglion; ex. md, attachment of extensor mandibulae; fl. md, attachment of flexor mandibulae; g.f, ganglion frontale; n, new gland or hypostigmatic gland; s.g, salivary gland; st, stomodium; sl.g, silk gland; tent⁽¹⁼²⁾, first and second tentorium; other letters as in preceding figures.

Figs. 92--93. Series of sagittal sections through head and thorax of embryo as in preceding figure. (Zeiss, D × 2).

Lettering as in preceding figures.

Fig. 94. Frontal section through head and thorax of more advanced embryo taken out on April 9th. (Zeiss, D × 4).

Lettering as in preceding figures.

Fig. 95. New gland at base of first stigma, (taken from larva of third stage). (Zeiss, A × 2).

g.a, abdominal ganglia; m, muscles; n, new gland or hypostigmatic gland; tr, trachea.

Fig. 96. New gland of full grown larva. (Zeiss, A × 2).

Figs. 67⁽¹⁻²⁾. Portion of the new gland, highly magnified. (Zeiss D × 2).

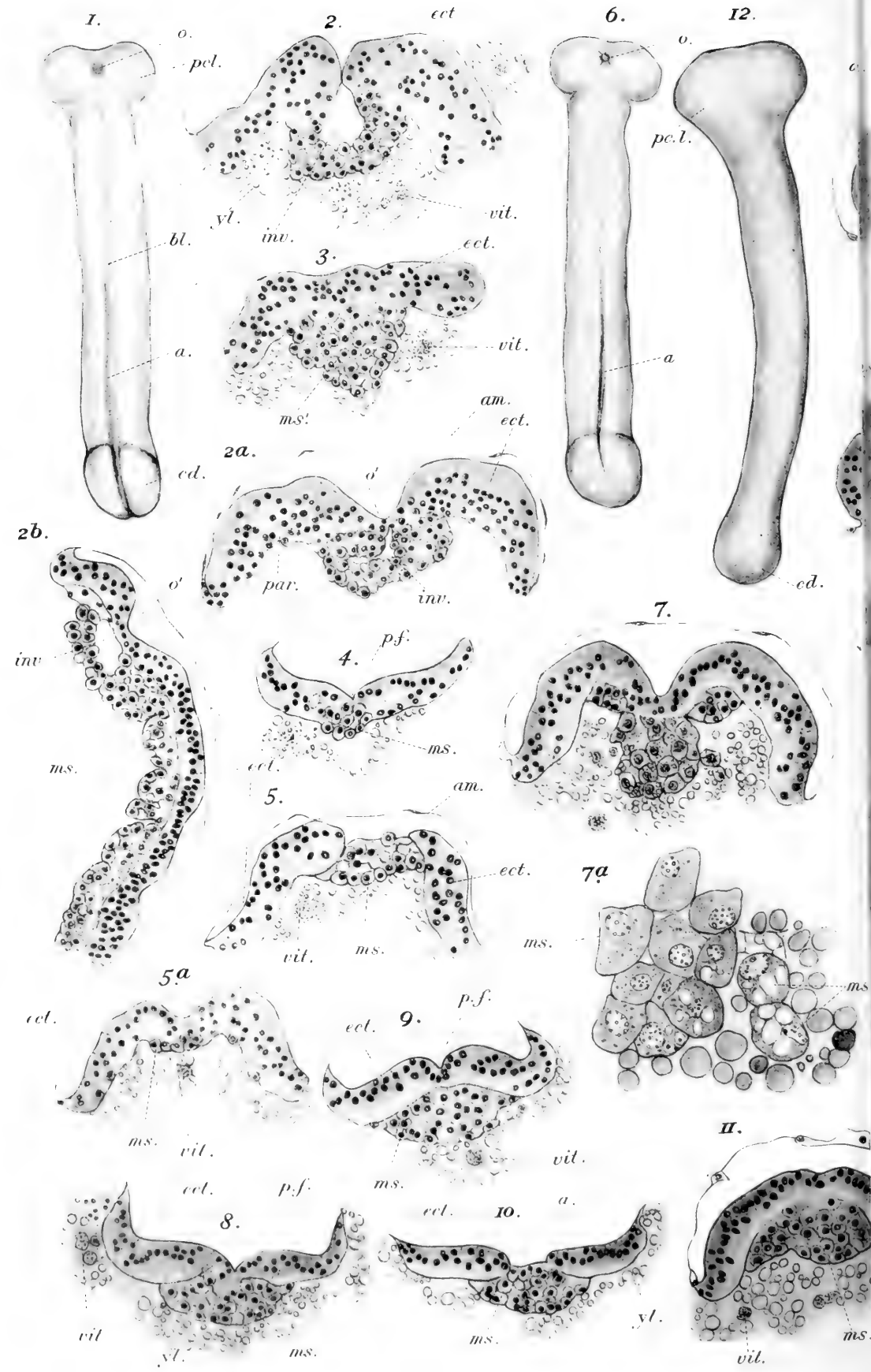
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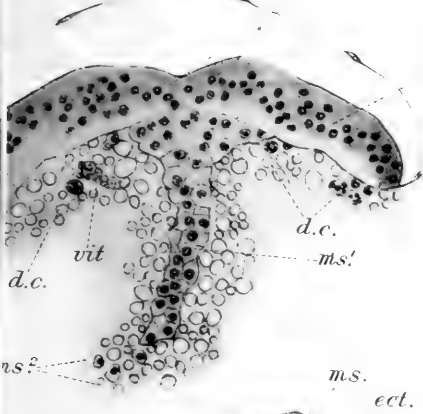
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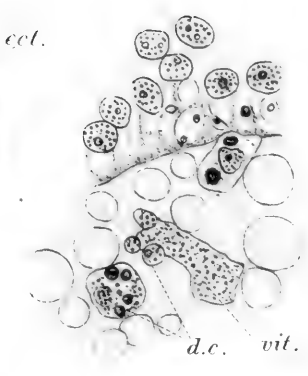




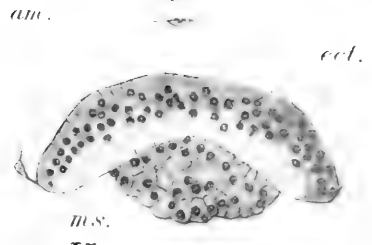
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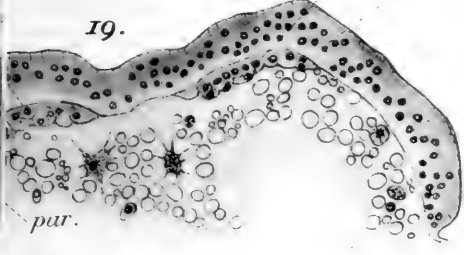
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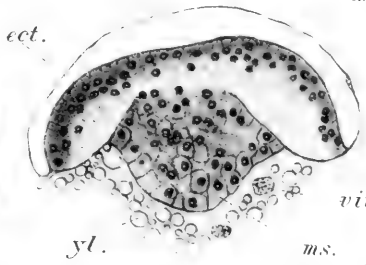
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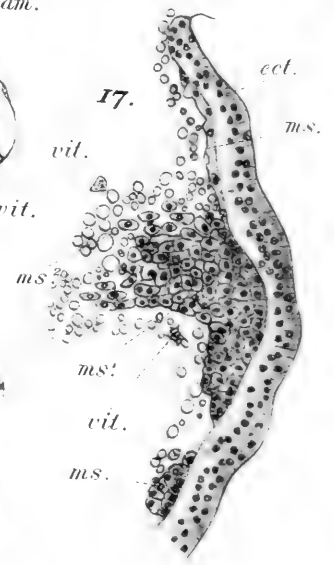
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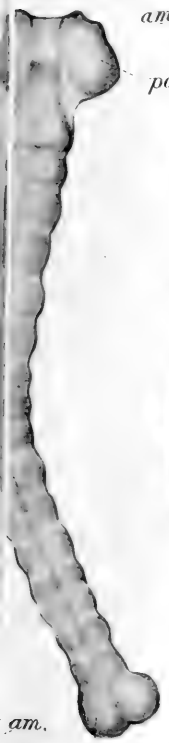
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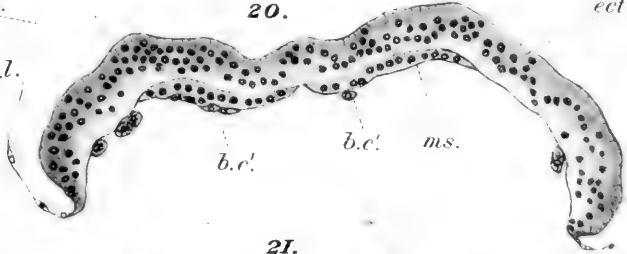
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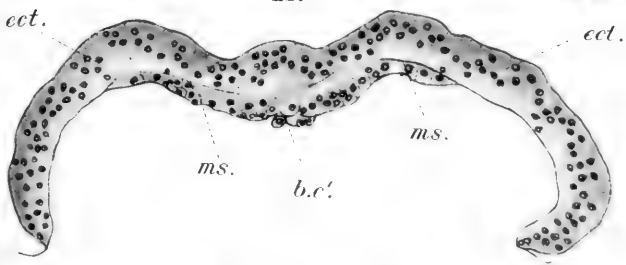
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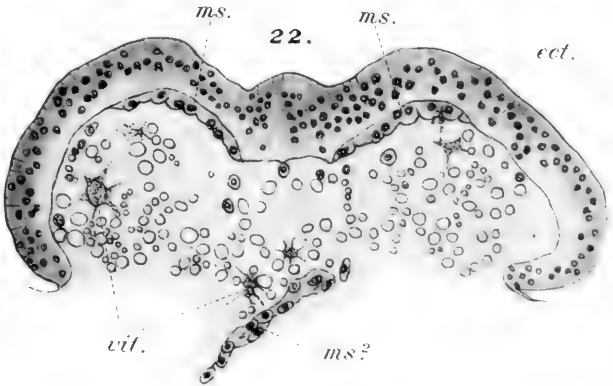
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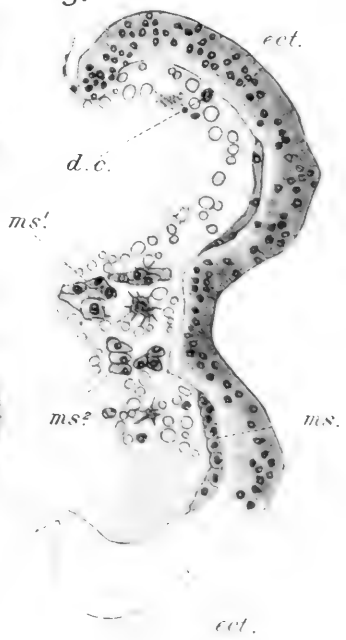
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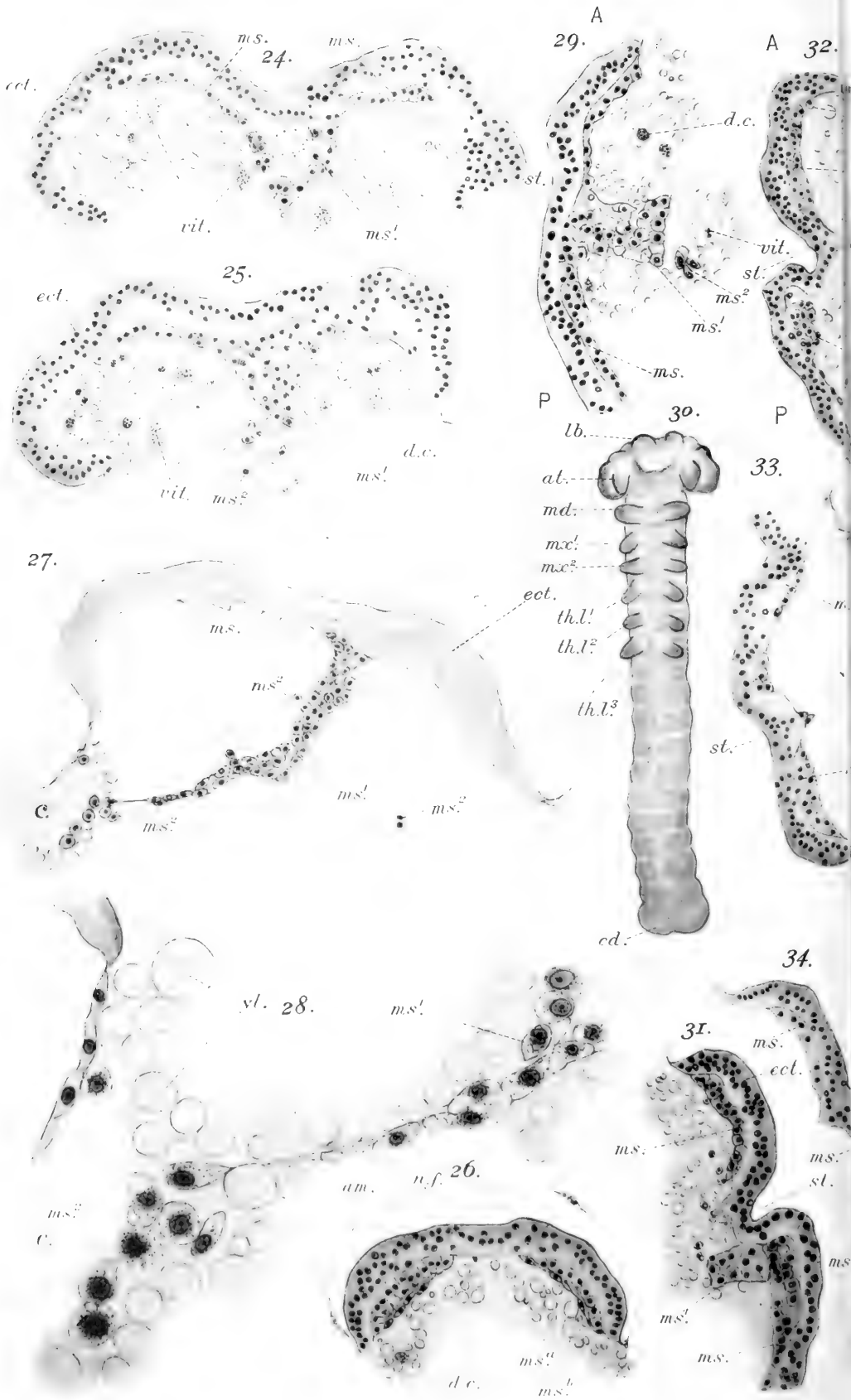
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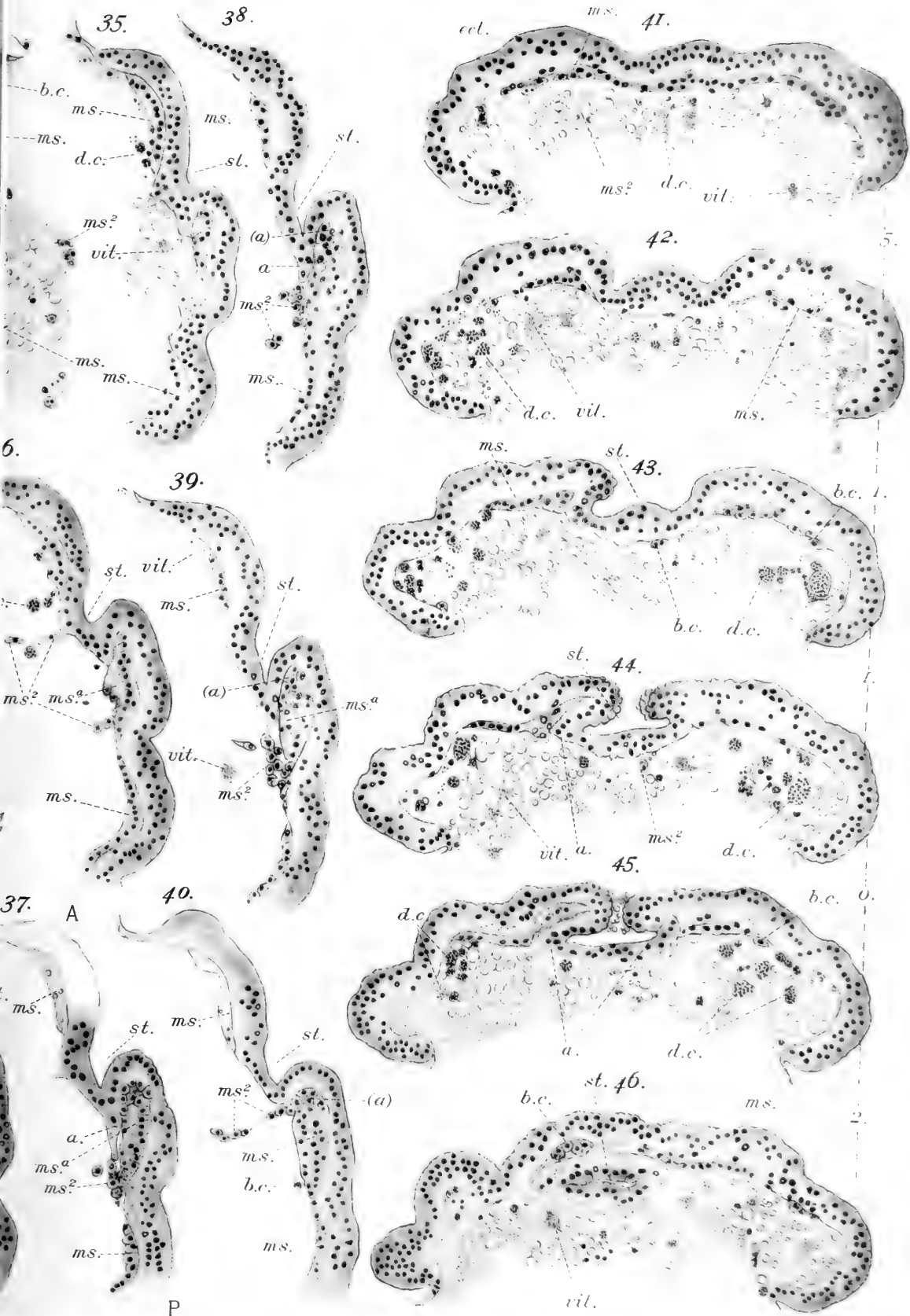
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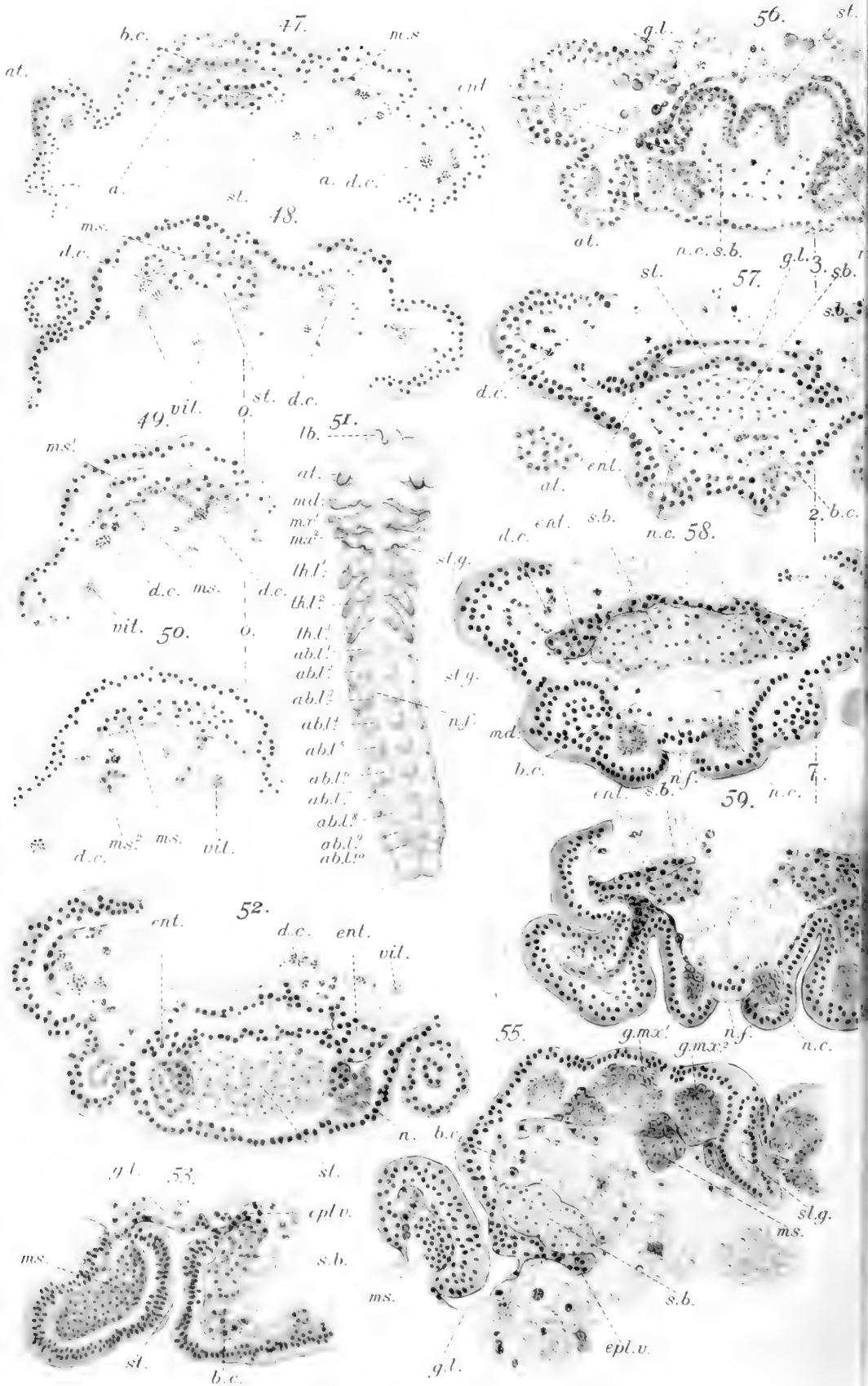






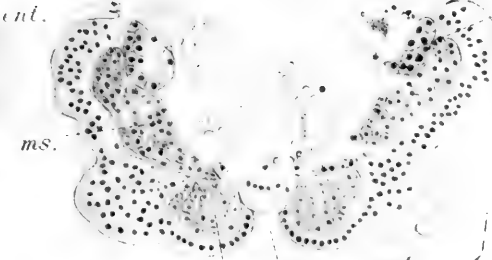




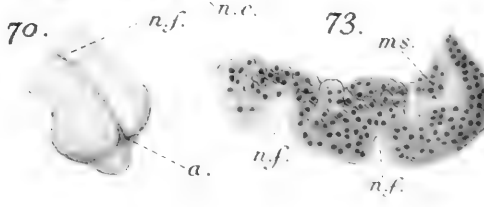
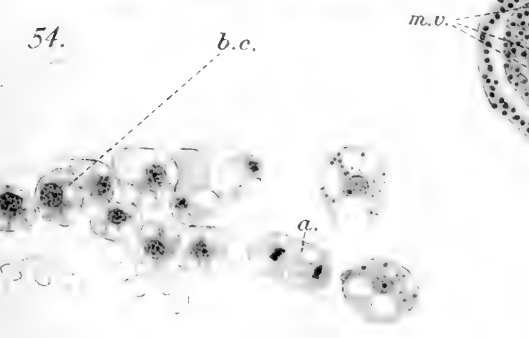
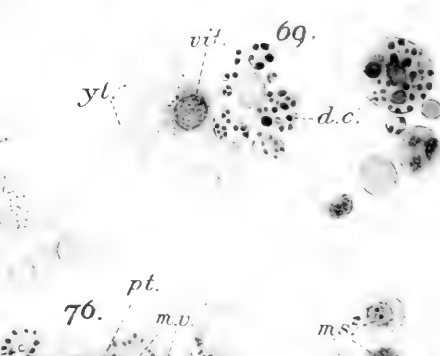
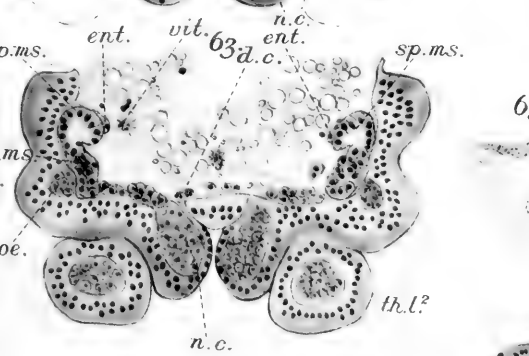
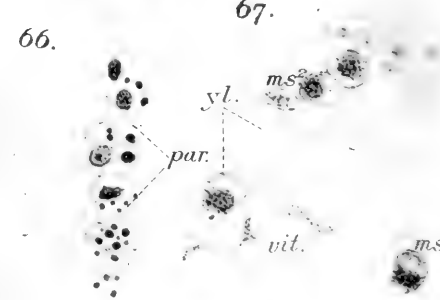
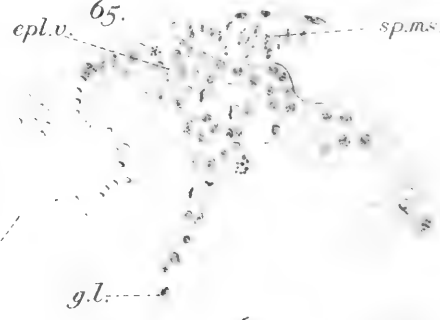
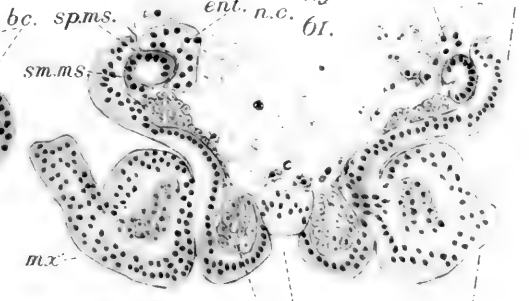
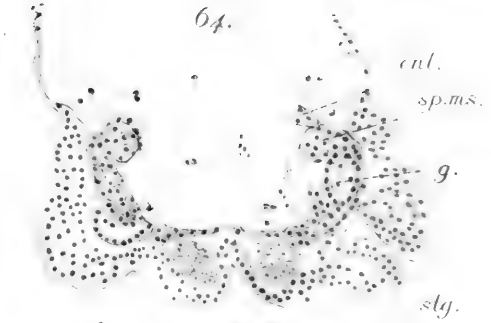


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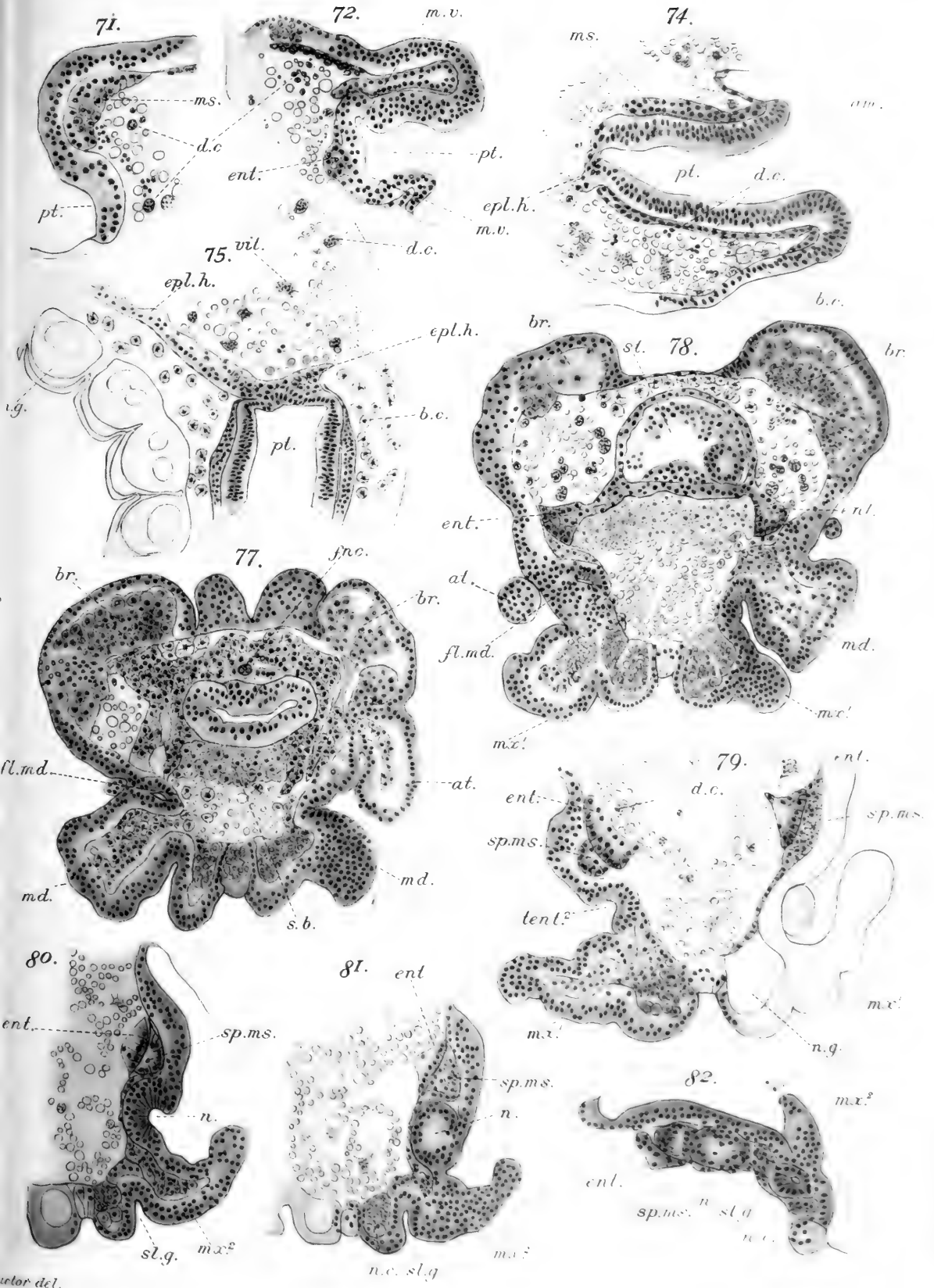
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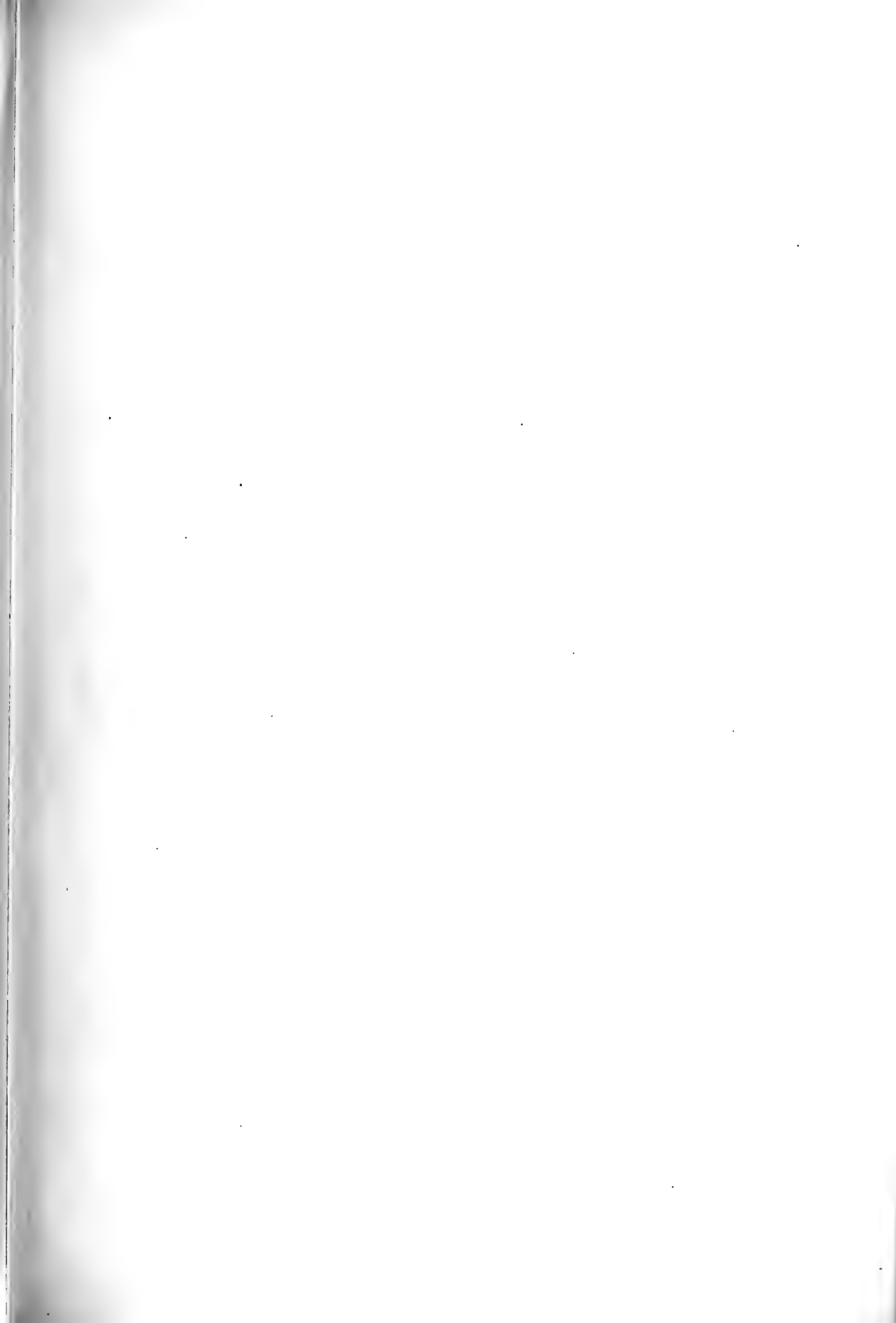
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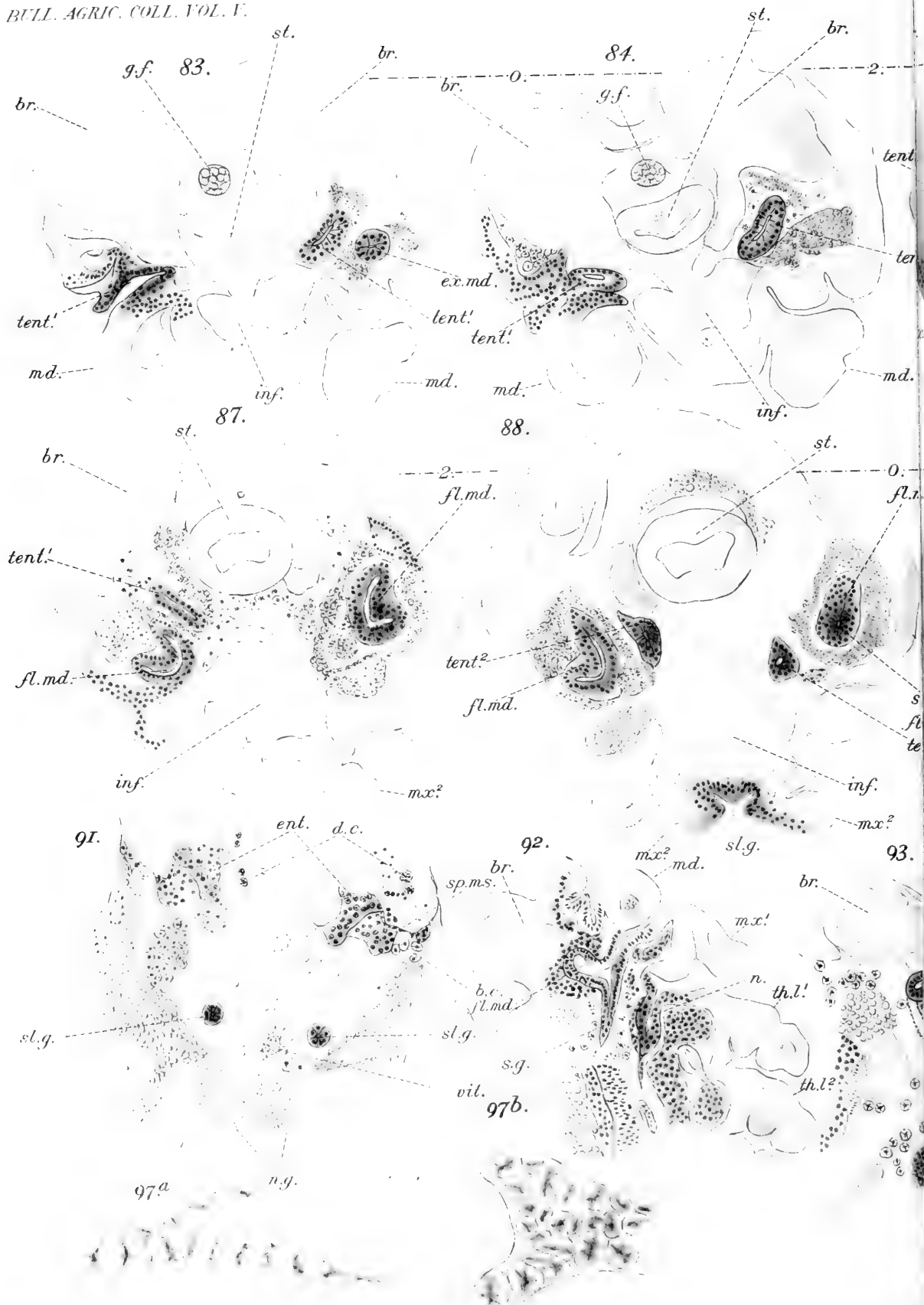


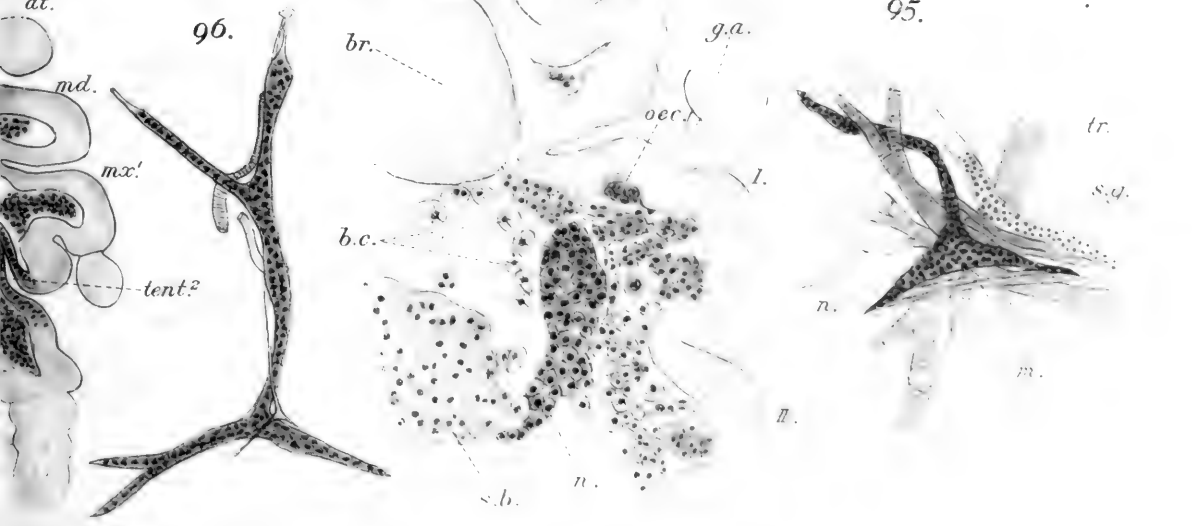
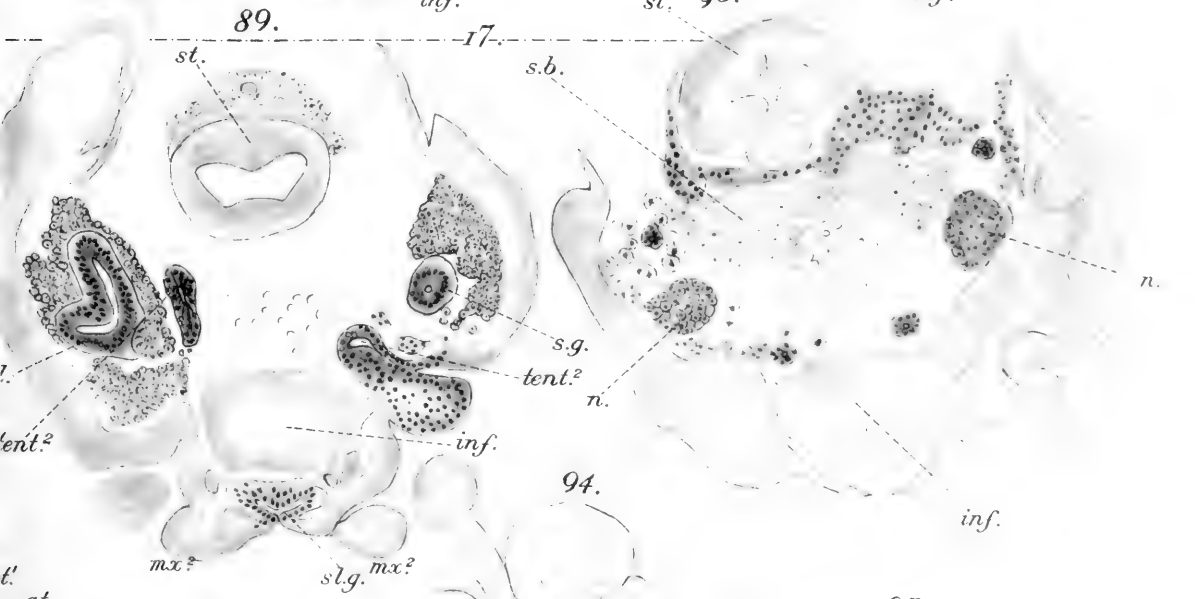
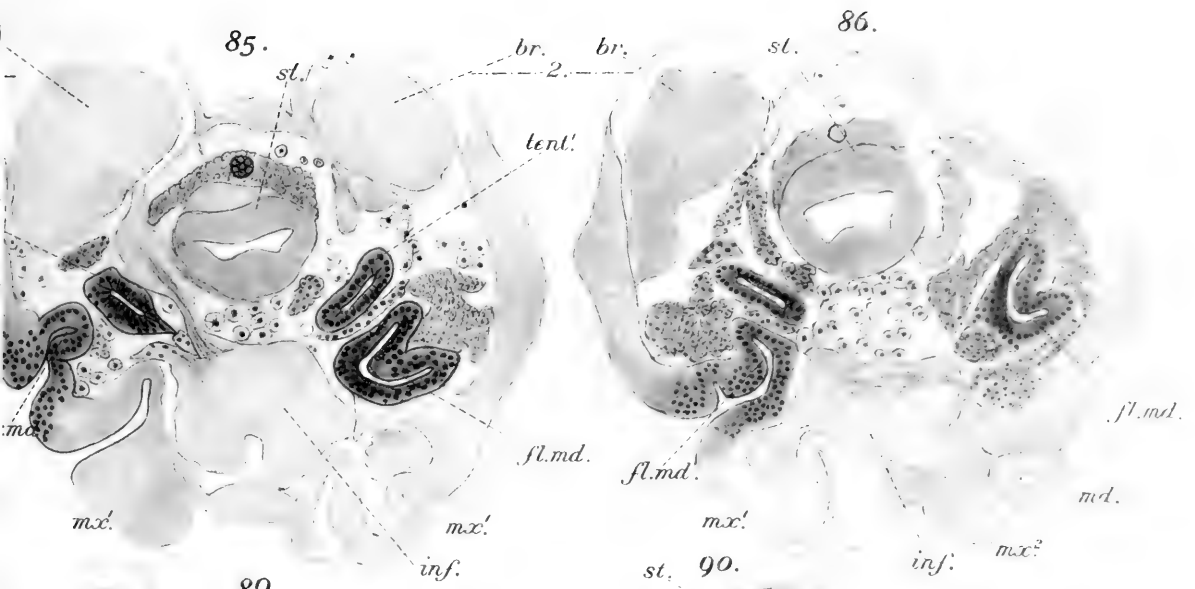


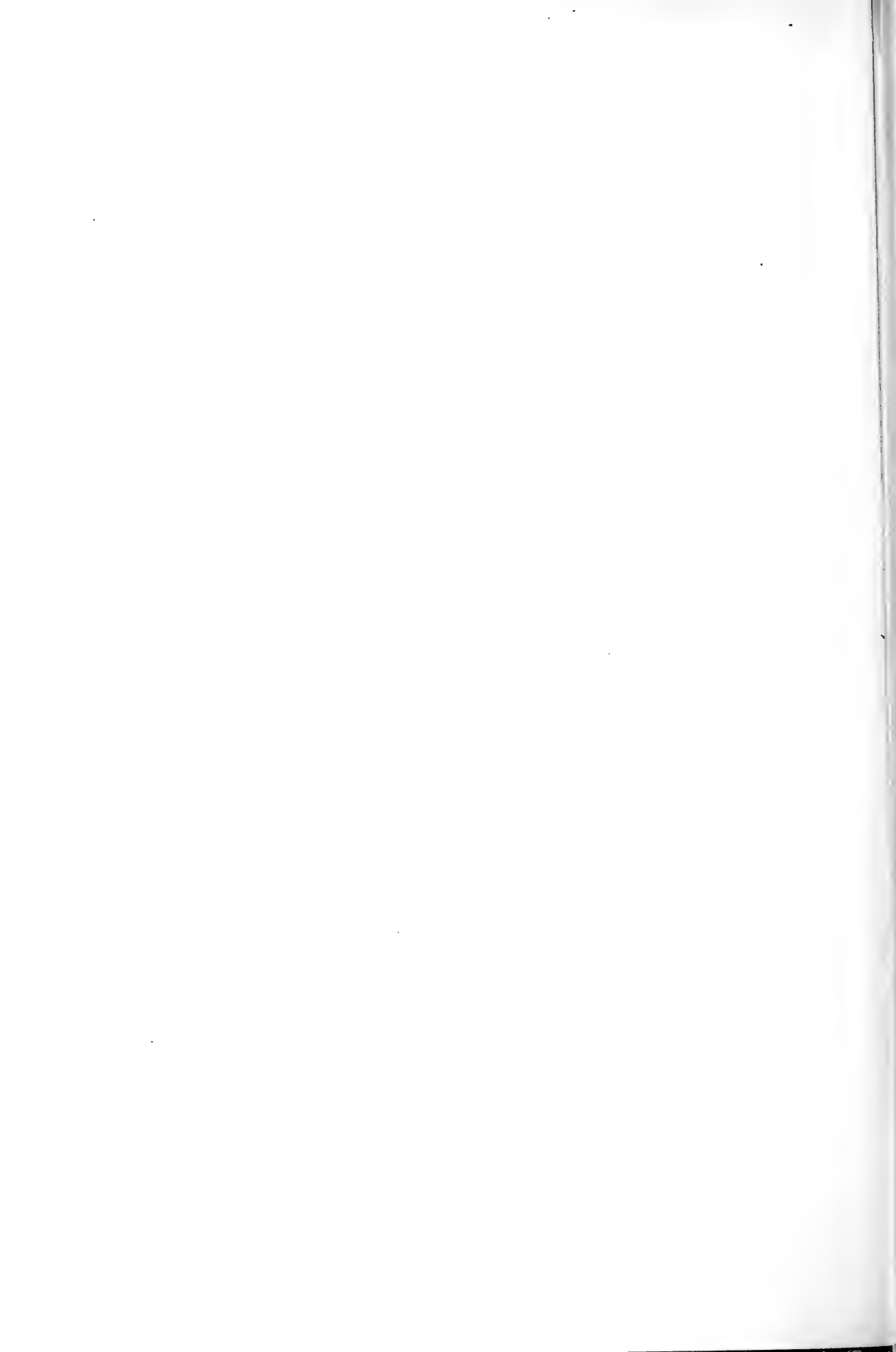












Ueber das wirksame Princip des Tuberculinum Kochii.

VON

N. Nitta.

Einleitung und Literatur.

Seit R. Koch sein Tuberculinpraeparat in Anwendung brachte, haben viele Forscher versucht, das wirksame Princip desselben zu isoliren. Koch selbst hat sein sogenanntes Reintuberculin aus dem Rohtuberculin oder gewöhnlichen Tuberculin mittelst 60% igen Alcohol dargestellt und zeigte, dass es im Wesentlichen die chemischen Eigenschaften einer Albumose besitzt. Nach W. Kühne ist dieses Produkt aber kein chemisches Individuum, sondern ein Gemisch von Proteinstoffen, das noch an 20% Aschenbestandtheile enthält. Aus Tuberkelbacillenculturen hat W. Kühne folgende verschiedene Proteinfractionen erhalten: Albuminat, Acroalbumose und Deuteroalbumone; Essigsäurefällung und Ammonsulfatfällung,¹ von denen einige beim Tierversuche eine noch energischere Wirkung als das „Reintuberculin“ Koch's zeigten. Trotzdem ist er der Meinung „dass keine dieser Substanzen mehr sei, als der Träger des Tuberculinum verum; sie sind dazu unter sich in chemischen Verhalten zu verschieden und uns aus dem Nährboden und als Bestandtheile des Handelspeptons nur allzu bekannt.“

Auch hat Helmann eine eingehende chemische und pharmacologische Untersuchung über das Tuberculin ausgeführt. Er fand als dessen Bestandtheile: 1) Albumose und zwar Protoalbumose und Deuteroalbumose, 2) mehrere Alkaloide, 3) Extractivstoffe, 4) Mucin, anorganische Salze, Glycerin und Farbstoffe. Ferner stellte er mit dem Alcoholniederschlag des Tuberculins(A) und mit dem Alcoholfiltrat(C) Tierversuche

¹ Letztere beide Fractionen wurden aus albumosefreier Peptonkultur erhalten.

an und fand, dass ersteres, welches den grössten Teil der Albumose enthält, starke, entzündliche Local-Reaction und kein oder nur geringes Fieber, dagegen letzteres, welches besonders die Salze und nur wenig Albumosen enthält, keine locale Entzündung, aber hohes Fieber verursacht. Helman hat über das von ihm aus Kartoffelkulturen, welche unter oder ohne Mitwirkung von Serum und Glycerin gewachsen waren, erhaltene Tuberculin, sowie über die Koch'sche Lymphe Studien veröffentlicht. Er sieht sich auf Grund seiner Beobachtungen zu dem Schluss gezwungen, dass der wirksame Körper nicht aus Albumosen allein besteht; das „Reintuberculin“ von Koch ist seiner Meinung nach eine Mischung von Albumosen und wirksamer Substanz; das Eiweiss reisse beim Ausfallen gewisse in der Flüssigkeit enthaltene Substanzen mechanisch mit nieder.

Matthes bekam durch Injection von gewöhnlichen Albumosen, also von Körpern, welche ohne jede spezifische bacterielle Thätigkeit aus Verdauungsgemischen isolirt werden, bei an Lupus erkrankten Menschen deutliche locale Reaction und hält die Tuberculinwirkung wenigstens zum Theil für eine Wirkung einer gewöhnlichen Albumose: daher empfiehlt er statt des Tuberculins, welches nach ihm ein theures, schwer haltbares Praeparat und noch dazu kein einheitlicher Körper ist, die gewöhnliche Deuteroalbumose zu benutzen, welche allerdings in etwas grösseren Dosen (0,05-0,075 g) angewendet werden muss. Die Deuteroalbumose ist ferner ein völlig reines Material, welches sich leicht vollkommen salzfrei darstellen lässt; sie erhält sich als weisses trockenes Pulver jahrelang unverändert und erlaubt eine absolut genaue Dosirung.

Petri und Maassen zeigten, dass gewöhnliche 10 proc. Peptonbouillon, welche für gesunde Meerschweinchen in Mengen von 4 ccm. eingespritzt ohne Nachtheil war, tuberculöse Tiere tötete. Bei der Section fanden sich in der Umgebung der tuberculösen Herde deutliche Erscheinungen einer Reaction. Nach Ansicht der Verff. ist diese Giftwirkung wesentlich dem hohen Peptongehalt der Nährbouillon zuzuschreiben; sie läugnen deshalb die Specificität der Tuberculinwirkung, womit auch mehrere andere Autoren übereinstimmen.

Von Stoffen, die dem Tuberculin ähnlich wirken, werden genannt:

ein proteinhaltiges Extrakt des *Bac. pyocyaneus* (Roemer), das Protein der Pneumobacillen oder des *Bac. prodigiosus* (Buchner), Proteine nicht pathogener Bakterienarten (G. Klemperer), Teucrin, ein Pflanzenextrakt (v. Mosetig), Kreatin, Kreatinin, Cystin, Allantoin und Tyrosin (Dixon und Zuill). Ausserdem noch eine grosse Anzahl der verschiedenartigsten Stoffe wie Thiophen, Benzol, Sulfoharnstoff, Sulfoäthylharnstoff, Aceton, Propylamin, Trimethylamin, Allylamin, Taurin, Cadaverin (Spiegler), kantharidinsäure Salze (Liebreich) u. s. w.

Nach der Untersuchung Viquerat's verhält sich Tuberculin, welches auf 150–200° c. erhitzt wurde, gegen tuberculöse Tiere wie nicht erhitztes; seine weitere Behauptung, dass bernsteinsäure Salze ebenso wirken wie Tuberculin, wurde von Hutyra sowie von mir selbst nicht bestätigt gefunden.

Ruppel untersuchte Filtrate von Massenculturen des Tuberkelbacillus auf ihre chemischen Bestandtheile. Die Filtrate enthielten an fällbaren Substanzen vorwiegend Deuteroalbumosen, neben primären Albumosen und Hemialbumosen; Acroalbumose ist nur dem Gehalt an Witte's Pepton entsprechend wenig vorhanden. Es gelang nicht, ein typisches und spezifisches Stoffwechselprodukt des Tuberkelbacillus zu isoliren; es liess sich nur feststellen, dass den Tuberkelbacillen ein tryptisches Verdauungsvermögen zukommt, indem sie in alkalischer Lösung Eiweisskörper bis zur Bildung von Pepton unter gleichzeitigem Auftreten von Tryptophan spalten.

Bei der grossen Divergenz der Meinungen verschiedener Forscher waren weitere eingehende Untersuchungen wünschenswerth, und ich habe deshalb viele Versuche angestellt, um einen kleinen Beitrag zur Aufklärung der Frage zu liefern.

Eigene Untersuchungen.

I. Herstellung des Tuberculins.

Das bei meinen vergleichenden Versuchen angewandte Koch'sche Rohtuberculin wurde von mir in meinem Laboratorium aus Menschen-

tuberkelbacillenkulturen hergestellt. Die Nährlösung bestand aus einer normalen Peptonrindfleischbouillon, die eine 1.6–3.3% Normalnatronlauge entsprechende Acidität besitzt und die ich mit 5% Glycerin versetzte. Die Kultur wurde im Brutofen bei einer Temperatur von 37–39° C. gehalten bis nach Ablauf von 1–2 Monaten die Entwicklung der Bacillen beendet war, worauf im Dampftopf $\frac{1}{2}$ –1 Stunde lang sterilisirt und durch sterilisirtes Filtrirpapier filtrirt wurde: das Filtrat wurde auf dem Wasserbade bei 95–100° C. in sterilisirter Porzellanschale bis auf den zehnten Theil ihres ursprünglichen Gewichts eingedampft. Die in dieser Weise gewonnene gelbbraune, syrupartige Flüssigkeit stellt das sogenannte Tuberculin (Rohtuberculin, gewöhnliches Tuberculin) dar. Sie reagirte schwach alkalisch und zeigte das spec. Gewicht 1.195 (bei 15° C.). Die Analyse ergab: Wasser 44.6375%. Stickstoff 2.4750%. Asche 6.3322%. Chlor 2.8609%. Das Glycerin wurde nicht bestimmt. Tuberculös gemachte Meerschweinchen zeigten nach Injection von 0,001 ccm. starkes Reactionsfieber (1–2° und darüber): bei hochgradig tuberculösen Tieren (4–8 Wochen nach der Impfung) genügt 0,1 ccm. Tuberculin zur Tötung innerhalb 6–30 Stunden. Die Lethaldose für die Tiere mit weniger fortgeschrittener Tuberculose ist 0,25–0,5 ccm.

II. Der mit 60% igem Alcohol erhaltene Niederschlag, das Reintuberculin Koch's.

Ein Theil des flüssigen Rohtuberculins wird mit nur $1\frac{1}{3}$ Volumenteilen absoluten Alcohols vermennt und 24–48 Stunden stehen gelassen: es bildet sich ein flockiger Bodensatz; die überstehende Flüssigkeit wird abgegossen, nun aber ein gleiches Volum nur 60% ige Alcohol zugesetzt, wieder absitzen gelassen und dies 3–4 Mal wiederholt, bis der über dem Niederschlag stehende Alcohol fast farblos erscheint: nach mehrmaligem Auswaschen mit absolutem Alcohol wird der Niederschlag bei 50–60° C. getrocknet, wobei eine schneeweiße leicht zerreibliche Masse resultirt. Dies ist das sogenannte Koch'sche Reintuberculin (Tuberculinum depuratum Koch). Tuberculöse Meerschweinchen reagiren auf Injection von 0,00005 g. dieses Praeparats.

III. Der in absolutem Alcohol unlösliche Teil des Rohtuberculins.

Anfangs modificirte ich die Koch'sche Methode auf die Weise, dass ich, statt sechzigprocentigem, absoluten Alcohol anwandte. Wird das Rohtuberculin mit dem mehrfachen Volumen absoluten Alkohols unter Umrühren vermischt, so bildet sich ein Niederschlag; nach 15-24 stündigem Stehenlassen wird die Flüssigkeit abgegossen und der zurückbleibende Niederschlag nochmals mit absolutem Alcohol versetzt und dieselbe Operation wird so lange wiederholt, bis der zugesetzte Alcohol vollkommen farblos erscheint. Nach Abpressen zwischen Filtrirpapier wurde der Niederschlag bei 50-60° C. getrocknet: es resultirt eine gelblich weisse leicht zerreibliche Masse. Eine Einspritzung von 0,0005 g. dieser Substanz bei tuberculösen Meerschweinchen ruft eine deutliche Steigerung der Körpertemperatur hervor. Es erwies sich schwächer als das Koch'sche Praeparat, wesshalb ich nun Ammonsulfat anwandte.

IV. Ammonsulfatfällung des Tuberculins.

Mein Tuberculinpraeparat wird mittelst Ammonsulfatfällung gewonnen. Sättigt man flüssiges Rohtuberculin mit Ammonsulfat, so scheidet sich eine gelbbräunliche zähe Masse ab, die nach 24 Stunden gesammelt und einmal mit gesättigter Ammonsulfatlösung ausgewaschen, dann zwischen Filtrirpapier gepresst, und in etwas destillirten chloroformhaltigen Wasser gelöst, hierauf so lange gegen strömendes Wasser dialysirt wird, bis die Lösung keine Reaction auf Schwefelsäure mehr zeigt. Die Flüssigkeit wird dann filtrirt, das Filtrat bei 50-60° C. eingedampft und schliesslich mit einem Ueberschuss von absolutem Alcohol unter Umrühren versetzt. Nach 24-48 Stunden wird der voluminöse gelblich weisse Niederschlag abfiltrirt, 2-3 Mal mit absolutem Alcohol ausgewaschen, zwischen Filtrirpapier gepresst und bei 50-60° C. getrocknet. Das dabei gewonnene grauweisse Pulver ist leicht löslich und enthält nur 1,5446% Asche, während Koch'sche Praeparate an 20% Asche enthalten. Die wässrige Lösung

dieser Substanz reagiert neutral und ist von bräunlicher Farbe. Dieselbe coaguliert nicht beim Kochen und wird durch Salpetersäure sowie durch Ferrocyankalium und Essigsäure gefällt; der dabei gebildete Niederschlag löst sich beim Erwärmen und scheidet sich beim Abkühlen wieder ab. Dieselbe wird auch durch Phosphorwolframsäure, Pikrinsäure, Gerbsäure, Sublimat und Ammonsulfat gefällt: Biuret, Millon'sche und Xanthoproteinreactionen fallen positiv aus.

Dass mein Präparat frei von peptonartigen Substanzen ist, geht daraus hervor, dass dasselbe vollständig durch Ammonsulfat gefällt wird und das Filtrat von der Ammonsulfatfällung keine Peptonreactionen gibt. Diese peptonfreie Substanz ist daher als Tuberculinalbumose zu bezeichnen.

Die wässrige Lösung meiner Tuberculinalbumose gibt bei Sättigung mit Chlornatrium sehr schwachen, beim weiteren Zusatz von etwas Essigsäure aber starken Niederschlag, was andeutet, dass meine Tuberculinalbumose hauptsächlich den Character einer Deuteroalbumose von Kühne trägt; sie enthält aber noch Spuren von Protoalbumose. Die Thatsache aber, dass eine wässrige Lösung meines Präparates mit verdünnter Essigsäure einen schwachen im Ueberschuss derselben löslichen Niederschlag erzeugt, deutet darauf hin, dass sie eine geringe Menge einer Albumose enthält, welche der Atmidalbumose Neumeister's analog ist.

V. Injectionsversuche.

a) Mit Meerschweinchen.

Die in destillirtem Wasser gelöste Tuberculinalbumose wurde in verschiedenen Dosen tuberculösen Meerschweinchen (Körpergewicht: ca 400–500 g.) subcutan eingespritzt. Die dabei gewonnenen Ergebnisse sind aus folgender Tabelle zu ersehen:

Nr. der Versuche.	Menge der Tuberculinalbumose g.	Körpertemperatur. Cels.°									Temperatursteigerung. Cels.°
		Vor Einspritzung.	Nach Einspritzung (Stunden).								
			1h	2h	3h	4h	5h	6h	7h	8h	
1	0,005	38,5	—	38,8	40,0	40,5	40,4	40,1	39,2	38,6	2,8
2	0,001	38,7	—	38,4	38,8	39,25	40,0	40,0	39,8	39,6	1,3
3	0,0005	37,25	37,35	37,95	39,25	39,75	40,05	40,05	39,85	—	2,8
4	0,0001	38,2	38,2	38,6	38,8	39,15	39,8	40,2	39,7	—	2,0
5	0,00005	38,5	38,9	38,9	39,1	39,7	39,5	39,4	39,3	39,0	1,2
6	0,00001	38,6	39,4	40,2	40,2	39,9	39,2	39,0	39,1	38,9	1,6
7	0,000005	39,1	—	39,1	39,3	39,5	40,1	40,1	40,0	39,6	1,0
8	0,000001	39,0	—	39,0	39,2	39,4	39,1	39,1	39,4	39,2	0,4

Bei Betrachtung der obigen Versuche erkennt man, dass die Tuberculinalbumose in Dosen von 0,00001 (—0,000005) g. bei tuberculösen Meerschweinchen die echte Fieberreaction zu erzeugen im Stande ist. Beim Vergleich der Wirkung des Rohtuberculins und des Reintuberculins von Koch mit meiner Tuberculinalbumose ergab sich folgender Unterschied:

- Rohtuberculin nach Koch..... 0,001 ccm.
- Reintuberculin nach Koch 0,00005 g.
- Tuberculinalbumose 0,00001 g.

Die Versuche bezüglich ihrer tödtlichen Wirkung auf tuberculöse Meerschweinchen (Körpergewicht: ca. 400-500 g.) hatten folgendes Ergebniss:

Nr. der Versuche.	Tage nach Virusimpfung.	Menge der Tuberculinalbumose. g.	Körpertemperatur. Cels.°							Bemerkungen.
			Vor Einspritzung.	Nach Einspritzung. (Stunden.)						
				2h	3h	4h	5h	6h	7h	
1	34	0,1	38,9	40,0	39,6	39,3	—	Todt.		Todt.
2	„	0,05	38,0	38,1	37,9	37,5	—	—	Todt.	„
3	48	0,01	38,3	38,6	39,0	40,2	40,0	39,7	37,3	Todt in der folgenden Nacht.
4	37	0,005	38,6	38,8	39,5	38,8	37,0	—	Todt.	Todt.
5	28	0,001	37,4	38,2	39,0	39,5	39,6	39,4	39,3	Todt in der folgenden Nacht.
6	37	0,0005	38,5	39,4	39,6	39,3	38,2	—	—	„
7	„	0,0005	38,4	38,6	39,3	40,1	40,3	40,6	39,9	Lebend.
8	„	0,0001	37,5	37,7	38,7	39,5	40,6	39,1	38,7	„
9	„	0,0001	38,2	39,5	39,6	40,2	39,8	38,6	39,3	„

Aus obigen Tabellen ersieht man, dass die minimale Lethaldose der Tuberculinalbumose für mittelgrosse Meerschweinchen 28–48 Tage nach der Impfung mit Tuberkelbacillen 1 Milligr. ist. Bei den Tieren, welche dieser Dose erlagen, finden sich bei den Eingeweiden, insbesondere an der Oberfläche der Milz und Leber, zahlreiche haemorrhagische Flecke von Mohnsamen- bis Pfenniggrösse. Diese Haemorrhagie wurde auch von mir bei der Rohtuberculinjection beobachtet: nach R. Koch ist dieser Befund ein charakteristisches Merkmal der Tuberculinwirkung.

Nach Koch erfordert die Tötung der Tiere von seinem Reintuberculin 5–10 Milligr: somit ist Giftwirkung meiner Tuberculinalbumose 5–10 fach so stark als die der Koch'schen.

Zum Vergleich habe ich auch einige Tierversuche mit den von mir aus Witte'schem Pepton hergestellten Albumosen ausgeführt. Die Injection von 0,1 g. dieses Praeparats hatte nur einige Zehntel Grade Temperatursteigerung im Gefolge und schädigte das Wohlbefinden der Tiere nicht im Geringsten, während von meiner Tuberculinalbumose schon 0,001 g. tödlich auf diese Tiere wirkt und 0,00001 g. schon eine Temperatursteigerung herbeiführt.

b) Mit Rindern.

Es dienten hier Rinder, welche ein Jahr vorher bei der Probeinjection des gewöhnlichen Tuberculins eine deutliche Fieberreaction gezeigt hatten, zu den Versuchen, deren Resultate in folgender Tabelle zusammengestellt sind :

VERSUCHE MIT TUBERCULINALBUMOSE.

Laufende Nummer.	Tiere.	Menge der Tuberculinalbumose, gr.	Körpertemperatur. Cels.°										Temperatursteigerung, Cels.°	
			Nach Einspritzung. (Stunden.)											
			Vor Einspritzung	8h	10h	12h	14h	16h	18h	20h	22h	24h		32h
1	13 jährige Kuh.	0,001	Nachm. 5	38,5	38,4	39,0	39,3	40,2	41,3	41,2	40,5	39,9	38,6	2,7
2	7 "	"		38,6	38,9	39,8	40,7	41,3	41,0	40,7	40,55	40,4	39,3	2,7
3	6 "	"		38,2	38,4	39,0	39,5	40,2	40,9	40,7	40,4	40,6	38,5	2,0
4	" "	"		38,4	38,6	38,9	40,1	40,8	40,8	40,4	40,4	40,15	39,35	1,75
5	7 "	"		38,6	38,6	39,0	40,0	41,5	41,2	40,6	40,2	40,6	39,0	2,8
6	5 "	"		40,7	41,1	41,0	41,2	40,7	40,3	39,7	39,7	39,5	38,4	2,1
7	2½ "	0,002		38,8	39,1	40,2	41,2	41,5	41,8	41,5	41,1	40,8	39,3	2,9
8	4 "	"		39,0	40,6	41,2	41,5	41,5	41,2	40,8	40,85	40,85	39,7	2,6
9	6 "	"		38,9	38,9	39,2	39,3	40,9	41,4	41,2	40,6	40,0	38,4	2,7
10	" "	0,003		38,5	38,5	38,6	39,7	41,1	41,2	40,6	40,2	39,65	39,0	2,5
11	5 "	"		38,9	38,9	40,0	40,5	41,1	40,7	40,7	40,9	40,0	38,8	2,6
12 ¹	" "	"		40,8	42,0	41,2	40,6	39,5	—	—	—	—	—	2,9
13	4 "	0,005		39,7	40,4	41,0	41,3	40,8	40,7	39,5	—	—	—	2,9
14 ²	" "	"		38,8	39,8	40,5	40,8	40,6	40,1	39,55	—	—	—	2,0

¹ 1-12, Versuche an Shorthorn-Kreuzung.

² 13 und 14, Versuche an Holsteiner Rasse.

VERSUCHE MIT ROHTUBERCULIN.

Laufende Nummer.	Menge des Tuberculin. ccm.	Körpertemperatur. Cels.°										Temperatur- steigerung. Cels.°
		Vor Einspritzung.					Nach Einspritzung. (Stunden.)					
		Vorm. 8	Mittags, 12	Nachm. 5	8h	10h	12h	14h	16h	18h	20h	
1	0.5	38,7	38,8	38,8	38,4	38,6	40,0	40,1	39,6	40,3	40,2	1,5
2	"	38,8	38,7	38,8	38,5	38,7	40,2	41,0	40,8	40,4	39,6	2,2
3	"	38,8	38,8	38,9	38,1	38,8	39,8	39,7	39,7	40,5	39,1	1,5
4	"	38,7	39,0	38,7	38,7	39,7	41,2	40,9	40,9	41,3	40,8	2,3
5	"	38,4	38,6	38,8	40,0	40,2	41,0	40,9	40,8	40,5	39,9	1,2
6	"	38,6	38,7	39,0	37,9	40,0	40,6	40,7	39,8	40,3	40,0	1,7
7	"	38,4	38,5	39,0	39,9	39,9	40,5	40,5	40,4	40,1	40,2	1,5
8	"	39,1	39,0	39,3	40,2	40,1	40,8	41,1	40,6	40,4	39,5	1,8
9	"	38,5	38,8	38,7	38,5	38,7	39,0	39,5	40,0	40,7	39,0	1,9
10	"	39,0	39,2	39,1	39,2	40,3	41,3	41,0	40,7	40,7	39,8	2,1
11	"	38,6	38,6	38,6	39,0	40,1	41,0	40,9	40,9	40,9	40,5	2,4
12	"	38,8	38,7	39,0	39,0	39,0	40,1	41,0	40,8	41,1	39,3	2,1
13	"	—	39,1	39,7	40,45	40,75	40,8	41,9	40,55	40,85	40,1	2,2
14	"	—	39,1	39,6	40,35	40,9	41,25	41,75	41,65	40,95	40,9	2,15

Bei diesen Fällen wurde keine Section ausgeführt; doch ist diese Nachprüfung hier entbehrlich, denn der vorliegende Versuch zielt nicht auf die Feststellung der Diagnose der Tuberculose ab, sondern er bezweckt, die Analogie der Wirkung zwischen beiden Praeparaten zu constatiren. Man erkennt, dass sämtliche Tiere, welche auf Rohtuberculinimpfung reagirt haben, eine starke Fieberreaction auf Einspritzung meiner Tuberculinalbumose zeigen.

Im Anschluss an diese Tierversuche habe ich noch mit anderen aus dem Rohtuberculin hergestellten Körpern und zwar mit dem Aetherextract, welches aus dem mit verdünnter Schwefelsäure versetzten Rohtuberculin gewonnen wurde, sowie mit dem direkt hergestellten Aetherextract an tuberculösen Meerschweinchen (mittelgross) Versuche gemacht: dabei liess sich aber nirgends eine tuberculinähnliche Wirkung beobachten. Ferner zeigten tuberculöse Meerschweinchen (mittelgross) nach Einspritzung von Bernsteinsäure (0,01–0,05 g.) oder von bernsteinsaurem Natron (0,01–0,1 g.) gar keine Steigerung der Körpertemperatur, was die Behauptung Viquerat's, dass diese Säure das active Princip im Rohtuberculin sei, widerlegt.

VI. Chemisches Verhalten meiner Tuberculinalbumose.

Eine 2% ige Lösung der Tuberculinalbumose zeigt folgendes Verhalten:

1. Alcohol 96%: Mit gleichem Volum fallen zarte, weisse Flocken aus.
2. Salpetersäure in der Kälte: Niederschlag, der sich in der Wärme rasch löst, in der Kälte aber wieder auftritt.
3. Gleiches Volumen concentr. Kochsalzlösung zu der mit Essigsäure angesäuerten Lösung gesetzt: mässiger Niederschlag, beim Erhitzen nicht löslich.
4. Sättigung der neutralen Lösung mit Kochsalz: Spur Trübung.
5. Verdünnte Kupfersulfatlösung: starke Fällung.
6. Essigsäure-Ferrocyankalium: starke Fällung, löslich beim Erwärmen.

7. Pikrinsäure: starke Fällung.
8. Metaphosphorsäure: Fällung, im Ueberschuss wieder löslich.
9. Trichloressigsäure: starke Fällung, in der Hitze löslich, in der Kälte wiederkehrend.
10. Jodquecksilberkalium: erst in saurer Lösung starke Fällung, welche sich im Ueberschuss von Salzsäure nicht löst.
11. Gerbsäure: starke Fällung, die sich in der Hitze nicht löst.
12. Millon'sches Reagens: weisse Fällung, beim Kochen Rothfärbung.
13. Xanthoproteinprobe: starke Fällung, welche sich im Ueberschuss von Salpetersäure löst, beim Erhitzen Gelbfärbung.
14. Adamkiewiez'sche Reaction: Violettfärbung.
15. Molisch'sche Zuckerprobe: schwach positiv, beim Erhitzen schwache Violettfärbung.
16. Biuretprobe mit Kupfersulfat: positiv.
17. Biuretprobe mit Nickelsulfat: beim Erwärmen schwache Gelbfärbung.
18. Kochen mit Alkali- und Bleiacetat: Schwarzfärbung.

Nach diesen Reactionen entspricht meine Tuberculinalbumose am nächsten der Deuteroalbumose Kühne's, ferner in einem gewissen Grade der secundären Albumose A von Pick; jedoch mit dem Unterschied, dass meine Albumose bei den oben unter 3 und 6 erwähnten Reactionen einen starken Niederschlag gibt, während die secundäre Albumose A Pick's hierbei nur Spur Trübung gibt; die secundäre Albumose B. und C. gibt hier gar keine Trübung.

VII. Hitzebeständigkeit der Tuberculinalbumose.

Eine 1% ige wässerige Lösung der Tuberculinalbumose wurde in kochendem Wasser je 10 Minuten, 30 Minuten und eine Stunde lang erhitzt, dann tuberculösen Meerschweinchen subcutan eingespritzt; folgende Tabelle zeigt die dabei gewonnenen Ergebnisse:

Nummer der Versuche.	Erhitzungsdauer.	Menge der Tuberculinalbumose, g.	Körpertemperatur, Cels.°								Temperatursteigerung, Cels.°
			Vor Einspritzung.	Nach Einspritzung. (Stunden.)							
				1h	2h	3h	4h	5h	6h	7h	
1	Zehn Minuten.	0,001	38,3	—	39,8	40,0	39,9	38,8	38,3	—	1,7
2	„	0,0005	38,0	—	39,2	40,0	40,3	39,9	38,8	—	2,3
3	„	0,0005	37,7	—	38,4	40,2	40,5	40,3	39,7	38,6	2,8
4	Dreissige Minuten.	0,0005	37,9	—	39,6	40,35	40,15	40,15	40,3	40,0	2,45
5	„	0,00025	38,4	—	39,4	38,8	39,5	40,15	40,0	39,8	1,75
6	Ein Stunde.	0,0005	37,75	—	39,8	40,4	40,25	39,0	38,8	—	3,0
7	„	0,00025	37,6	—	39,6	40,2	40,1	40,0	40,2	40,0	2,6
8	Controllösung.	0,0005	37,7	—	38,1	40,3	40,2	40,0	39,6	38,8	2,6
9	„	0,0005	37,4	—	38,9	40,45	40,7	40,25	39,5	38,25	3,3

Es unterliegt also kaum einem Zweifel, dass die Tuberculinalbumose gegen Hitze sehr widerstandsfähig ist. Eine 1 stündige Erhitzung bei 100° C. ist nicht im Stande, die Wirksamkeit der Tuberculinalbumose im Geringsten zu schädigen.

VIII. Verhalten der Tuberculinalbumose gegen Pepsin und Trypsin.

Je 0,1 g. Tuberculinalbumose wurde einerseits in 10 ccm. 0,2% iger Salzsäure und andererseits im gleichen Volum 0,2% iger Sodalösung (Na₂CO₃) gelöst und dort etwas Pepsin, hier aber ebensoviel Trypsin zugesetzt. Bei Gegenwart von etwas Chloroform wurden beide Lösungen wohlverschlossen 2 Tage lang im Brutofen bei 37–38° gelassen. Hierauf wurden sie im Dampftopf 10 Minuten lang erhitzt und tuberculösen Meer-schweinchen subcutan eingespritzt; gleichzeitig wurden Controllösungen (Tuberculinalbumoselösungen in 0,2% iger Salzsäure und in 0,2% iger Sodalösung ohne Pepsin resp. Trypsin) geprüft: Die Ergebnisse sind in folgenden Tabellen zusammengestellt:

Nummer der Versuche.	Tuberculinalbumose.	Menge der Tuberculinalbumose. g.	Körpertemperatur. Cels.°								Temperatursteigerung. Cels.°
			Vor Einspritzung.	Nach Einspritzung. (Stunden.)							
				1h	2h	3h	4h	5h	6h	7h	
1	Mit Pepsin.	0,001	38,0	—	38,8	38,9	38,7	38,5	38,3	38,3	0,9
2	„	0,0005	37,4	—	37,7	38,25	38,4	37,75	38,0	37,9	1,0
3	„	0,0001	37,7	—	38,0	38,45	38,6	38,6	38,3	38,0	0,9
4	Ohne Pepsin.	0,001	38,1	—	38,2	40,0	39,9	39,5	39,3	39,3	1,9
5	„	0,0002	37,7	—	38,0	40,0	40,3	40,1	39,7	39,5	2,6
6	Mit Trypsin.	0,001	38,0	—	37,9	38,3	38,6	38,5	38,0	38,0	0,6
7	„	0,0005	38,3	—	38,2	38,5	38,7	38,8	39,1	38,9	0,8
8	„	0,0001	38,3	—	38,8	39,1	39,0	39,1	38,8	38,25	0,8
9	Ohne Trypsin.	0,001	37,4	—	38,2	39,0	39,5	39,6	39,4	39,3	2,2
10	„	0,0002	37,9	—	37,9	38,3	39,6	39,4	39,5	39,1	1,7

Die Tiere verhalten sich also bei Behandlung mit verdauter Tuberculinalbumose indifferent; Pepsin und Trypsin hatten die Tuberculinalbumose verändert.

IX. Sind gewisse labile Atomgruppen die Ursache der Giftwirkung?

Die schon erwähnte Thatsache, dass die Tuberculinalbumose bei einstündigem Erwärmen nicht im Geringsten an Wirksamkeit einbüsst, liess es von vorneherein wenig wahrscheinlich erscheinen, dass die Wirksamkeit auf besonders labilen Atomgruppen beruhe. Nichtsdestoweniger wurden einige Versuche angestellt mit Körpern, welche sehr leicht in labile Amido- und Aldehyd- oder Keton- gruppen eingreifen.

Je 5 ccm. 20% ige wässrige Lösung des Rohtuberculins wurde mit 25 ccm. der folgenden Lösungen vermischt (in jedem Falle zwei Kölbchen).

1. Controllösung.
2. 1% Natriumnitritlösung: unmittelbar vor Gebrauch mit einigen Tropfen verdünnter Essigsäure versetzt.

3. 5% Formaldehydlösung.
4. 1% Hydroxylaminchloridlösung: unmittelbar vor Gebrauch mit Natriumcarbonat neutralisirt, (entspr. 0,47 freiem Hydroxylamin).

Die Mischungen wurden mit etwas Chloroform versetzt und 24 Stunden bei Zimmertemperatur stehen gelassen, dann mit absolutem Alcohol ausgefällt, der Niederschlag wiederholt mit absolutem Alcohol ausgewaschen und nach Pressen zwischen Filtrirpapier in wässrigen Lösungen tuberculösen Meerschweinchen subcutan eingespritzt. Die dabei gemachten Beobachtungen zeigt die folgende Tabelle:

Nummer der Versuche.	Reagentien.	Menge des Tuberculins, cem.	Körpertemperatur, Cels.°								Temperatursteigerung, Cels.°
			Vor Einspritzung.	Nach Einspritzung. (Stunden.)							
				1h	2h	3h	4h	5h	6h	7h	
1	Controllösung.	0,04	38,6	—	39,0	40,8	40,6	40,4	40,0	37,0	2,2
2	Natriumnitrit.	0,2	38,4	—	38,8	39,2	39,9	39,7	39,7	39,6	1,5
3	„	0,1	37,8	—	38,65	38,8	40,0	39,7	40,0	39,6	2,2
4	„	0,04	38,5	—	39,0	39,3	39,7	39,4	39,0	39,0	1,2
5	Formaldehyd.	0,2	37,9	—	37,9	40,0	40,3	40,0	40,0	40,0	2,5
6	„	0,1	38,2	—	38,1	39,4	39,6	39,5	39,2	39,2	1,4
7	„	0,04	38,5	—	38,2	39,4	40,2	40,2	39,8	39,5	1,7
8	Hydroxylaminchlorid.	0,2	37,9	—	38,0	38,3	39,5	39,7	39,7	40,2	2,3
9	„	0,1	38,0	—	38,15	39,2	40,0	40,5	40,3	40,2	2,5
10	„	0,04	38,6	—	39,0	39,7	40,1	40,4	40,1	39,7	1,8

Hieraus ersieht man, dass oben erwähnte Reagentien keinerlei schädigenden Einfluss auf das Tuberculin hatten, dessen Natur demnach wohl verschieden ist von derjenigen der gewöhnlichen Enzyme; denn diese werden durch 5% Formaldehyd nach 24 Stunden stehen leicht unwirksam.

Schlussfolgerungen.

Die Resultate meiner Untersuchungen sind wie folgt:

1. Die Ammonsulfatfällung des Tuberculins äussert bei Meerschweinchen und Rindern sich qualitativ wie das Koch'sche Reintuberculin; es reichen jedoch von meinem Praeparat weit geringere Mengen ($\frac{1}{3}$) hin, dieselbe Reaction zu erzeugen.
2. Die Ammonsulfatfällung besteht wesentlich aus einer Deuteroalbumose nebst Spuren Prot- und Amidalbumose (Tuberculinalbumose Nitta's).
3. Unter der Einwirkung von Pepsin und Trypsin verliert meine Tuberculinalbumose ihre spezifische Wirkung.
4. Nach einstündigem Erhitzen auf 100° C. behält die wässrige Lösung der Tuberculinalbumose ihre spezifische Wirkung.
5. Die spezifische Wirkung des Tuberculins verändert sich nicht im Geringsten unter der Einwirkung von Natriumnitrit (1%), Formaldehyd (5%) und Hydroxylamin (0,47%).
6. Bei Tierversuchen zeigt die Ammonsulfatfällung von Witte'schem Pepton nicht die gleiche Wirkung wie das Tuberculin resp. die Tuberculinalbumose.
7. Das wirksame Princip des Tuberculins ist eine Albumose (Deuteroalbumose).
8. Die Wirkung der Tuberculinalbumose, des wirksamen Principes des „Tuberculins,“ ist ganz specifisch, und durch gewöhnliche Albumosen nicht herbeizuführen. Die Kühne'sche, Hunter'sche und Helmann'sche Ansicht bezüglich des wirksamen Principes des Tuberculins ist unrichtig.

Zum Schlusse halte ich es mir eine angenehme Pflicht, Herrn Prof. Dr. O. Loew und Herrn Prof. Dr. Y. Kozai für ihre gütige Unterstützung meinen ergebensten Dank auszusprechen.

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Ueber Ernährungsverhältnisse beim *Bacillus prodigiosus*.

VON

O. Loew und Y. Kozai.

Da aus mehreren Beobachtungen die Bildung eines bacteriolytischen Enzyms beim *Bac. prodigiosus* wahrscheinlich wurde, stellten wir einige Versuche an, welche Aufklärung darüber geben sollten, inwieweit die Bildung eines solchen Enzyms mit Ernährungsverhältnissen bei diesem Microben zusammenhängt. *Fermi* beobachtete, dass Zucker mit Ammoniaksalzen die Bildung eines proteolytischen Enzyms verhindert, dagegen Glycerin mit Ammoniaksalzen dieselbe begünstigt. Ob dieses proteolytische Enzym zugleich ein bacteriolytisches ist oder neben dem ersteren ein bacteriolytisches vorhanden ist, wurde nicht untersucht. Die Existenz eines bacteriolytischen Enzyms in Culturen des *B. prodigiosus* schien aus der Beobachtung von *Freudenreich*² hervorzugehen, dass diese Culturen sehr entwicklungshemmend auf einige andere Bacterienarten wirken.

Bei unserer ersten Versuchsreihe verwendeten wir folgende Lösungen :

1. Pepton 1% mit Glycerin 0.1%.
2. „ „ „ „ 1%.
3. „ „ mit essigsauerm Natron 0.2% und Asparagin 0.2%.
4. Pepton 0.2% mit essigsauerm Natron 1%.
5. Asparagin 0.2% mit Glycose 1%.
6. Harnstoff 0.2% mit Glycose 1%.
7. Natriumnitrat 0.2% mit Glycose 1%.
8. Bouillon.

Die zugesetzten Mineralsalze bestanden hier aus :

¹ Arch Hyg. Bd. 14 ; S. 16 und 30.

² Jahresber. f. Bakt. 1889, S. 531.

Secundärem Kaliumphosphat.....	0.2%
Natriumsulfat	0.1%
Magnesiumsulfat	0.01%

Die Proben wurden zuerst zwölf Tage bei 10-15^o, dann noch fünf Tage im Brutkasten gehalten. Es ergab sich dann folgendes Resultat:

Loesung 1 und 2: Viel Bacteriensediment, und wenig Farbstoff.

3: Nach anfänglich reichlicher und rot gefärbter Vegetation fast völlige Wiederloesung.

4. 5 und 6: Geringe Entwicklung.

7: Gar keine Entwicklung.

8: Mässige Entwicklung, rotes Sediment,¹ welches selbst nach acht weiteren Wochen nicht wieder gelöst war.

Die Combination von Pepton mit essigsauerm Natron und Asparagin hatte sich der Bildung von Farbstoff und bacteriolyschem Enzym am günstigsten erwiesen. Wo das stickstoffhaltige Material gegenüber dem stickstofffreien vermindert war, fand nur geringe Entwicklung statt, bei Natriumnitrat als Stickstoffquelle gar keine.

Bei unsrer zweiten Versuchsreihe ersetzten wir das schwefelsaure Natron durch Chlornatrium, machten ferner die Loesungen schwach alkalisch und machten geringe Zusätze von Stoffen, welche bei höheren Concentrationen giftig wirken, um zu beobachten, ob eine günstige Einwirkung auf Wachstum und Enzymbildung stattfinden würde.²

Unsre Controlloesung hatte die folgende Zusammensetzung:

Pepton	0.5%
Glycerin	0.1 „
Dikaliumphosphat	0.1 „
Natriumbicarbonat	0.1 „
Natriumchlorid	0.2 „
Magnesiumsulfat.....	0.01%

¹ Nach *Kuntze* sind Magnesia sowohl wie Schwefelsäure wesentlich für die Farbstoffproduction. Jedenfalls existiren aber hier auch noch manche andere Einflüsse, auch solche, welche der Production entgegenwirken.

² Nach *Hüppe's* biologischem Grundgesetz wirken Gifte bei sehr hoher Verdünnung als Reizmittel.

In Loesung 2 war das Chlornatrium durch die aequivalente Menge (0.25%) Natriumsulfat, in 3 durch die aequivalente Menge Natriumnitrat (0.30%) ersetzt. In 4. war der Normalloesung noch 0.01% Jodkalium, in 5. ebensoviel Fluornatrium, in 6. ebensoviel Ferrocyankalium zugesetzt worden. Die dreimal sterilisirten Loesungen wurden am 16. Januar inficirt und bei 6-15° C. stehen gelassen. Am 27. Januar war der Stand folgender:

1. (Control): Keine Haut, nur Trübung und ein Ring am Rande.
2. (Na₂SO₄): Roter Ring, Spur Haut, Trübung.
3. (NaNO₃): Loesung klar, keine Haut, nur schwacher weisser Ring.
4. (NaJ): Trübung, schwache rote Haut.
5. (NaF): „ „ „ „
6. (Kfcy): Trübung, stark entwickelte rote Haut.

Am 3. Februar wurde bemerkt, dass die Färbung am intensivsten in 5. war, diese verblasste aber später wieder; die Vegetation war am üppigsten in 6.

Am 18. Februar war der Stand folgender:

Bei 3. Trübung, geringer weisser Bodensatz,

1 und 5: Mässiger Bodensatz, kaum gefärbt.

2 und 4: Etwa ebenso starkes Wachsthum als bei 1 und 5, aber mehr Farbe.

6: Der Bodensatz betrug hier, dem Volumen nach abgeschätzt, mindestens des *vierfache* der in 1. gebildeten Masse; es war also eine *Reizwirkung des Ferrocyankaliums* auf die Wachstumsintensität unverkennbar.

Während in der ersten Versuchsreihe das Natriumnitrat als untaugliche Stickstoffquelle erschien, hat es sich in dieser als sehr hemmend selbst bei Anwesenheit von Pepton erwiesen.¹ Fluornatrium und Jodkalium haben

¹ Da die Hemmung von Anfang an vorhanden war, so kann sie nicht etwa die Folge von erst gebildetem Nitrit sein. Die Ursache ist nicht leicht anzugeben, es mag aber darauf hingewiesen werden, dass Nitrate auch hemmend auf die Entwicklung der Leguminosenbakterien wirken (*Marchal*, Compt. rend. 133 p. 1032) sowie auf die Kohlensäureassimilation der Meeresalgen (*Arber*, Botan. Centralbl. 1902 p. 120) und schliesslich auch auf die katalytische Wirkung der Katalase.

in der angewandten Verdünnung das Wachstum nicht gefördert, wenigstens nicht in direct erkennbarem Maasse.

Die auffallende Wirkung des Ferrocyankaliums veranlasste uns zu einem weiteren Versuch, in welchem diese Wirkung auf den *B. prodigiosus* mit der auf andere Microbenarten verglichen wurde. Als Nährloesung diente hier Bouillon, je 8 cc. in einer Eprouvette.

Nach drei Tagen bei 35° war das Resultat folgendes:

Microbenart.	Bouillon.	
	Ohne Zusatz.	Mit 0.01% Ferrocyankalium.
<i>B. prodigiosus.</i>	Kein Bodensatz, schwacher roter Ring.	Starker Bodensatz, dicker roter Ring.
<i>B. pycnococcus.</i>	Dicke Haut.	Schwache Haut.
<i>B. mesenter. ruber.</i>	Starke Haut.	Schwache Haut.
<i>B. megatherium.</i>	Haut.	Nur Trübung und Ring.
<i>B. Zenkeri.</i>	Trübung u. Flocken.	Trübung und Flocken.
<i>B. cyanogenus.</i>	Haut und Bodensatz. Dunkle Färbung nahe der Oberfläche.	Geringe Haut und Bodensatz, schwächere Färbung.
<i>B. capsulatus.</i>	Trübung, starker Rand.	Trübung, schwacher Rand.
<i>B. acidi lactici Hüppe.</i>	Starker Bodensatz.	Schwacher Bodensatz.
<i>B. subtilis.</i>	Dicke Haut.	Dünne Haut.
<i>B. typhi mur.</i>	Trübung.	Trübung.

Es hat sich also eine stimulirende Wirkung nur beim *B. prodigiosus* erkennen lassen, in den andern geprüften Fällen ergab sich meistens eine deutliche Schädigung. Dieses Resultat veranlasst uns zur Annahme, dass beim *B. prodigiosus* es sich um eine Spaltung des Ferrocyankaliums handelt, wobei einerseits die schädigende Cyanwasserstoffsäure sofort

weiter zersetzt wird, andererseits das in den Zellen freiwerdende Eisen in einer lockeren salzartigen Verbindung eine fördernde Wirkung ausübt.

Fassen wir unsre Beobachtungen am *B. prodigiosus* kurz zusammen, so ergibt sich Folgendes :

1. Eine für die Production von Farbstoff und bacteriolytischem Enzym günstige Nährstoffcombination besteht aus Pepton 1%, essigsaurem Natron 0.2% und Asparagin 0.2%.
 2. Eine beträchtliche Vermehrung stickstofffreien Materials gegenüber stickstoffhaltigem übt auf die Entwicklung einen ungünstigen Effect aus.
 3. Natriumnitrat ist nicht nur unfähig, als Stickstoffquelle zu dienen, sondern hemmt sogar die Entwicklung bei Gegenwart von Pepton.
 4. Jodkalium und Fluornatrium in einer Verdünnung von 0.1 p.m. üben keine deutliche Reizwirkung aus.
 5. Ein Ferrocyankaliumzusatz von 0.1 p.m. befördert die Entwicklung, was bei andern Microben nicht zutrifft.
-

Ueber die Vertheilung des Kalks im thierischen Organismus.

VON

M. Toyonaga.

Die Wichtigkeit des Kalks für alle thierischen Organismen ist seit lange anerkannt. Ungefähr drei Viertel sämtlicher Mineralstoffe der höheren Thiere besteht aus Tricalciumphosphat, da dieses Salz die Hauptmasse der Knochen und der Zähne ausmacht. Aber abgesehen von diesem mehr in die Augen fallenden Vorkommen finden sich noch Kalkverbindungen in sämtlichen Organen vor, ja höchst wahrscheinlich existiert auch bei den niedersten thierischen Organismen keine Zelle, die frei von Kalkverbindungen wäre. Auch die grosse Wichtigkeit für das Blut ist in neuerer Zeit anerkannt worden. Blut verliert seine Gerinnbarkeit, wenn durch Zusatz von etwas Natriumfluorid oder Natriumoxalat der Kalkgehalt des Blutes in die unlöslichen Formen Calciumfluorid oder Kalkoxalat übergeführt wird. Auch mag darauf hingewiesen werden, dass in kalksalzfreier Lösung auch das Casein der Milch auf Labzusatz nicht gerinnt, wohl aber wird dabei das Casein so verändert, dass nun auf Zusatz von Kalksalzen ein Niederschlag von den Eigenschaften des frischen Käses entsteht. Cavazzani hat es ferner wahrscheinlich gemacht, dass Kalksalze auch für die Gerinnung des Muskelplasmas von Bedeutung sind. Im Harn findet sich Kalk regelmässig gelöst, ferner ausnahmsweise in Form von Concretionen.

Die Anwesenheit von Kalk in den Nahrungsmitteln ist bei dem Bedürfniss von Thier und Mensch für Kalk von grösster Wichtigkeit. Junge Thiere leiden bei kalkarmer Nahrung an Rachitis ähnlichen Veränderungen, aber auch ausgewachsene Thiere leiden in Folge eines solchen Mangels, da die Kalkausscheidung durch die Niere regelmässig fort dauert. Bunge hat deshalb auf die Gefahr hingewiesen, wenn man die

an Kohlehydrat reichen Vegetabilien der menschlichen Nahrung durch bloßen Rohrzucker ersetzt. Erstere enthalten verschiedene mineralische Nährstoffe wie Phosphate, Kalk, Magnesia und Eisen-Salze, während dem Rohrzucker des Handels, der reinen Saccharose, alle diese Stoffe mangeln, besonders aber fällt der Mangel von Kalk und Eisen ins Gewicht. Bunge glaubt, dass Anämie und Zahncaries auf den mit Zuckergenuss verbundenen Kalk- und Eisenmangel zurückzuführen sind und er schlägt deshalb vor, das Bedürfniss der Kinder nach Süßigkeiten, statt mit bloßem Zuckerpräparaten, mit süßen Früchten, frisch oder gekocht, zu befriedigen. Auch für unsere Hausthiere ist es von sehr grosser Bedeutung, dass bei Zusammensetzung einer Futterration der Kalk dieselbe Berücksichtigung als andre Nährbestandtheile findet. Vielfach ist Knochenbrüchigkeit des Rindsviehs auf eine ungenügende Ernährung mit Kalk zurückzuführen. Die Thiere werden unter solchen Umständen matt und magern ab und bei den geringsten Veranlassungen treten Knochenbrüche ein. In manchen Gegenden mit kalkarmen Boden zeigt sich in Folge des kalkarmen Grases die Knochenbrüchigkeit sehr häufig beim Rind, welches auf diese Nahrung angewiesen ist. *Katsuyama* hat gefunden, dass Kaninchen beim Hungern anfangs etwas weniger Kalk ausscheiden, die Menge steigt aber vom vierzehnten Hungertage an langsam bis zum Tode. Die Magnesiawerthe dagegen zeigen ein continuirliches Sinken. *Gerhart* und *Schlesinger* fanden, dass die Ausscheidung des Kalks im Harne beim Gesunden und beim Diabetiker der Ammoniakausscheidung parallel geht und wie diese durch Alkalizufuhr herabdrückbar ist. Unter abnormaler Säurebildung im Thier nimmt auch der Kalkgehalt des Harnes abnorm zu. Eine Vermehrung des Kalkgehaltes des Harnes oder der Faeces findet nach *Babeau* auch in der Entwicklungsperiode der Rachitis statt. Gelöste Kalkverbindungen sind ferner nach *Halliburton* für die Herzthätigkeit von grosser Bedeutung, was von *Jaques Loeb* bestätigt wurde. Es ist daher von grösstem Nachtheil für die regelmässige Herzthätigkeit, wenn der Kalk des Blutserums durch Fluornatrium oder oxalsaures Natron³ in

¹ Zeitschrift f. physiol. Chemie 26, S. 542.

² Fluornatrium hat übrigens auch noch andre Wirkungen, die nicht auf der Kalkfällung beruhen.

unlösliche Verbindungen übergeführt wird. In neuester Zeit hat *Friedenthal*¹ es sehr wahrscheinlich gemacht, dass die Giftwirkung der Seifen (oxelsaures Natron) bei Injection in das Blut auf der kalkfällenden Wirkung derselben beruht. Er sagt richtig: „wie hätte man vermuthen können, dass eine Substanz, die in grossen Dosen verfüttert werden kann fast wie ein Nahrungsmittel, die in kleinen Mengen einen normalen Bestandtheil der Gewebe und des Blutes bildet, in die Blutbahn eingeführt schon in Dosen von 0,1 g. pro Kilo Thier den Tod im Augenblick der Einführung herbeiführen könne. *Munk* hat gezeigt, dass es nicht etwa das aus den Seifen freiwerdende Natron ist, welches die Giftwirkung bedingt. Dieses würde ja auch bei dem Kohlensäure gehalt der Blutes sofort in Carbonat übergehen.

Obwohl nun jede thierische Zelle Kalksalze benöthigt, so existirte doch bis in die neuere Zeit herein keine befriedigende Theorie der Funktionen des Kalks für die thierischen Zellen. Auch Pflanzen mit Ausnahme der niedersten Formen bedürfen des Kalks. Was nun diese Funktion des Kalks in den Pflanzen betrifft, so hat *O. Loew* aus der Giftwirkung des neutralen oxelsauren Kalis, welche er unter dem Mikroskop verfolgte, geschlossen, dass sowohl die Chlorophyllkörper als auch der Zellkern aus Kalkverbindungen von Nucleoproteiden aufgebaut sind. Die Funktion der Magnesia in den Pflanzen dagegen bestehen nach ihm² in der Ermöglichung der Assimilation der Phosphorsäure bei der Bildung von Lecithin und Nucleoproteiden, weil die Phosphorsäure am leichtesten von allen in den Pflanzen sich findenden Phosphaten aus dem sekundären Magnesiumphosphat abgespalten werden kann. Er hatte die merkwürdige Giftwirkung von oxelsauren Salzen, welche nur für höhere Thiere und einige Pflanzen bekannt war, nicht nur bei verschiedenen Phanerogamen,³ sondern bei den höheren Algen und niedersten Thieren beobachtet. In der Wirkung der Oxalate auf niedere Wasserthiere liessen sich nun grosse Unterschiede erkennen, der Tod trat bei einigen Arten (Asseln, Copepo-

¹ Archiv für Anatomie und Physiologie 1901.

² Flora 1892, 368-394.

³ Von Interesse ist hier die Thatsache, dass jedoch geringe Mengen löslicher Oxalate normalerweise in manchen Pflanzen vorkommen können.

den, Rotatorien) sehr bald, bei anderen (Wasserkäfern, Wassermilben und Nematoden) aber weit später ein, was wohl mit der verschiedenen Schnelligkeit zusammenhängen mag, mit der jene Salze zu den wichtigeren Organen vordringen können.

In 0.5 proc. Lösungen neutralen Kalium- oder Natrium-Oxalats sind Asseln, Copepoden und Rotatorien in 30-50 Minuten todt, dann folgen Egel und Planarien, hierauf Insektenlarven und Ostracoden, während nach 24 Stunden noch leben Wasserkäfer, Wassermilben¹ und einzelne Nematoden. In einem Controlversuch mit neutralem weinsaurem Kali lebten fast alle jene Organismen noch nach 24 Stunden, viele noch nach mehreren Tagen.

In einer 0.1 proc. Lösung neutralen oxalsauren Kalis starben Asseln, Copepoden und Rotatorien nach 3-4 Stunden, kleine Planarien nach 3 Tagen und Ostracoden waren darin noch nach 8 Tagen lebendig.

Fadenlagen, wie *Zygnema*, *Mougeotia*, *Vaucheria*, *Sphaeroplea*, *Cladophora*, *Oedogonium* sterben binnen 24 Stunden unter Verquellung der Chlorophyllkörper in einer 0.5 proc. Lösung von neutralem oxalsaurem Kali ab. Bei Spirogyren lässt sich sehr gut beobachten, dass zuerst der Zellkern angegriffen wird. Derselbe quillt in einer 0.5 proc. Lösung nach einiger Zeit auf und wird öfter zu einem unregelmässigen zackigen Gebilde. Lässt man aber eine 2 proc. Lösung auf diese Algen einwirken, so gewahrt man schon nach 5 Minuten, dass die Kerne sich auffallend stark contractiren und nach 10 Minuten kein einziger Kern mehr intact geblieben ist. Das Cytoplasma ist allem Anschein nach noch völlig unverletzt, doch erholen sich die Zellen nicht wieder, wenn sie nach 10 Minuten wieder in kalkhaltiges Quellwasser zurückversetzt werden, die Zellen sind nach 24 Stunden in allen Theilen abgestorben. Der Einfluss der 2 proc. Oxalatlösung macht sich bei den Chlorophyllbändern der Spirogyren in ca. 30 Minuten geltend, wobei eine Veränderung der Conturen durch Verquellung sichtbar wird.²

¹ Wassermilben starben erst nach 20-22 Stunden in einer 1 proc. Lösung oxalsauren Natrons.

² Bei Controlversuchen mit ebenso starken Lösungen von schwefelsaurem oder weinsaurem Kali blieben jene Erscheinungen aus.

Von Wichtigkeit ist es, bei der Thierfütterung solche Pflanzen als Nahrung auszuschliessen, welche lösliche oxalsaure Salze in geringen Mengen enthalten; es wird einerseits der lösliche Kalk im Verdauungstractus ausgefällt und durch den mangelhaften Kalkgehalt entwickelt sich Knochenbrüchigkeit und andererseits treten durch Resorption ins Blut andre krankhafte Erscheinungen auf, die raschen Verfall und Tod herbeiführen. In den Rübenblättern ist z.B. die Oxalsäure theils an Natron, theils an Kalk gebunden. Als Endresultat vieler Versuche ergab sich: Oxalsäure enthaltendes Futter ist in geringer Menge, wenn es nur kurze Zeit gegeben wird, als unschädlich anzusehen. Werden jedoch die Bedingungen für die Unschädlichkeit des Futters (Zusatz von Calciumcarbonat) nicht erfüllt, so entwickeln sich die Symptome der chronischen Oxalsäure-Vergiftung, wobei zunächst die Nieren und Knochen in Mitleidenschaft gezogen werden.

Diese allgemeine Giftwirkung neutraler oxalsaurer Salze bei niederen, sowohl wie höheren Thieren erklärt sich am einfachsten, wenn wir die bei Pflanzen sich ergebenden Schlüsse, dass die Zellkerne zu ihrer Organisation Kalkverbindungen von Nucleoproteiden bedürfen,¹ auch auf die thierischen Zellen anwenden. *Loew* hat den Satz aufgestellt: „Je grösser die Zellkernmasse in einem Organ, desto mehr Kalk enthält sie.“ Dieser Satz hat aber noch keine Berücksichtigung bei den Physiologen gefunden. Vergleicht man jedoch bei verschiedenen thierischen Organen den Kalkgehalt mit der relativen Grösse der Zellkerne, so findet man eine auffallende Uebereinstimmung mit jener Folgerung, soweit die bis jetzt vorliegenden Analysen Material zu Vergleichen liefern. Leider sind aber manche Organe noch gar nicht im Bezug auf ihre Mineralbestandtheile quantitativ untersucht, wie z. B. die Lunge, die peripheren Nerven, die Nebenniere, die Speicheldrüsen und die Hoden: Von einigen Organen sind erst in neuester Zeit Aschenanalysen bekannt geworden.

Aus dem obigen Satz würde z. B. folgen, dass Drüsen kalkreicher sein müssen, als Muskeln, weil sie grössere Zellkerne haben, ferner dass Muskeln niederer Thiere mehr Kalk enthalten, als die der Warmblüter.

¹ Ausgenommen sind nur die niedersten Algen- und Pilzformen.

da erstere ebenfalls grössere Zellkerne besitzen. *Loew*¹ hat einige von *Katz*² vor einigen Jahren publicierte Analysen des Muskelfleisches höherer und niederer Thiere verglichen sowohl unter sich, als auch mit dem vorliegenden Analysenmaterial von Drüsen und fand seine Folgerung bestätigt. Wir wollen im Folgenden sämtliche Analysen von *Katz* berücksichtigen. Dieser Autor fand

in 1000 Th. frischen Muskels von Warmblütern:

	Calcium.
Menschenfleisch	0,0748
Schweinfleisch	0,0806
Rindfleisch	0,0211
Kalbfleisch... .. .	0,1444
Hirschfleisch	0,0959
Kaninchenfleisch	0,1832
Hundefleisch	0,0685
Katzenfleisch	0,0846
Hühnerfleisch	0,1051
Durchschnitt	0,0954

Diese Zahl ist etwas höher als die von *Bunge*³ schon früher gefundenen Zahlen 0,086 und 0,072 CaO, entsprechend 0,061 und 0,053 Ca. Andererseits fand *Oidtmann* in der Leber, dieser grössten Drüse der Säugethiere, 0,284 Theile Calcium in 1000 Theilen des Organs oder nahe $3\frac{1}{2}$ mal so viel als jener Durchschnittgehalt beim Muskel beträgt. Bei Kaltblütern fand *Katz* in 1000 Theilen Muskel:

	Calcium.
beim Frosch... .. .	0,1566
Schellfisch	0,2202
Aal	0,3913
Hecht	0,3977
Durchschnitt	0,2913

¹ The physiolog. rôle of mineral Nutrients, Bulletin No. 18, U. S. Department of Agriculture, Washington 1899.

² Jahresbericht f. Tierchemie 1896, p. 479.

³ Zeitschrift f. physiolog. Chemie 9, p. 60.

Wir finden somit, dass der Kalkgehalt der Muskeln von Batrachiern und Fischen weit grösser ist als der bei den Muskeln von Säugethieren, in Uebereinstimmung mit obiger Folgerung. Für den Magnesiumgehalt ergibt sich umgekehrt, dass derselbe bei den Muskeln von Säugethieren grösser ist als bei denen von Batrachiern und Fischen; nämlich

in 1000 Th. frischer Substanz:

	Magnesium
Menschenfleisch	0,2116
Schweinfleisch	0,2823
Rindfleisch... ..	0,2434
Kalbfleisch... ..	0,3044
Hirschfleisch	0,2906
Kaninchenfleisch	0,2869
Hundefleisch	0,2370
Katzenfleisch	0,2863
Hühnerfleisch	0,3713
Durchschnitt	0,2793

In Uebereinstimmung damit fand schon *Bunge* für 1000 Theile Fleisch 0,412 und 0,381 Magnesia, oder 0,249 und 0,230 Magnesium. Vergleichen wir hiermit den von *Katz* gefundenen Magnesiagehalt der Muskeln von Batrachiern und Fischen, nämlich:

In 1000 Theilen:

	Magnesium
Froschfleisch	0,2353
Schellfishfleisch	0,1670
Aalfleisch	0,1782
Hechtfleisch	0,3102
Durchschnitt	0,2227

so sehen wir, dass der Magnesiagehalt hier geringer ist als dort, wenn auch der Unterschied für Magnesium geringer ist als der gegentheilige für Calcium. *Oidtmann*¹ fand auf 3,62 Theile Kalk in der Leber der Säugethiere nur 0,19 Theile Magnesia, oder auf 0,284 Theile Calcium in 1000

¹ Citirt von *Halliburton*, S. 538 seiner chemischen Physiologie.

Theil Organ nur 0,017 Theile Magnesium. Es ist somit der Kalkgehalt weit grösser als der Magnesiagehalt, während beim Muskel umgekehrt der Kalkgehalt geringer ist als der Magnesiagehalt. *Gossmann*¹ fand ähnliche Zahlen für die Pancreasdrüse und Niere wie *Oidtmann* für die Leber.

In 1000 Theilen Organ sind enthalten:

		Pancreas	Niere
Rind ...	Ca	0,2451	0,1184
	Mg	0,0383	0,0410
Mensch	Ca	0,3958	0,2008
	Mg	0,0833	0,0472

Seither sind weitere Analysen einiger thierischer Organe erschienen. So hat *Lüning* die anorganischen Bestandtheile der Pancreasdrüse von zwei alten Frauen quantitativ bestimmt und fand 2,56% Calcium und 1,48% Magnesium (wahrscheinlich in 100 Theilen² der Asche). *Ribaut*³ hat ferner die Milz in dieser Beziehung untersucht, wobei er jedoch die Pulpa vom Bindegewebe durch Auspressen trennte. Die Pulpa betrug 41,5; 60,4 und 73,0% der getrockneten Organe. Er erhielt folgende Werthe auf Trockensubstanz bezogen:

	Calcium.			Magnesium.			Ca : Mg.		
	I. %	II. %	III. %	I. %	II. %	III. %	I.	II.	III.
Ganze Milz	0,129	0,153	0,141	0,054	0,058	0,055	2,38	2,62	2,56
Pulpa	0,247	0,183	0,158	0,070	0,082	0,067	3,51	2,24	2,36
Bindegewebe	0,046	0,108	0,098	0,026	0,025	0,023	1,76	4,34	4,26

Auch *Aloy*⁴ hat gefunden, dass in Milz, Pancreas und Niere, ferner in Knorpel und Bindegewebe der Kalkgehalt grösser ist als der Magnesiagehalt. Derselbe fand, dass in Gehirn und Muskel das Verhältnis $\frac{\text{Ca}}{\text{Mg}}$ kleiner als 1 ist, während er für Milz, Pancreas und Niere die Verhältnisse fand 6.79; 4.05; und 1.84. *Aloy* scheint keine Kenntniss von den oben bereits discutirten Beziehungen zwischen Kernmasse und Kalkgehalt

¹ Jahresbericht f. Thierchemie 30, S. 410.

² Ibid, S. 386.

³ Ibid, S. 492.

⁴ Jahresbericht f. Thierchemie 30, S. 492.

gehabt zu haben, sonst wäre er von seinen Resultaten weniger überrascht worden.

Was meine eigene Untersuchung betrifft, so habe ich zunächst die weisse und graue Gehirnsubstanz separat auf Kalk- und Magnesiumgehalt untersucht. Diese beiden Gehirnsubstanzen unterscheiden sich bekanntlich sehr bedeutend in mehreren Beziehungen. Die graue Substanz besteht aus Nervenzellen mit einem wohl entwickelten Nucleus, während die weisse Substanz lediglich aus Nervenfasern besteht, welche aus Nervenzellen hervorgehen und nicht wie diese wohl ausgebildete Kerne haben. Obwohl zahlreiche Untersuchungen beider Substanzen vorliegen, was die organischen Bestandtheile betrifft, existirt doch noch keine Analyse der Asche von jeder dieser Substanzen für sich. Trotzdem mussten solche Analysen von hohem Werth sein, mit Rücksicht auf die grosse Zellkernmasse der grauen Substanz. Der totale Aschengehalt des Gehirns variiert nach verschiedenen Autoren von 0,1 zu 1,0%, und nach *Geoghegan*¹ enthalten 1000 Theile Total- Gehirn:

Ganze Asche	K	Na	Mg	Ca	Cl	PO ₄	CO ₂	Fe(PO ₄) ₂
2,95-7,08	0,58-1,77	0,45-1,11	0,017-0,072	0,006-0,022	0,43-1,32	0,95-2,01	0,24-0,79	0,01-0,09

Während *Geoghegan* das Lecithin und damit eine beträchtliche Menge Phosphorsäure vor dem Einäschern des Hirns mit Aether entfernte, somit auch weniger Totalasche erhielt als andere Autoren, führt auch das directe Einäschern des Hirns zu Fehlerquellen, da viel Phosphorsäure aus dem Lecithin frei wird, die nun während des Einäscherns mit der glühenden kohligen Masse zu lange in Berührung bleibt.² Ich habe, um Verluste zu vermeiden, und den Verbrennungsprocess zu erleichtern, bei dem Einäschern eine bestimmte Menge Soda (meist 5 g.) zugesetzt und die Menge nach dem Veraschen und Wiegen wieder abgezogen. Allein auch diese Methode liefert keine fehlerfreie Bestimmung der Gesamt-

¹ Citirt in *Halliburton's Chemical Physiology* 1891, p. 517. Nach Z. f. physiol. Chem. Bd. I. S. 335.

² *Geoghegan* zeigte übrigens, dass diese freie Phosphorsäure auch Carbonate zersetzt, welche beim Einäschern des Gehirns erhalten werden. Ein von Lecithin befreites Gehirn liefert nämlich nach ihm eine Kohlensäure haltige Asche.

asche, weil während des Veraschens, ein Theil der zugestzten Soda in Folge der Bindung der Lecithinphosphorsäure Kohlensäure verliert. Doch mir kam es lediglich auf die Bestimmung der Kalk- und Magnesia-mengen für 1000 Theile frischen Gehirnes an und ich habe deshalb das Problem, eine möglichst einwandfreie Bestimmung der Gesamtmineralstoffe im Gehirn zu liefern, noch einstweilen bei Seite gelassen.

Ich habe sowohl das Gehirn vom Pferde als auch vom Kalb untersucht, nachdem ich die weisse Substanz so gut als möglich von der grauen trennte.¹ Eine frisch abgewogene Portion wurde zunächst im Wasserbade eingetrocknet, zuletzt im Luftbade. Nach Zusatz von Soda wurde eingäschert, nach Extraction mit Wasser die Asche weissgebrannt und nun Kalk und Magnesia in üblicher Weise bestimmt. In gleicher Weise bestimmte ich ferner diese Basen in den peripheren Nerven und der Lunge des Pferdes. Die Resultate sind aus folgender Tabelle ersichtlich.

	Angewand- tes Gewicht an frischer Substanz, g.	Asche g.	Kalk.		Magnesia.		
			In der Asche g.	In 1000 Thl. frischer Substanz.	In der Asche g.	In 1000 Thl. frischer Substanz.	
Kalb	Graue Hirnschubstanz.	89,50	0,8440	0,0329	0,368	0,0227	0,254
	Weisse Hirnschubstanz.	158,00	1,3918	0,0092	0,058	0,0094	0,060
Pferd	Graue Hirnschubstanz.	23,970	0,2794	0,0261	1,089	0,0111	0,463
	Weisse Hirnschubstanz.	68,106	1,0350	0,0045	0,052	0,0138	0,203
Periphere Nerven des Pferdes.	39,720	0,2011	0,0315	0,794	0,0239	0,602	
Lunge des Pferdes.	61,050	0,2035	0,0313	0,513	0,0274	0,449	

Es hat somit die Analyse in Analogie mit den obenerwähnten Verhältnissen auch hier für die graue Substanz mit ihren zahlreichen Zellkernen einen grösseren Kalkgehalt ergeben als für die weisse, an Zellkernen weit

¹ Die graue Substanz macht 37,7-39,0%, die weisse 61,0-62,3% des Gesamthirns aus.

ärmere¹. Ferner überwiegt in der grauen Substanz der Kalkgehalt über den Magnesiagehalt, während in der weissen das Umgekehrte der Fall ist. Der Nucleingehalt für die graue und weisse Substanz ist noch nicht separat chemisch bestimmt worden. Nach *Jaksch* enthält des Totalhirn in 1000 Theilen nur 3 Theile Nuclein, nach *Geoghehan* gar nur 1,34-1,62. Wenn nun auch die Grundlage dieser Berechnungen keine ganz sichere ist, so bleibt soviel jedenfalls richtig, dass der Nucleingehalt nur gering ist. Damit würde also auch der relativ geringe Kalkgehalt des Totalhirns stimmen. Doch dürfte immerhin die von *Geoghehan* hierfür gefundene Zahl 0,006 Ca auf 1000 Theile Gesamthirn auf einer mit Fehlern behafteten Analyse beruhen.

Magnesia und Kalkgehalt des Gehirns scheinen beträchtlichen Schwankungen unterworfen zu sein; wahrscheinlich üben Art und Alter der Thiere, sowie der wechselnde Fett- und Wassergehalt² hierauf, wie auf den Procentsatz an Gesamtasche einen wesentlichen Einfluss aus. Wie schon erwähnt, fanden verschiedene Autoren den Aschegehalt des Gesamthirns zu 0.1-1.0 Procent; ferner wurde das specifische Gewicht der grauen Substanz von 1.029-1.039 schwankend gefunden. Bei krankhaften Zuständen ferner werden weit grössere Schwankungen eintreten können. Um nur ein Beispiel zu erwähnen, kann bei Erkrankungen von Blutgefässen (Aorta) der Kalkgehalt bis zu dem 20 fachen des normalen ansteigen.³

¹ Auch wenn wir den verschiedenen Fett- und Wassergehalt berücksichtigen, bleibt dieses richtig. Es ergibt sich für 100 Theile trockne fettfreie graue Substanz beim Kalb = 0,260 Thl. CaO; dito weisse Substanz 0.075 Thl. CaO.

² In *Hammarsten's* Lehrbuch der physiologischen Chemie, III Auflage, S. 353, heisst es: „die Menge des Wassers im Gehirn ist grösser bei jüngern Individuen als bei Erwachsenen.“ Wie aus der darauf folgenden Tabelle ersichtlich ist, gibt es Ausnahmen.

³ *Gazert*, Jahresbericht für Thierchemie, 1900, S. 511.

Zusammenfassung.

In Uebereinstimmung mit der von *O. Loew* gezogenen Folgerung, dass der Kalkgehalt mit der Masse der Zellkerne wächst, steht das Resultat meiner Untersuchung, dass die graue Hirnsubstanz relativ kalkreicher ist, als die weisse.

On the Digestive Power of the Intestinal Canal.

BY

S. Sawamura.

After the experiments of *Thiry* and *Quince* the digestive function of the main part of the lower intestins was regarded to be of minor importance, but recent investigations disproved this view. Besides the accelerating action on the enzymes of the pancreatic juice observed by *Schepowalnikow*¹ the variety of the enzymes found in the intestinal juice is of special interest. A diastatic enzym was observed in the human intestinal juice by *Demant*,² in the small intestines of the swine by *Brown* and *Heron*,³ in the small intestine of the dog by *Krüger*,⁴ *Grünert*,⁵ and *Schepowalnikow*,⁶ and in the coecum and colon of various animals by *Ludwig Vella*.⁷ On the other hand, an enzym acting on inulin was proved to be absent in the human intestinal juice by *Demant*.⁸ Sucrase was shown to exist in the human intestinal juice by *Demant*,⁹ in the small intestines of the swine by *Brown* and *Heron*,¹⁰ in the intestines of the rabbit by *Pazyr*,¹¹ in the coecum and colon of various animals by *Vella*,¹² in the small intestines of the dog by *Grünert*,¹³ *Krüger*,¹⁴ and *Bastianelli*,¹⁵ and in the small intestines of man by *Miura*.¹⁶ Maltase was observed in the small intestines of the swine by *Brown* and *Heron*,¹⁷ in the intestines of

¹ and ⁶ Jahresbericht für Tierchemie vol. XX, XI, p. 370.

² ⁸ and ⁹ ibid. vol. IX, p. 222.

³ ¹⁰ and ¹⁷ ibid. vol. X, p. 77.

⁴ and ¹⁴ ibid. vol. XX, VIII, p. 337.

⁵ and ¹³ ibid. vol. XXI, p. 273.

⁷ and ¹² ibid. vol. XV, p. 297.

¹¹ " ibid. vol. XIV, p. 294.

¹⁵ Jahresbericht für Agrikulturchemie, 1890, p. 523.

¹⁶ Jahresbericht für Tierchemie, Vol. XXV, p. 288.

the rabbit by *Parry*,¹ in the caecum and colon of various animals by *Vella*,² and in the small intestines of dogs and cattle by *Pautz* and *Vogel*;³ Lactase, in the intestines of various young animals by *Römann* and *Lappe*,⁴ *Pautz* and *Vogel*,⁵ *Portier*,⁶ *Orban*⁷ and *Weinland*.⁸ *Pautz* and *Vogel*⁹ found an enzym which acted upon raffinose in the intestines of the dog and cattle. As to the occurrence of cytase, the opinions differed, but it was finally decided in the negative.

As to the behavior of digestive juices to various hemicelluloses, our knowledge is still imperfect. *Hauber*¹⁰ in *Voits* laboratory, in experimenting with a dog to investigate the digestibility of mucilages, found that they were digested and absorbed. He also observed that the glycerin-extracts of the stomach and pancreas of the same animal transformed mucilages into sugars, while ptyalin had no such effect. *Stone* and *Jones*,¹¹ and *Lindsey* and *Holland*¹² observed the digestibility of pentosan by the rabbit, *Weiske*¹³ by the sheep and *König* and *Reinhartd* by man.^{14 15} But *Nilson*¹⁶ observed that no sugar was formed from lichenin by digesting it with the gastric and pancreatic juices at 36°C, for 24 hours. As to the digestion of fat by the intestinal juice *Vella*¹⁷ observed that it is only emulsified by them, while the absence of a special enzym was

¹ Jahresbericht für Tierchemie, vol. XIV, p. 294.

² and ¹⁵ Ditto, vol. XV, p. 297.

^{3 5} and ⁹ Ditto, vol. XXIV, p. 304.

⁴ Ditto, vol. XXV, p. 286.

⁶ Ditto, vol. XX, VIII, p. 723

⁷ Ditto, vol. XX, IX, p. 384.

⁸ Ditto, vol. XXIX, p. 382.

¹⁰ Ditto, vol. IV, p. 375.

¹¹ Jahresbericht für Agrikulturchemie, 1893, p. 373.

¹² and ¹³ Ditto, vol. 1895, p. 431.

¹⁴ Ditto, 1893, p. 53.

¹⁵ Chemisches Central-Blatt, 1902, vol. 1, p. 673.

¹⁶ Herbivora can digest pentosan better than omnivora, and as it is always hydrated by digestion, there must be present a special kind of cytase in the intestines.

Zeitschrift für physiol. Chem., Vol. 36, p. 65-66.

¹⁷ Jahresbericht für Tierchemie, vol. XV, p. 297.

proved by *Demant*¹ in the secretion of the intestinal tract, and by *Schepowalnikow*² in the intestines of the dog. A proteolytic enzyme was proved to be present in the intestinal secretion of the dog by *Grünert*³ *Gachet*,⁴ and *Schepowalnikow*,⁵ but *Demant*⁶ (in the human intestines) and *Krüger*⁷ (in the intestines of the dog) questioned its presence.

Although various kinds of enzymes were hitherto observed in the intestines, little attention has been paid to the special parts of the intestines in which they are produced. Since both, *Lieberkühn's* and *Brunner's* glands, decrease in numbers gradually towards the rectum and especially in some animals the latter glands are found only in the part of the small intestines near the stomach, there must be some difference in the production of enzymes between the small and large intestines.⁸ I have directed my attention to the occurrence of enzymes that can saccharify mannan and galactan in the different parts of the intestines. In Japan a preparation consisting chiefly of mannan and derived from the root of *Conophallus Konyaku* and further the common agar are consumed generally by the people and since those hemicelluloses are digested we have to infer the occurrence of mannase and galactase in the intestines. The question seemed to me of some importance whether such enzymes are produced in all the various parts of the intestines. I compared in this regard the small intestines, caecum and colon, testing these three parts at the same time separately upon the production of the above enzymes as well as of others also.

The intestines serving for these experiments were taken from a horse, well washed with water, and exposed to the air for a day. 50 grs. of the small intestines, caecum and colon cut into pieces, were digested with about five times their weight of dilute alcohol (35%) for a week. The filtered alcohol extracts were precipitated with ether-alcohol, the precipitate

and ⁶ Jahresbericht für Tierchemie vol. IX, p. 222.

² and ⁵ Ditto. vol. XX, IX, p. 379.

³ Ditto. vol. XXI, p. 273.

⁴ Ditto. vol. XX, VII, p. 377.

⁷ Ditto. vol. XXVIII, p. 357.

⁸ *Ellenger*. Histologie der Haussäugethiere, p. 694.

dissolved in a 0.25% solution of sodium carbonate, and with the addition of some thymol served for the following experiments with crude fibrin, neutral olive oil, starch solution, cane-sugar, mannan and cellulose (filter-paper). The results observed after three day's digestion at 36°C were as follows:—

Materials used	Extract from the small intestines	Extract from the caecum	Extract from the colon
Fibrin	A little attacked	Not dissolved	Not dissolved
Oil	No acid reaction	No acid reaction	No acid reaction
Starch	Much sugar	Some sugar	Little sugar
Mannan	Sugar reaction positive	Sugar reaction positive	Sugar reaction positive
Canesugar	Inverted	Not inverted	Not inverted
Cellulose	No sugar	No sugar	No sugar

The identity of the sugar produced with mannose was proved by the production of the difficultly soluble characteristic phenylhydrazon.

The enzym-production is therefore not quite the same in the various parts of the intestines. As to the enzyme which acts upon mannan,—the mannase—it exists in all three divisions of the intestines.

A second experiment was performed with the intestines of a swine. The extracts were prepared by digesting respectively 50 grs. of the duodenum, caecum, colon and pancreas (as a control) in 200 cc. of 20% alcohol for a week. The experiments were carried on with the addition of some thymol. The results observed after digestion at 36° C. for three days were as follows[†]:—

[†] The extract was proved to be free from any reducing sugars by testing with *Fehling's* solution.

	Extract from the duodenum.	Extract from the cœcum.	Extract from the colon.	Extract from the pancreas.
Fibrin	Not attacked	Not attacked	Not attacked	Dissolved completely
Oil	No acid reaction	No acid reaction	No acid reaction	Acid reaction
Starch	Sugar reaction positive	Sugar reaction positive	Sugar reaction positive	Sugar reaction positive
Mannan	Sugar reaction positive	Sugar reaction positive	Sugar reaction positive	Sugar reaction positive
Galactan	Sugar reaction negative	Sugar reaction negative	Sugar reaction negative	Sugar reaction negative
Saccharose	Inverted	Not inverted	Not inverted	Not inverted

The sugar produced from mannan was proved to be mannose by testing with phenylhydrazine.

As will be seen in the above table proteolytic and lipatic enzymes were absent in the intestines, while diastase and mannanase were present in all the parts of the intestines.¹

Contrary to our expectation the absence of galactase was proved in this experiment.² We may conclude from these result as follows:—

1. In the intestines and pancreas of the higher animals, there is present *mannase* besides the other enzymes already observed.

2. The enzym-production is different in the small intestines, cœcum and colon, the most notable being the absence of *sucrase* in cœcum and colon in the case of the horse and swine, and of *trypsin* in the case of the horse.

¹ Diastase was also present in the rectum.

² A mucilage consisting of mannan, galactan, and araban was digested with the extract of the intestines of the horse and swine, and it was found that some arabinose was formed in both these cases, as was proved by phloroglucin and hydrochloric acid in the alcoholic extracts of the digested mucilage.

3 *Cytase* was not observed in this experiment.

4 The function of the intestines in digestion must be regarded to be very important, since they secrete enzymes such as *sucrase* that are not contained in the pancreatic juice.

On the Action of Manganese Compounds on Plants.

BY

O. Loew and S. Sawa.

With Plate XII.

The almost universal occurrence of manganese in the ashes of plants is a fact known long since, but as plants have been raised in water-culture to perfection in absence of manganese, this element is considered to be without any intrinsic value for the life of the plants. But it is nevertheless of interest to note the relatively large quantity occurring in the plants, exceeding often that of the related and so important iron. Thus, in the ash of beech leaves was found in one case 11.25% Mn_3O_4 and only 1.07% Fe_2O_3 .¹ It was found in the most different organs, even in the pollen-grains,² further also in the ash of parasitic fungi feeding on the sap of trees. Young shoots and leaves are especially rich in it.³

It deserves to be pointed out that in one case mangano manganic oxid, Mn_3O_4 , formed even the chief constituent of a plant ash. This being really an extraordinary case we mention the composition of the said plant ash. T. Schröder⁴ found on analysis of the ash of various parts of a pine tree (*Pinus abies*,) among other things the following result :

	Leaves	Bark
Pure ash.....	3.064%	1.805%
In 100 parts of pure ash :		
K_2O	14.48%	20.46%
Na_2O	0.67	0.38
CaO	11.42	14.72

¹ Wolff's Tables of Plant Ashes, I, p, 121.

² Ramann found it in the pollen grains of the pine, Botan. Centrallbl. 1868.

³ Fichard, Compt. rend ; vol 126, p. 550.

⁴ Forstchemische und pflanzenphysiologische Untersuchungen, Tharand, 1878. Also: Jahresbericht für Agriculturchemie 1878.

MgO	8.46.....	7.14
Fe ₂ O ₃	4.94.....	3.61
Mn ₃ O ₄	35.53	41.23
P ₂ O ₅	9.59.....	6.73
SO ₂	8.68.....	2.69
SiO ₂	6.33.....	3.04

The manganese content, as Mn₃O₄, for the dry matter of these leaves was therefore 1.08% and that of the bark 0.66%, corresponding to 0.69 and 0.42% Mn respectively.—Animal organs contain much less manganese than vegetable organs. *Riche* found only 0.5 milligrams Mn₃O₄ in one kilo of blood and according to other authors it is often completely absent. *Wurzer* (1833) observed it in the ash of the liver and teeth, *Weidenbusch* in the bile, *Horsford* (1851) in urine, *Pollacci* (1871) in milk and eggs, *Maument* (1883) in hairs and bones, *Pichard* (1898) in molluscs, crabs, sardines, pigs blood and hens eggs. The general occurrence in animals forms apparently a contrast to the highly poisonous properties it shows on subcutaneous and intravenous injections. Eight milligrams of manganous oxid in the form of sodium-manganese citrate represents according to *Kobert* the letal dose per kilo body weight of a dog. On introduction into the stomach even large doses of manganese compounds prove harmless, on account of deficient absorption by the intestinal walls.¹

In regard to the behavior of plants towards manganese compounds, but few experiments have been made and these show, that manganese cannot replace the related iron in regard to the production of chlorophyll, and that manganous and manganic phosphate suspended in culture solutions can exert an injurious effect.² Recently *Giglioli*³ applied peroxid of manganese as an addition to various manures on fields and observed in

¹ In recent times manganese compounds commenced to play a rôle in therapeutics. A manganese-iron pepton preparation is frequently recommended for certain disorders.

² Cf. *Blenker* and *Lucanus*, Landw. Versuchs-Stat. vol. 8, p. 128 and *Wagner*, *ibid.* vol. 13, p. 69 and 278.

³ Ann. Della R. Scuola Sup. di Portici 1900. The experiments were made with wheat. The peroxid of manganese was applied in the proportion of 1,14 ctw. per ha. Cf. also *Centralbl. f. Agricultur-chemie* 1902, No. 3.

some cases a moderate increase, in others a decrease of the harvest. The result was not decisive, which will not create surprise since peroxid of manganese is a compound which hardly can be attacked and dissolved by the rootlets.

The influence of manganese compounds in high dilutions has not been studied, although some action or other might be expected, considering the chemical properties of these salts, so closely related to the salts of iron, considering further the occurrence of manganese in the ash of oxidizing enzymes (*Bertrand*) and in that of certain nucleoproteids (*Asō*).¹

In order to observe the character of the injuries caused by manganese, young pea plants, 16-17 cm. high were placed in a solution of 0.25% manganous sulphate but this concentration injured the plants within five days so considerably that no characteristic symptoms could be observed. Most leaves had lost their turgor, some had even perfectly dried up, and no trace of new rootlets became visible. The control plants, however, remained perfectly healthy and had commenced to develop water rootlets. In the following experiment young barley plants 15—18 cm. high were placed in a 0.1 percent solution² of the same salt and kept in a heated room near a window. In this dilution of the manganese compound the injury developed more slowly. After seven days, however, a gradual change from green to yellow was evident, and this phenomenon became very marked two days later. Some water roots had developed, although much smaller ones and fewer as in the control case. A checking influence of the manganese was quite evident. On the ninth day further observation was given up and comparative tests were made as to the color reactions upon oxidizing enzymes. Five grams of the upper halves of the leaves were finely triturated with addition of pure quartz sand and gradual addition of 50 cc. water. This liquid had a weak acid reaction but this was still weaker in the control case. A portion of the colorless filtrate (f) was exposed for several hours to the air, whereby it assumed a reddish hue which was not observed in the control case. One cc. of the filtrate (f) was diluted with 20 cc. distilled water and five drops of a 2% alcoholic guaiac solution added

¹ Bull. College of Agriculture, Tokyo; vol. 4 No. 3.

² Such data refer in this article to the anhydrous compound, not to the crystallized salt.

whereby the blue color produced was much more intense than in the control case. A considerable difference in the intensity of the guaiac reaction for peroxidase after killing the oxidase by heating to 75°C., and adding some hydrogen peroxid was also observed. But still more striking were the differences in the tests with guaiacol and with paraphenyldiamine in presence of hydrogen peroxid. One cc. of the filtrate (f) was diluted with 20 cc. of water and five drops of a 1% aqueous solution of guaiacol and three drops of dilute hydrogen peroxid added. A red-brown color of great intensity set in at once, while in the control case a much weaker coloration was slowly developed. A colorometric test fifteen minutes after adding the reagents showed that in the latter case the intensity of color was less than one half that of the former. In an analogous manner the test with paraphenyldiamine hydrochlorid (to which a little sodium acetate had been added) and hydrogen peroxid was carried out. The green color in the control case was but half as intense as in the case of the manganese plants.

The undeniable fact that the reactions on the oxidizing enzymes are more intense with the manganese plants than the control plants can give as also an account for the fading out of the green color of the leaves. Our result corroborates the statement of *Bertrand*¹ that the oxidizing enzymes act more powerfully in presence of manganese compounds than in their absence, and that iron compounds cannot produce an effect of the same intensity.² The effect of the manganese seems to be the same, as an increase of the oxidizing enzymes by certain stimulants secreted by parasitary insects (*Aphides*) and fungi. The yellow spots thus produced on leaves yield according to *Albert R. Woods* more intense reactions upon oxidase and peroxidase than an equal surface of the healthy leaves. *Woods* also observed a higher content of oxidases in etiolated shoots, than in normally green ones.³

¹ Compt. rend., vol. 124, p. 1032.

² There may exist cases in which oxidizing enzymes are not associated with manganese or iron but they will be less powerful in that case. Cf. also *Sarthon*, Journ. Pharm., Chem. vol 11, p. 583 [1900.]

³ C. Bakt. H. Abt. 5, S. 745 [1899.]

In our next experiments¹ the manganous sulphate was applied in a much higher dilution in the expectation to diminish the injurious effects to a minimum. At the same time the mineral nutrients were offered to the plants in the following proportions ;

Calcium nitrate	0.04%
Magnesium sulphate	0.01%
Potassium nitrate	0.03%
Monopotassium phosphate	0.02%
Ammonium sulphate	0.01%

To one portion was added 0.01% ferrous sulphate (control solution), to another 0.02% manganous sulphate and to a third 0.01% ferrous sulphate plus 0.02% manganous sulphate.

Shoots of barley and soy bean were placed in these solutions and kept near a window in a room whose temperature ranged from 4—12°C. during the first three weeks of observation. After a series of days the shoots in the solution containing manganese and iron jointly *exhibited an increased growth*. The measurement revealed even considerable differences (see Table) ; gradually however these shoots *turned yellowish*, their assimilation power was consequently depressed and in further consequence of this the decreased nutrition led to a relaxation in the speed of growth, as seen from the measurements of the soy bean plants.

Experiment with Barley.

Two shoots were placed in each of the solutions above mentioned
March 27.

¹ We also have made experiments with algae but they failed on account of development of parasites.

		Date of Measurement, cm.			Increase after 25 days. %
		March 27	April 9	April 22	
Mn.	A	34.0	46.0	51.5	51.4
	B	38.0	47.2	55.5	46.5
Mn. Fe.	A	35.5	50.0	60.0	71.8
	B	34.0	51.0	58.0	70.6
Fe.	A	35.0	43.4	52.1	48.5
	B	35.0	50.3	57.7	64.9

Experiments with Soy bean.

Three shoots were placed in each solution, March 25.

		Date of Measurement, cm.				
		March 25.	April 8.	April 22.	April 30.	May 10.
Mn.	A	9.3	26.0	37.0	40.0	45.0
	B	10.2	22.0	25.4	35.0	40.5
	C	7.0	22.5	31.0	31.6	dead.
Mn. Fe.	A	6.6	27.0	40.0	48.1	56.5
	B	6.8	26.0	40.2	43.5	43.5
	C	7.5	23.5	38.2	46.0	51.5
Fe.	A	7.8	24.1	39.5	43.5	56.5
	B	9.3	21.0	39.0	38.5	45.0
	C	5.1	11.2	35.0	43.5	40.0

The gradual increase in the speed of growth up to April 22 and the following diminution of that speed with the plants that received iron and manganese is still clearer seen from the average :

	March 25.	April 8.	April 22.	April 30.	May 10.
Mn + Fe	8.9	25.3	39.4	45.8	50.5 cm.
Fe	8.5	23.1	33.5	42.5	50.2 cm.

From the yellowing of the leaves under the influence of manganese might be inferred that this is a strong poison for plants. But this conclusion would not be justified, since our further observations have demonstrated beyond any doubt that at summer temperature the plants are capable to overcome the yellowing effects of the manganese, if this is present only in small quantities. It appears that by the increased activity of the protoplasm a part of the dissolved manganese in the cells is transformed into insoluble compounds¹, a process, that probably takes place in nature, when plants absorb manganese compounds from the soil. Thus it may be rendered possible that only such small quantities remain dissolved in the cells as can exert a beneficial effect.

Experiment with Rice in Soil Culture.

In the following experiment with rice in soil culture the yellowing was not observed at all. The soil came from the experiment grounds of our College of Agriculture. Each pot containing eight kilo air dry soil was manured with 16 g. superphosphate, 10 g. potassium carbonate and 16g. sodium nitrate. Pot I received no further addition. Pot II was watered with 200 cc. of a 0.1 per cent solution of ferrous sulphate, Pot III with this solution and further with 200 cc. of a 0.1 per cent solution of manganous sulfate. The seed was sown on May 24 and the number of shoots reduced four weeks later to seven about equally large ones. The rice was cut on Nov. 10 with the following result :

¹ The observation of *Asp* (l.c.) that a nucleoproteid contained besides iron also manganese may furnish us a clue in the right direction, *Z.*

	I. Control,	II. Fe SO ₄	III. Fe SO ₄ + Mn SO ₄
Number of stalks	19	20	18
Average length	58.6 cm.	59.7 cm.	64.6 cm.
Weight of straw	45.7 g.	46.5 g.	48.7 g.
Weight of grains	5.7 g.	7.0 g.	11.2 g.

These numbers show that ferrous sulphate alone exerted a manuring effect, an observation also formerly made by others. Soils which contain iron in a difficult soluble condition will respond in this manner. Much more striking was here however the effect of the manganese. Although the number of stalks was smaller, the weight of straw and especially that of the grains was larger than in the control cases I and II. The stimulating effect of the absorbed manganese was exhibited in an unmistakable manner. Nevertheless the inference would not yet be justified that every soil would respond in this manner. Probably many soils with a high *natural* fertility contain manganese in an easily absorbable condition, in which case a further supply of manganese salts would be of no avail.¹ It would be of interest, however, if in the analysis of soils more attention would be paid to the manganese content the determination of which is often wholly neglected.

Experiment with Pea in Soil Culture.

The soil was here the same as in the last experiment. The main and the control pot held this time 2300 g. soil and each was manured with 3 g. sodium nitrate, 3 g. potassium carbonate and 4.6 g. common superphosphate. On Febr. 21 fifteen seeds were sown and later on the young plants reduced to five equally large ones in each pot. The main pot received

¹ One of us (L.) has mentioned two years ago a case, in which tobacco plants showed no increase of the oxidizing power of the oxidases, after being irrigated with 0.1 per mille Mn SO₄ solution (Report No. 65 of the U. S. Dep't of Agriculture, p. 22).

highly diluted solution of manganous sulphate on March 11 and 25 ; April 14, 21, and 28 ; and on May 6. The total amount of this salt was 0.036 g.; the first two times also 0.001 g. ferrous sulphate dissolved in 100 cc. water was added.

The plants commenced flowering on April 22, the ripe fruits were harvested on June 2. A photograph, taken on May 17 (see Plate XII.), shows the more luxuriant development under the influence of manganese very distinctly.

The ripe fruits were weighed in the fresh state, further the peas isolated and weighed well dried at summer temperature. The straw was weighed in the air dry state. The results were as follows ;

	Manganese plants.	Control plants.
Weight of the fresh fruits71.060.5
Weight of air dry seeds29.123.2
Weight of the air dry straw15.910.7

These results leave no doubt of a stimulating action having been produced by manganese on the development of the pea plant, but the differences in the seed harvest were not so large as in the case with rice. The nodules on the roots were rather scanty with both the pea plants.

Experiment with Cabbage.

A small plot of 12,5 squ. Meter received 3 g. manganous sulphate (anhydr.) dissolved in 15 Liter water.¹ The land had received the previous year twice barn-yard manure and had served for cultivation of barley and radish. This year it received only ammonium sulfate at the rate of 50 g. to 12,5 square Meter. Cabbage seed was sown on a part of this plot on April 25. Germination proceeded very slowly and many seeds failed to germinate. In the beginning of June, however, the difference in develop-

¹ Two grams on April 24 ; 1 g. on May 21.

ment of the plants on the manganese plot compared with the control plants became very striking. On June 14 numerous plant lice made their appearance and damage by beetles threaten, hence the plants were collected, the roots washed and by gentle pressure freed from the adhering water. All control plants larger than 12 cm. from the base of the trunk, thirteen, weighed united in the fresh state, 56,0 g., while the same number of the manganese plants weighed 94,9 g. Hence a very favourable influence of the manganese is also here evident.

The question may be raised: How is the remarkable stimulus of growth by manganese to be explained? Can some light be expected by reviewing the characteristic properties of manganese compounds? The fact that in many other cases it seems almost impossible to find an explanation for the stimulant action ought not to deter us in every new case from searching for a clue. Now, it is well known that light retards growth. This hitherto unexplained phenomenon forms a strange contrast to the great chemical work the light performs with the aid of the protoplasm in the chlorophyll bodies. One and the same agency then increases the organic food on the one hand and suspends the utilization of that food on the other. It is in the absence of light that growth proceeds and the products of the sun's work are chiefly consumed. The absence of light has therefore the same effect as the presence of manganese. It seems as if under both these conditions a check is removed which the sun's rays exert. This check might be due to the action of certain noxious compounds produced in the cells under the influence of light. Such compounds (Hemmungs-Stoffe, Ermüdungs-Stoffe) are frequently produced in the course of the metabolism.¹ It is the office of the oxidizing enzymes, as one of us (*L.*) has suggested, to destroy noxious by-products of the benzene series, a view verbally expressed as follows²:

“The writer's view on this subject is that as the living protoplasm can oxidize carbohydrates and fat, but does not attack or attacks only with difficulty compounds of the benzene group, and, on the other hand, as

¹ Cf. *Fr. Reinitzer*, *Berichte d. botan. Gesellschaft*, Vol. 11, p. 531 [1893].

² Report No. 59 of the U. S. Dep't. of Agriculture, Washington 1899, p. 27.

just the opposite takes place with the oxidizing enzymes, it may be inferred that there exists between the protoplasm and the oxidizing enzymes a certain division of labor, the former oxidizing the compounds of the methane series and the latter those of the benzene series. The former provides for the kinetic energy of the cells; the latter destroys by partial oxidation noxious by-products. The oxidations in the former case are generally complete, but in this latter only partial."

If the checking compounds are gradually changed by partial oxidation, without being produced anew in darkness, we can understand the increased growth in the absence of light. Since, however, as we have seen above, the presence of manganese increases the oxidizing power of the oxidizing enzymes, the destruction of the checking compounds may be accomplished as quickly as they are formed and thus an explanation can be furnished why in presence of manganese the growth proceeds day and night while in absence of manganese only at night time. Future investigations will show whether this explanation is the correct one.

It was in this connection of some interest to note that fungi show no enhancement of growth under the influence of small quantities of manganese although traces of other salts, as zinc salts (*Richards*) and copper salts (*Ono*) can exert a stimulant action. A remarkable stimulant influence on the growth of *Aspergillus* under the influence of traces of sodium fluorid was also observed by *Ono*. These observations are in full accordance with *Hüppe's* biological law. The different behavior of fungi towards manganese in this regard seems to indicate that the enhancement of growth of phænogams under the influence of manganese is not due to a direct stimulation of the protoplasmic activity, not to an irritation as it is observed by poisons in very high dilutions, but it must be accounted for by a different cause. The explanation just given by one of us (*L*) would agree with this inference.

The first experiments on the influence of manganese salts on fungi were made by *H. Molisch*.¹ He concluded that manganese cannot enhance the development of fungi like iron salts can, the latter even being

¹ Wiener Akad. Ber., October 1894.

indispensable. Recently Mr. *Takahashi* from this College made some further experiments in the same direction. As culture solution served a sake wort prepared from boiled rice by the action of *Aspergillus oryzae*. To this wort was added after sterilization a sterilized solution of manganous sulfate, to a second flask ferrous sulphate and to a third ferrous and manganous sulphate jointly, while a fourth served as control. These liquids were infected with a pure culture of sake yeast while in a second series the flasks were infected with a trace of spores of *Aspergillus oryzae*. Both series were kept in diffuse day light. After three weeks the fungus mass was collected on a weighed filter and dried. The result was as follows :

Salts added.	Weight of yeast.	Weight of <i>Aspergillus</i> .
Manganous sulphate 0.1 p.m.	0.764	1.122
Ferrous sulphate 0.1 p. mille.	0.778	1.394
Manganous and ferrous sulphates 0.05 p. mille each.	0.467	1.216
Control.	0.813	1.285

These results agree well with those of *Molisch*, there is no distinct stimulant action of manganese on fungi. It is true that the weight of the yeast failed also to show an increase by the ferrous sulphate, but here it must be born in mind that the original culture solution contained already some iron.

Summary.

Manganese exerts in moderate quantity an injurious action on plants, consisting in the bleaching out of the chlorophyll. The juices of such plants show more intense reactions for oxidase and peroxidase than the healthy control plants. Manganese exerts further a promoting effect on the development, still observable in high dilution, while the injurious effects disappear under this condition. It is probable that soils of great natural fertility contain manganese in an easily absorbable condition, and that this forms one of the characteristics of such soils.



I.

II.

Table showing the influence of manganese on tea. I Manganese plant; II Control plant. To page 160.



Ueber die Wirkung des Urans auf Pflanzen.

VON

Oscar Loew.

— — —
Tafel XIII.
— — —

Die Lichtempfindlichkeit der Uransalze¹ liess es von Interesse erscheinen, die Wirkung derselben auf grüne Pflanzen zu verfolgen, da möglicherweise Spuren von Uran im Chlorophyllkorn die Umwandlung von Licht in chemische Energie befördern und damit die chemische Leistung vermehren konnten. Es wurde von *Seckamp* beobachtet, dass Bernsteinsäure unter Vermittlung von Uransalzen durch Licht in Propionsäure und Kohlensäure gespalten wird. Ferner bilden die Fluorescenzerscheinungen und die in neuester Zeit beobachtete Radioactivität² mancher Uranverbindungen interessante Beziehungen zum Lichte.

Bis jetzt scheint nur ein einziger Versuch über die Wirkung von Uranverbindungen auf Pflanzen ausgeführt zu sein und zwar durch *Knopf*,³ welcher Uranylphosphat als Aufschwemmung in der Nährloesung verwendete. *Knopf* zog aus diesem Versuch den Schluss, dass wegen der grossen Schwerloeslichkeit des Uranylphosphats dasselbe ohne jede Einwirkung sei. Sorgfältige Vergleiche mit Controlpflanzen scheint er nicht angestellt zu haben. Zwar gehört das Uranylphosphat mit zu den schwerlöslichsten Phosphaten, doch geben Uransalze in einer Verdünnung von 0.1 pro mille keine Fällung mehr mit Monokaliumphosphat, sondern nur eine schwache Opalescenz. In solcher Verdünnung könnte

¹ Bekanntlich werden Uransalze auch in der Photographie verwendet.

² Radioactive Substanzen wirken im Dunkeln auf die photographische Platte ein und wenn nach Monaten diese Eigenschaft erlischt, so kann sie durch Belichtung mit Kathodenstrahlen wieder hervorgerufen werden. Die Radioactivität scheint Beziehungen zu den Becquerelstrahlen und zur Phosphorescenz zu haben.

³ Jahresber. f. Agricultur-Chem. 1884, S. 139.

demnach wohl Uran auch in Gegenwart der Phosphate des Bodens von den Wurzeln aufgenommen werden.

Zunächst wollte ich einige Daten betreffs des Giftigkeitsgrades¹ sammeln, dann die Wirkung bei sehr grosser Verdünnung beobachten. Auf junge Erbsenpflanzen wirkte Uranylнитrat schon in 3 Tagen sehr giftig ein, als diese in eine 0.2 procentige Loesung gesetzt wurden. Wurde diese Loesung bis auf 0.05% Uranylнитrat verdünnt, und junge Zwiebelpflanzen eingesetzt, so war nach sieben Tagen das Hauptblatt fast überall von der Spitze abwärts bis nahe zur Hälfte der Länge gelb geworden und partiell verdorrt; die jüngeren Blätter wurden erst später afficirt.² Die Wurzeln hatten eine gelbliche Färbung angenommen, keine neuen Zweige, keine Wasserwurzeln waren erschienen, während bei den Controlpflanzen in blosem Wasser dieses der Fall und überhaupt das ganze Ansehen noch ein normales war. Ganz ähnlich war die Wirkung auf junge Gerstenpflanzen von 12-18 cm. Höhe. Diese Pflanzen hatten versucht, neue Wasserwurzeln zu treiben, diese waren aber schon als kurze Stummeln wieder abgestorben, während die Controlgerstepflanzen in blosem Wasser in sieben Tagen neue Wurzeln von bis zu 2 cm. Länge getrieben hatten.

Wurden nun Erbsen- und Gerstenpflanzen in Nährloesung gesetzt, welcher 0.01 per mille Uranylнитrat zugesetzt war, so liess sich selbst nach Wochen keine schädliche Wirkung mehr wahrnehmen.

Nun wurde (Febr. 16) ein Topfversuch mit Erbsenpflanzen ausgeführt, denen bis zur Beendigung der Blütenperiode sechsmal je zwei Milligramme Uranylнитrat in 100 cc. Wasser gelöst, gegeben wurde.³ Die jungen Pflanzen im Haupt- und Controltopf wurden auf fünf möglichst gleich grosse reducirt. Die Blütenperiode dauerte vom 21 April bis zum 12 Mai. Am 17 Mai wurde eine Photographie aufgenommen welche auf Tafel XIII reproducirt ist und die üppigere Entwicklung der Uranpflanzen deutlich

¹ Auf Thiere wirken bekanntlich Uransalze sehr giftig und rufen Diabetes, Degeneration der Leber und Paralyse hervor.

² Ein Vergleich mit Manganoxydulsulfat zeigte, dass dieses weit weniger schädlich wirkte als das Uranylнитrat.

³ Dieser Versuch wurde gleichzeitig mit dem Manganversuch an Erbsen, und unter denselben Bedingungen angestellt (siehe den vorhergehenden Artikel).

erkennen lässt. Auffallend war, dass die Uranpflanzen zur Zeit der Reife der Früchte neue Zweige aus dem Boden trieben, welche Blüten bildeten, was bei keiner der andern zur selben Zeit beobachteten Pflanzen der Fall war.¹ Am 2. Juni wurde geerntet, die Samen enthülst und diese sowohl als das Stroh lufttrocken gewogen. Die Wurzeln hatten in beiden Fällen nur wenige Knöllchen entwickelt, doch die Controlpflanzen immerhin etwas mehr als die Uranpflanzen.

Das Resultat der Wägung war wie folgt :

	Fünf Uranpflanzen.	Fünf Controlpflanzen.
Samen... ..	29,5 g.	23,2 g.
Stroh	17,0 g.	10,7 g.

Ein stimulirender Einfluss des Uranyl-nitrats mit Vermehrung nicht nur des Strohs sondern auch der Samen ist demnach zweifellos. Es ist dieses eine interessante Thatsache, doch verbietet der hohe Preis der Uransalze deren praktische Verwendung.

Zugleich mit diesem Versuche mit Erbsen wurde unter ganz gleichen Verhältnissen ein Versuch mit Hafer angestellt. Ernte am 3. Juli, Stroh und Samen (unenthülst) wurden im lufttrocknem Zustande gewogen, mit folgendem Resultat :

	Uranpflanzen.	Controlpflanzen.
Zahl der Halme... ..	11	0
Stroh, g.	49.5	45.2
Körner mit Hülsen	26.7	21.4

Die fördernde Wirkung des Urans ist somit auch hier unverkennbar.

¹ Ausser mit Mangansulfat waren Pflanzen mit hoch verdünnten Loesungen von KJ und NaF behandelt worden (siehe in diesem Heft Mr. *Asch's* und *Sizuki's* Artikel).







I.

II.

Die Tafel zeigt den Einfluss des Urins. I. Boden erhielt 0,012 g. Urea-Nitrat II. Controlpflanze. Zur Seite 174.



On the Physiological Influence of Manganese Compounds on Plants.

BY

K. Asō.

With Plates XIV—XVII.

It is a well known fact, that plants can develop normally in absence of every trace of manganese in water culture and further that manganese which is of frequent occurrence in plants can not replace iron in the production of chlorophyll. But since certain metallic salts, as those of zinc, cobalt and nickel¹ can exert a stimulating effect on the growth of fungi when applied in high dilution, it seemed of interest, to observe also the action of manganese, so frequently present in the soils, on the growth of agricultural plants.

Experiment with Radish.

On Nov. 26, 1901, shoots of radish, 5-5 cm. high, were placed, two shoots in each flask, in the following solutions :

- A. 0.02% Mn SO₄ + trace of Fe SO₄
- B. 0.02% Mn SO₄ + 0.02% Fe SO₄
- C. 0.02% Fe SO₄

Each flask contained further the following nutrients :

Ca (NO ₃) ₂	0.2 %
KNO ₃	0.15 %
KH ₂ PO ₄	0.05 %
Mg SO ₄	0.05 %
(NH ₄) ₂ SO ₄	0.05 %

¹ Cf. Ōno, Journal of the College of Science, In p. Univ., Tōkyō. Vol. XIII, part I., also *Revue de* and others.

In a second series, (a), (b), (c), the above solutions (A), (B) and (C) were diluted with ten times the volume of water, while the mineral nutrients were present in one fifth the quantity as in the first series. These shoots were kept in a cold room with a winter temperature of 0°-6° C. After two weeks, the difference in development was very striking, as will be noticed from the accompanying photograph (Plate XIV) taken on Dec. 12. On Dec. 14, the following determinations were made:

	Number of leaves.	Length.	Fresh weight.
A.....	4	11.2 cm.	1.2 gm.
	4	10.0 "	
B.....	4	8.4 "	0.65 "
	4	7.2 "	
C.....	3	6.8 "	0.35 "
	3	6.0 "	
a.....	4	11.5 "	1.3 "
	4	10.5 "	
b.....	4	9.8 "	0.9 "
	4	8.8 "	
c.....	3	8.2 "	0.45 "
	4	8.3 "	

This result shows doubtless a most remarkable stimulating effect of the manganese. An undesirable feature was the gradual yellowing of the leaves, which however turned gradually again to a normal green, on transferring the plants to a heated room. A fungus now appeared on the roots, hence the experiment had to be terminated.

Since *Bertrand* has repeatedly observed that the ash of oxidizing enzymes contains manganese and that in presence of manganese compounds the oxidizing effect of these enzymes is considerably increased, it was of interest to compare here those effects. Pieces of leaves of (a) and (c) of equal surface (5 × 7 m.m.) were well crushed in a mortar with addition of

10 c.c. of water. This extract (2 c.c. in each case) served for the following tests:¹

1. Upon addition of one drop of a 2% guaiac tincture, the blue color produced was more intense in the case (a), than in that of (c), and this difference became much more marked after one minute.
2. On addition of one drop of guaiac tincture and two drops of a 1% hydrogen peroxid solution, after killing the oxidase by heating, the blue reaction for peroxidase appeared, but the difference was not so striking as in the former case with oxidase proper.
3. On addition of 1 c.c. of a 1% guaiacol solution and two drops of hydrogen peroxid, the red reaction produced was more intense with (a) than with (c).
4. On addition of a few drops of dilute sodium acetate, paraphenylenediamine hydrochlorid and hydrogen peroxid, a green reaction of much higher intensity was produced in (a) than in (c).
5. This difference was also noticed with the violet reaction which was obtained with tetramethyl-paraphenylen-diamine and hydrogen peroxid.

Furthermore, two sections of equal size (4 × 6 m.m.) of the leaves of (A), (B) and (C) were ground with 15 c.c. of water. The tests (with the exception of 5) carried out as just mentioned, showed also in this case that the plant containing manganese (A and B) yield a juice which exerts a more powerful oxidative power than the plants without manganese (C).

Experiment with Barley.

On Nov. 26, barley shoots (7-8 cm. long) were placed in solutions of the same composition as in the experiment with radish. The influence of manganese was here noticed not so early as with radish, but was very marked nevertheless, as seen from the following table, containing the determinations made on Dec. 14, and from the photograph taken, Dec. 12, (Plate XV).

¹ Cf. also the paper of the writer, "On oxidizing Enzymes in the Vegetable Body," in this Bulletin.

	Number of leaves.	Length.	Fresh weight.
A.....	2	19.5 cm.	—
	3	11.6 „	—
B.....	2	17.2 „	—
	2	14.2 „	—
C.....	2	14.1 „	—
	2	12.0 „	—
a.....	2	23.3 „	0.70 grm.
	3	15.0 „	
b.....	2	17.0 „	0.65 „
	2	16.5 „	
c.....	2	13.5 „	0.55 „
	2	13.6 „	

The series (A), (B), (C), was no further observed, since some shoots commenced to show injury, and demonstrated beyond a doubt as also did the above described experiments of *Loew* and *Sawa*, that barley in water culture suffers—at least at the low winter temperature—from the concentration of 0.02% manganese salt. But in regard to the series (a), (b), (c) in which manganous sulphate was applied in a concentration of only 0.002%, the observations were continued, after the plants were transferred (Dec. 14) from the cold room to a heated room.

The following table shows the results :

	Jan. 9.		Febr. 2.		Febr. 15.		March 3.		March 15.		April 14.	
	No. of leaves.	Length.	No. of stalks.	Length.	No. of stalks.	Length.	No. of stalks.	Length.	No. of stalks.	Length.	No. of stalks.	Length.
a	8	cm. 27.0	4	cm. 27.1	4	cm. 30.5	5	cm. 30.5	5	cm. 31.0	8	cm. 35.5
b	7	20.2	3	20.2	3	26.0	5	32.0	6	41.0	7	52.5
c	8	18.5	2	22.0	5	23.4	7	33.0	7	39.0	8	57.1

The fresh weight.

	Febr. 2.	March 3.	April 15.
a	4.0 grm.	9.7 grm.	16.8 grm.
b	1.5 „	6.5 „	16.7 „
c	2.1 „	8.5 „	17.0 „

On Febr. 2, a difference in the color of the leaves was not yet noticed, but the yellowing of leaves in (a) was clearly observed on Febr. 15. The solutions were renewed on Febr. 3, March 3, and March 15. The roots in the manganese culture solutions turned gradually brown, so also did the lower leaves in (a), whereby the brown color was more especially concentrated in certain points, which had also been noticed in the series (A), (B) and (C). On microscopical examination the membranes of the epidermis cells, and here and there also what seemed to be the nuclei proved to be deeply brown. For barley in water culture an addition of 0.01 per mille $Mn SO_4$ seems to be the highest concentration which is applicable without injury. The colorimetric tests for oxidizing enzymes were here made with equal weights of the fresh leaves of (a) and (c), and thus ascertained as in the case of radish that the yellowish leaves of the manganese plants gave reactions of higher intensity than the green leaves of the control plants. But the difference was here not so great as in the case of the radish shoots.

Experiment with Wheat.

At the same time at which the barley experiment was begun, one with wheat shoots, 6-7 cm. long, was started under the same conditions. The observations on the series (A), (B), (C), are contained in the following table :

	Dec. 20.			Febr. 2.	
	Number of stalks.	Length.	Fresh weight.	Number of stalks.	Length.
A	3	23.0 cm.	0.90 gm.	8	25.0 cm.
	3	25.0 ..			
B	3	23.0 ..	0.80 ..	1	17.0 ..
	3	24.5 ..			
C	3	14.7 ..	0.50 ..	1	11.9 ..
	3	18.8 ..			

The leaves of this series gradually turned yellowish, and since one of the two plants in B and C died off, the observations on this series was discontinued, while those on the series a, b, c were continued until May 14. The data relating to this series are shown in the following table.

	Dec. 20.			Febr. 2.			Febr. 15.		
	No. of stalks.	Length.	Fresh weight.	No. of stalks.	Length.	Fresh weight.	No. of stalks.	Length.	Fresh weight.
a	3	cm. 23.5	gm. 0.85	8	cm. 28.8	gm. 6.6	8	cm. 33.0	gm. 9.1
	4	24.5							
b	3	23.2	0.75	6	27.8	6.0	7	30.8	9.1
	3	25.0							
c	3	22.1	0.75	5	23.0	4.9	6	28.5	8.2
	3	21.2							

	March 3.			March 15.		March 29.			April 14.	
	No. of stalks.	Length.	Fresh weight.	No. of stalks.	Length.	No. of stalks.	Length.	Fresh weight.	No. of stalks.	Length.
a	8	cm. 38.5	gm. 19.5	8	cm. 43.7	8	cm. 60.2	gm. 34.5	9	cm. 67.0
b	8	39.0	21.8	9	44.7	10	62.0	46.5	10	72.7
c	9	36.5	19.0	10	45.0	10	59.8	40.5	10	62.5

The ears developed in the following number :

	May 1.	May 3.	May 6.	May 7.
a	2	6	6	7
b	3	6	6	7
c	0	0	0	1

On May 6 a photograph was taken (see Plate XVI). The leaves of the plants (a) were paler than those of (b) and (c) and many of the lower leaves turned brownish¹ what was less the case with (b) and not at all with (c). Also the roots of the manganese plants turned gradually brown.² The solutions were renewed on Febr. 3, March 3, 15, 29, April 14 and 28, increasing the amount of $MnSO_4$ from March 3 to April 14 to 0.004%.

Since now a parasitic fungus appeared on the leaves, the experiment was terminated on May 14. The final observations were as follows :

	Length.	Number of ears.	Fresh weight of ears.	Total weight of dried straw.	Weight of living leaves.	Weight of dead leaves.	Weight of dried roots.
a	cm. 59.08	7	gm. 2.4	gm. 5.6	gm. 3.2	gm. 2.4	gm. 1.2
b	59.09	8	3.5	8.0	7.0	1.0	3.0
c	48.48	4	1.0	7.4	6.5	0.9	2.2

The stimulant effect of manganese on the wheat plant becomes therefore very evident, when the plants (b) are compared with the plants (c). The plants (a) suffered evidently from the want of sufficient amount of iron, which was more evident in the later stage of development. It deserves to be pointed out especially, that the wheat plant can overcome the injurious effect of manganese much more readily than the so closely related barley plant.

¹ The brown points did not here appear as distinctly as in the case with barley.

² In comparing (A) with (B) and (a) with (b), it appears that the increase of iron had a counteracting effect upon the influence of manganese, not only in regard to the yellowing of the leaves but also in regard to the stimulating effect, produced by the manganese salts. This same inference can be drawn from the observations made on the radish shoots (see above p. 178). The brown color of the roots was due to some adhering MnO_2 .

Experiment with Pea.

On March, 8, shoots of pea (3-4 cm. long) germinated in saw dust were placed in the following solutions and observed during the first period of development, that is, until the mineral and organic nutrients of the cotyledons were consumed. The plants were kept in a warm room. Of mineral salts, ferrous and manganous sulphates only were applied.

- a. 0.002% MnSO_4
- b. 0.002% MnSO_4 + 0.002% FeSO_4
- c. 0.002% FeSO_4

The following observations show the growth of the shoots :

	Length.					Fresh weight.
	March 22.	March 27.	March 31.	April 7.	April 20.	
a	cm. 15.0	cm. 18.0	cm. 23.0	cm. 30.0	cm. 39.2	gm. 1.6
b	11.6	14.0	16.5	21.5	30.5	1.2
c	11.6	13.8	17.5	23.0	33.0	1.1

The accompanying photograph (see Plate XVII) was taken on March 31. It deserves mentioning that the yellowing of the leaves, observed with radish and barley, did here not make its appearance in this first state of development, which may be explained by the presence of a sufficient amount of iron in the reserve stores.

Conclusions.

1. Manganese salts exert on the one hand an injurious action and on the other a stimulant influence on plants; with increased dilution the former diminishes while the latter increases. Thus a dilution can be reached in which only the favorable action of manganese becomes obvious.
2. Manganous sulphate added in a dilution of 0.002% to culture solutions¹ exerted a stimulant action upon radish, barley, wheat, and pea. Iron seems to counteract to a certain degree the action of manganese.
3. The intensity of the color reactions of the oxidizing enzymes of the manganese plants exceeds that of the control-plants.

¹ This was of course transformed into phosphate in the culture solution.





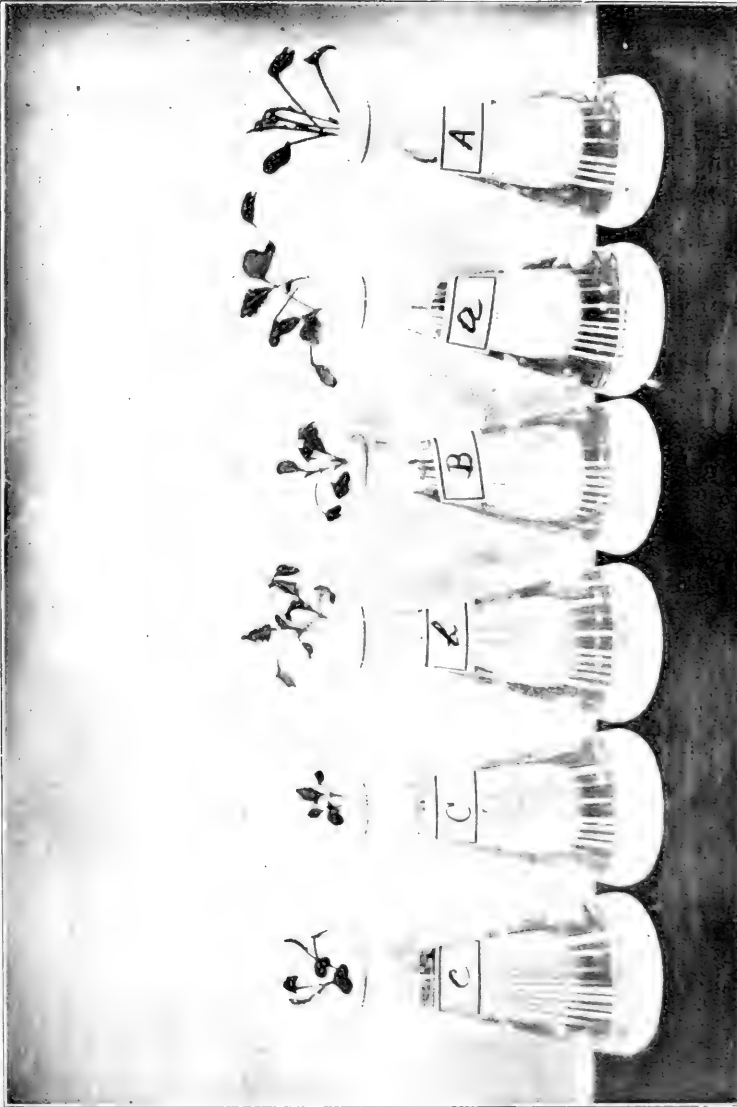


Plate showing the influence of manganese on radish shoots. To page 178.



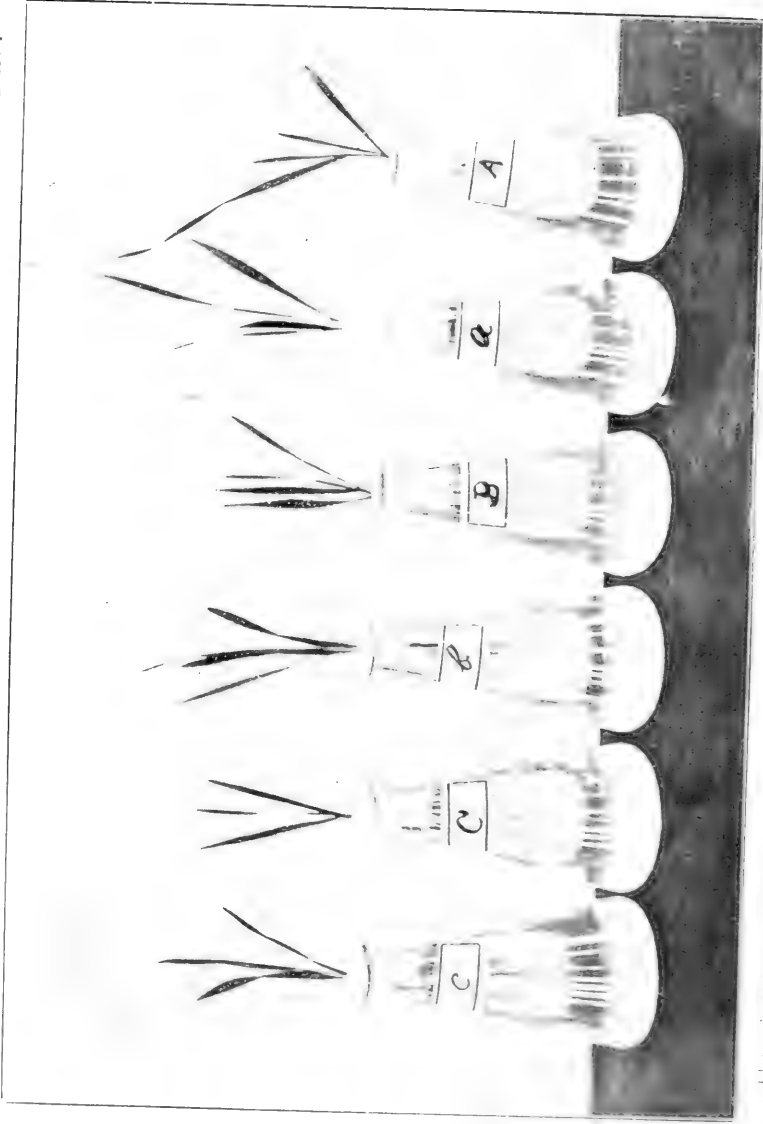


FIG. 1. 1000 ft. alt. 1000 ft. alt. 1000 ft. alt. 1000 ft. alt. 1000 ft. alt. For page 170.

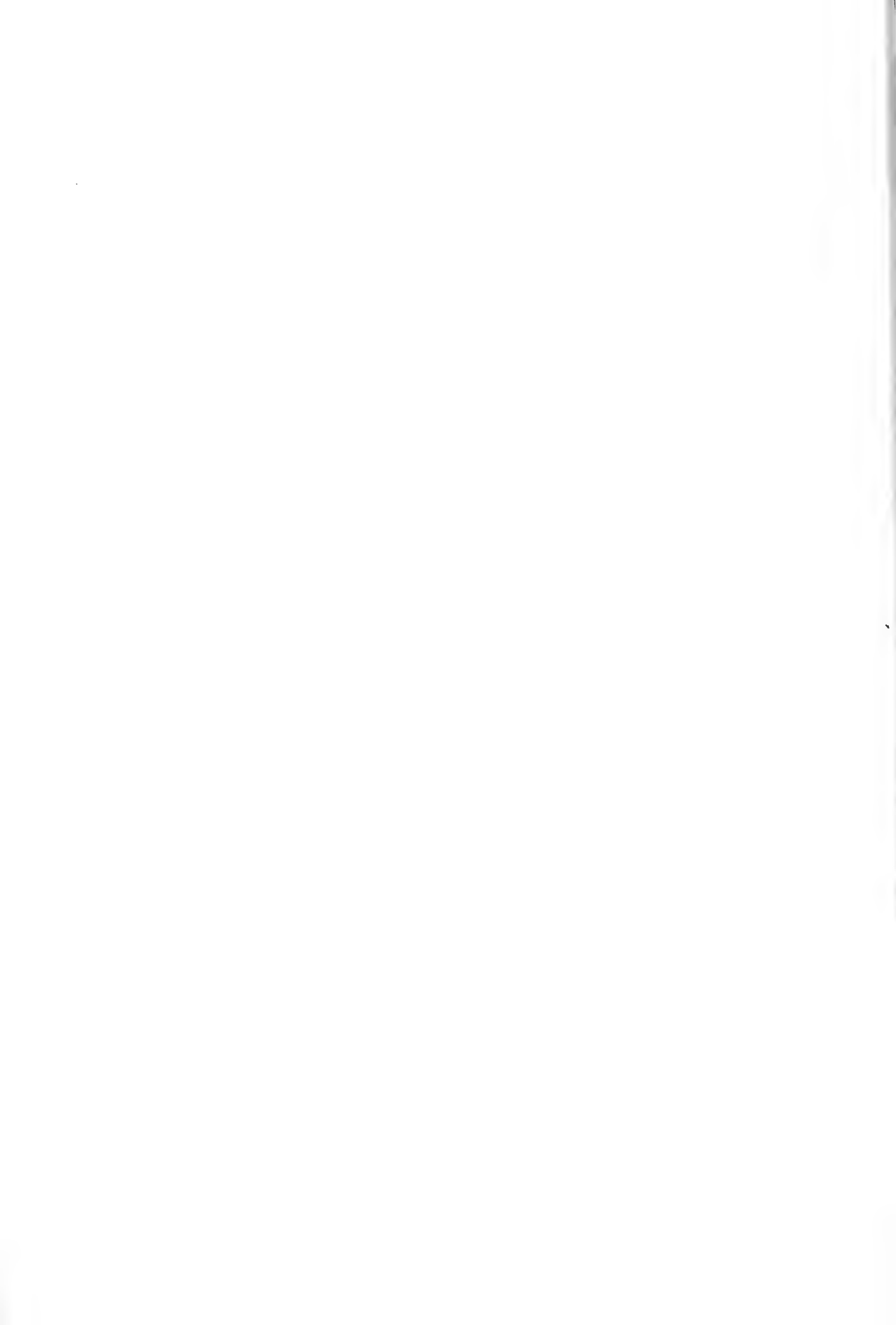




Plate showing the influence of manganese on wheat. 187





Plate showing the influence of manganese on pea shoots. To page 18



On the Action of Sodium Fluorid upon Plant Life.

BY

K. Aso.

With Plates XVIII—XIX.

Although it is very well known that sodium fluorid exerts a poisonous action on plants and animals, the writer has made some further experiments with the view of determining the point of dilution at which that poisonous action disappears and a stimulating action sets in.

Ono has in this regard observed that sodium fluorid in a dilution of 0.000.03% has a stimulating action on the development of algæ.¹

Experiment with Seeds.

Seeds of rice, wheat and mustard (50 of each kind) were steeped in solutions of 1%, 0.5%, 0.25%, 0.1% and 0.05% NaF for 48 hours and then, superficially dried, left to germination in purified sand. The number of germinated seeds were counted after 6 days with the following result :

NaF.	Rice.	Wheat.	Mustard.
1 %	20	1	0
0.5 %	28	2	0
0.25%	34	2	0
0.1 %	38	2	6
0.05%	44	7	14
Control.	45	16	23

It will be noticed that the rice seed had more resistance power toward the poison than that of the wheat and mustard, what is probably due to

¹ Journ. College of Science ; Imp. Univ., Tokyo. Vol. XIII, part I, (1900). With fungi, a stimulating action was observed by him in the concentration of 0.005% NaF. Yeast is injured by 0.01% NaF.

the thicker cover, preventing the ingress of so much poison as entered in the other cases. The shoots were largest in the control case but these were closely followed by the shoots from the seeds treated with the 0.05% NaF solution. Very striking, however, was the rapid falling off in length between these and the shoots from the seeds treated with the 0.1% and the stronger solutions.

In a second trial, seeds of soy-bean were compared in regard to the resistance power with seeds of wheat. They were steeped in solutions of NaF of the following concentrations :

0.05 %
0.01 ,,
0.005 ,,
0.001 ,,

After 24 hours, the solutions were poured off and the seeds left to germination, 20 in each case. After 9 days, the following data were observed :

Sodium fluorid.	Number of germinated seeds.		Average length of plants.		
	Wheat.	Soy-bean.	Wheat.		Soy-bean.
			plumule.	radicle.	
0.05 %	15	12	7.0cm.	8.2cm.	3.0cm.
0.01 ,,	18	20	7.0 ,,	9.6 ,,	6.5 ,,
0.005 ,,	17	20	8.5 ,,	11.0 ,,	8.0 ,,
0.001 ,,	19	20	8.5 ,,	11.0 ,,	6.5 ,,
Control ,,	18	20	7.0 ,,	10.5 ,,	9.0 ,,

Also in this case, therefore, like in the first, sodium fluorid in the concentration of 0.05% acted injuriously upon the seeds.

Experiment with Soy-bean Shoots.

Shoots of soy-bean were (March 18) placed in solutions of sodium fluorid of the following concentrations :

	NaF.	Length of shoots.
a	0.1 %	10 cm.
b	0.05 „	10 „
c	0.01 „	10 „
d	0.005 „	10 „
e	Control „	9 „

After three weeks, the shoots (a) and (b) had withered while with (c) and (d), the leaves remained green and perfectly healthy and the height of the shoots did not essentially differ from that of the control case. The cotyledons (c) and (d), however, had lost their green color and their turgor, and had become yellow. A slight touch sufficed to cause their dropping off. This indicates that the poison was retained to such an extent in the cotyledons that only traces reached the leaves above.

Experiment with Pea.

On March 6, 1902, pea shoots 2 cm. long were placed in solutions of 0.01, 0.001 and 0.0001% sodium fluorid. The results are shown by the following table :

	Length.			Fresh weight dete on April 22.
	March 31.	April 14.	April 22.	
0.01 %	17.0 cm.	20.4 cm.	20.4 cm.	1.2 gms.
0.001 „	19.0 „	26.5 „	32.5 „	1.3 „
0.0001 „	20.8 „	30.3 „	36.5 „	1.4 „
Control.	20.2 „	29.0 „	37.2 „	1.4 „

A poisonous action on pea shoots is therefore produced by 0.01 and 0.001% NaF, but it is not any more noticeable when the dilution reaches 0.0001%.

Experiments with Barley Shoots.

I. Shoots of barley were placed (March 25) in a culture solution to which was added NaF in the following proportions :

a	0.05 %
b	0.01 „
c	0.005 „
d	0.001 „
e	Control case.

The result is seen from the following table :

	Length, ¹				Number of stalks.		
	March 25.	April 4.	April 8.	April 16.	April 25.	April 8.	April 16.
a	29.3 cm.	29.2 cm.	28.2 cm.	27.0 cm.	4	4	4
b	37.5 „	37.2 „	37.5 „	40.0 „	4	4	4
c	30.0 „	31.0 „	40.0 „	53.0 „	4	4	7
d	28.2 „	30.2 „	33.5 „	43.5 „	4	7	7
e	25.2 „	29.7 „	35.5 „	41.5 „	3	3	3

On March 30, the leaves of (a) lost their turgor while with (b) the tips of the leaves became yellowish. On April 1, the lower leaves (a) had died, while with (b) they were injured and with (c) they showed yellow tips. No new rootlets had appeared with (a), while a few with (b) and more with (c) and (d). On April 4, the general appearance of shoot (d) was perfectly normal.

In this case, therefore, a stimulating effect of sodium fluorid in regard to the increase of the number of stalks of barley at a dilution of 0.005 and 0.001% was quite evident.

II. On October 15, 1901, barley shoots 10—12 cm. long were placed in a culture solution to which were added :

¹ The measurement relates only to that part that still was healthy.

a	0.01	%	Sodium fluorid.
b	0.001	,,	,,
c	0.0001	,,	,,
d	Control.		

The following table shows the observations made :

	Length.			Number of stalks.		Fresh Weight.
	Oct. 31.	Dec. 12.	Febr. 6.	Dec. 12.	Febr. 6.	Febr. 6.
a	15.3 cm.	26.1 cm.	27.0 cm.	5	9	11.5 gms.
b	16.8 "	30.3 "	37.0 "	7	10	11.8 "
c	19.5 "	39.0 "	—	5	—	—
d	17.5 "	34.1 "	37.0 "	5	7	20.2 "

The plant in (c) which developed most was injured by the severe cold in the winter.

Very many root-hairs and rootlets appeared in (c), also they developed very well in (b), but poorly in (a). The remarkable effect of sodium fluorid in very high dilution upon an increase of the stalks was also here observed like in the former case with barley as the table shows. The accompanying photograph (Plate XVIII) was taken on Dec. 12. During this experiment, the solutions were renewed on Nov. 5, 25, and Dec. 17.

Experiment with Wheat Shoots.

On May 18, shoots of wheat 6.5—7 cm. long, were placed (3 in each case) in solutions of 0.001% NaF (a), and 0.0001% NaF (b) to which the necessary mineral nutrients were added. Towards the end of May, it became evident that the growth of the plants (a) was much slower than that of (b) and of the control plants. On June 12, the following data were observed :

	Average number of leaves of one shoot,	Average length of one shoot,	Total fresh weight of 3 shoots.
a	3	21.1 cm.	0.8 gm.
b	4	29.4 ,,	1.6 ,,
c	4	29.1 ,,	1.6 ,,

A poisonous action of sodium fluorid in the dilution of 0.001% was therefore quite evident, while in the 10 times higher dilution the poisonous character was not observable. Since a fungus commenced now to show on the leaves this experiment had to be terminated.

Experiment with Rice.

I. Shoots of rice 8—10 cm. long were placed in solutions of 0.01% and 0.05% of NaF, but in the latter solution no development took place and the tips of the leaves turned yellow after a few days. In the 0.01% solution, some growth was observed, but less than in the control case, demonstrating the injurious influence at the dilution of 0.01% upon rice.

II. On June 12, shoots of rice 22—25 cm. long were placed in the same solutions as the wheat shoots (2 shoots in each flask). After five days, some difference of development was noticed, especially with the roots.

After 10 days, the following data were observed:

	Total number of new rootlets,	Total number of leaves,	Length.
a. 0.001 % NaF	32	10	32.0 cm.
b. 0.0001 ,,	37	8	30.4 ,,
c. Control.	20	6	33.0 ,,

A certain stimulant action is therefore noticeable in the case (a) and it appears therefore that rice is not so easily injured by NaF as wheat.

Experiment with Flower Buds.

On Febr. 4, three plum branches bearing 5, 7 and 10 buds respectively were placed in solutions of NaF of various concentration. The following data show the result:

	Number of flowers developed.		
	Febr. 24.	Febr. 25.	Febr. 27.
a. 0.01 % Sodium fluorid.	0	0	0
b. 0.001 ,, ,,	3	4	6
c. 0.0001 ,, ,,	2	3	3
d. Control. ,,	0	2	3

There had no bud opened in the solution of 0.01% NaF, which shows a poisonous influence in this concentration. Very striking was the different size of the flowers; the petals in the control case were much larger than those in (b) while those in (c) had an intermediate size. Hence we observe on the one side a stimulating action of sodium fluorid as to the time of development of the flowers, but on the other, a diminishing effect on the size of the petals, a notable parallelism to the case of the barley shoots (see above).

Experiment with Leaf Buds.

Branches of *Cornus macrophylla* Wall, of equal length and size bearing 4—5 winter buds were placed (March 4) in solutions of sodium fluorid of the same concentrations as just mentioned. The result after 37 days was as follows:

	Original number of buds.	Number of buds opened.			Remarks.
		March 26.	March 27.	April 10.	
a. 0.01 % Sodium fluorid	5	0	0	1	Leaves yellow.
b. 0.001 ,, ,,	4	4	4	4	
c. 0.0001 ,, ,,	5	4	5	5	
d. Control.	4	3	3	4	

The size of leaves was largest in (c), smaller in (d), and smallest in (a) and (b).

Here also a certain accelerating influence of sodium fluorid in very high dilution upon the development of leaf-buds was evident.¹

¹ It may be mentioned here that also some experiments were made with injections of a 0.2% solution of sodium fluorid into young branches and buds, but besides the death of the tissue around the point of injection no striking effect was noticed.

Experiment with Pea in Soil Culture.

Two pots each holding 2.3 k. soil served here for the experiments with peas, of which 15 seeds were sown on Febr. 19. The young plants were reduced however, on March 27, to five equally large ones in each case. Each pot had received the following manures :

4.6 gm.	Common superphosphate.
3.0 ,,	Potassium carbonate.
3.0 ,,	Sodium nitrate.

While the plants in one pot were treated with sodium fluorid, the other pot served for control. The pots were kept in the green-house almost all the time. The amount of sodium fluorid supplied on each application was only 0.001 gm., dissolved in 100 c.c. water. The days of application were as follows : March 11, 25, April 14, 21, 28 and May 6. The total amount of sodium fluorid was therefore 0.006 gm. and still in spite of this small quantity, a stimulating effect was gradually noticed and was finally also recognized by the weight of the seeds produced. The formation of flowers commenced on April 22, and was ended on the 16th of May. One day afterwards a photograph was taken (see Plate XIX), which shows that under the influence of sodium fluorid the plant had reached a greater height than the control plants.¹

Up to the flowering period, almost every day 300 c.c. water for irrigation was applied, later on the quantity was increased to 500 c.c. The fruits had ripened on the 2nd of June, and were weighed in the fresh state, while the straw was weighed in the air dry state. The results were as follows :

¹ On April 23, some plant-lice made the appearance on the leaves and from now careful search was kept up and every louse noticed killed by touching the insects with a little brush moistened with a 1% carbolic acid solution.

	Sodium fluorid.	Control.
Weight of fresh fruits.	71.7 gm.	60.5 gm.
Weight of air dry seeds	27.2 „	23.2 „
Weight of straw.	17.7 „	16.7 „

This result doubtless shows that a stimulating action by this small quantity of fluorid had taken place.



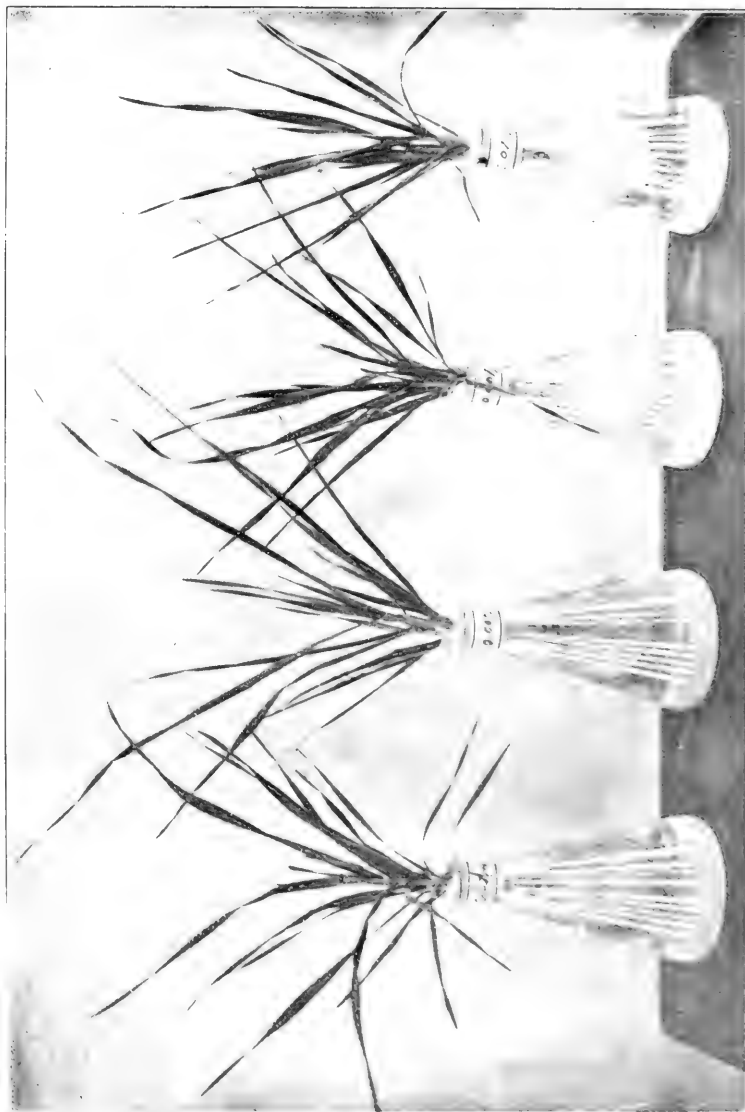


Plate showing the influence of sodium fluoide in different concentration on barley. To page 101





I.

II.

Plate showing the influence of sodium fluoride on pecan. I Fluoride
II control plant. To page 104.



On the Action of Sodium Silico-fluorid upon Plants.

BY

K. Aso.

The foregoing observation with sodium fluorid made it very probable, that sodium silico-fluorid would also prove a poison even in a considerable dilution. In order to observe however whether this salt would in still higher dilution exert a stimulant action, the following experiments were made with shoots of soy-bean and barley (about 25 cm. long). To the culture solutions were added :

- a. 0.05 % Sodium silico-fluorid.
- b. 0.01 ,, ,, ,,
- c. 0.005 ,, ,, ,,
- d. 0.001 ,, ,, ,,
- e. Control

The observations were as follows :

	Soy-bean.	Barley.
March 27.	Shoots (a) and (b) lost turgor.	
„ 28.	Shoots (c) lost turgor.	
„ 30.	Shoots (d) stationary. Considerable development in the control case.	The tips had all turned yellow with the shoots (b) and (c), while with (a), all leaves had withered. Leaves with (d) still normal.
April 1.	Shoot (d) lost turgor and withered.	Almost all leaves with (b) and (c) are dead; only shoot (d) and the control shoot were still healthy.
„ 8.	Only control shoot still alive.	Control shoot normal, has grown to cm. shoot (d) still alive, but stationary.

It will be noticed that sodium silico-fluorid is a stronger-poison than sodium fluorid when the result of the foregoing article is compared with these. In the solution containing even only 0.005% sodium silico-fluorid, the barley as well as soy-bean shoots had been killed almost completely in 6 days. But a remarkable effect was noticed with barley shoots (d), i.e. where the dilution of sodium silico-fluorid was 0.001%.

While here growth in height was very sluggish there had developed up to the 8th of April from the originally three stalks as many as seven new stalks, while not a single new one had started in the control case! This forms a third instance of this kind of action of a fluorine compound, since sodium fluorid produced the same phenomenon with barley shoots in the two cases described in the foregoing article.

It deserves notice, further, that soy-bean is more easily injured by highly diluted sodium fluorid and silico-fluorid than barley.

On the Action of Highly Diluted Potassium Iodid on Agricultural Plants.

BY

S. Suzuki.

With Plate XX.

Since it became known that in certain glands, especially in the thyroidea, occur peculiar proteins containing iodine, the inference had to be drawn that the vegetable food for animals and man contains a small amount of iodine compounds. The analyses of the ashes of crops thus far made ignore the presence of iodine completely, but a few instances are known of iodine having been found in other phænogams than agricultural plants. *Bourcet*¹ found iodine in ashes of plants growing on soil containing for 100 kgr. 0.83 mg. iodine. *Liliaceæ* and *Chenopodiaceæ* took up comparatively more iodine from this soil than *Solanaceæ* and *Umbelliferæ*. Long ago, however, it was known that marine algæ contain iodine.² *Stanford*³ observed in the dry matter of such an alga, *Laminaria digitata*, 0.453% iodine in average, in that of *Fucus serratus* 0.085% and in that of *Fucus vesiculosus* 0.029%. In which form the iodine is present in these plants is not yet fully decided, but it is very probable that it forms a constituent of peculiar proteins. If this amount of iodine would be present in form of a soluble iodid it would probably act poisonously, at least, if the cell sap would be of an acid reaction. Algæ with a neutral cell sap, as *Spirogyra*, show considerable resistance to sodium iodid. It has first been observed by *Dirks*⁴ that potassium iodid even in

¹ Compt. rend. Vol. 129 [1899]. Also Bot. Centralbl. 1900. No. 23.

² The ashes of these algæ served long since for the manufacture of iodine.

³ Quoted in *Musprat's* Technical Chemistry.

⁴ J. B. f. Agric. Chemie. 1868 p. 289.

a dilution of 0.0625 per mille proved very injurious for phænogams. Young maize plants died after two weeks and soon afterwards also buckwheat plants. Cress resisted longer but did not produce any ripe seeds.¹ *Loew*² has also observed a highly poisonous action of sodium iodid on buckwheat seedlings. Recently also *A. Voelcker*³ made some experiments in this direction. Sodium iodid at the rate of 200 cwts. per acre killed wheat, barley and red clover, peas were slightly benefited. At the rate of 1 cwt. per acre it injured wheat and barley. A top dressing at the rate of $\frac{1}{2}$ cwt. per acre injured also wheat and barley. Soaking the seed in a 1 per cent sodium iodid solution increased however the yield of wheat and barley, grain and straw, and benefited the red clover. In water culture sodium iodid proved highly poisonous; thus even in a dilution of 1 : 43700 it caused the roots to be "quite dwarfed." Since the general occurrence of iodine in agricultural products must be assumed and consequently also the occurrence of iodine traces in every soil, it seemed to me of some interest to observe the effect of a small increase of traces originally occurring in the soil, since poisonous compounds can in very high dilution often produce a stimulating action (*Hüppe's* biological law).

I have tried, therefore, the culture of pea (*Pisum*) in the soil of our College farm under the influence of small doses of potassium iodid. Each pot contained 2300 gr. air dry soil and was manured with 3 gr. NaNO_3 , 3 gr. K_2CO_3 and 4.6 gr. common superphosphate. Fifteen seeds were sown on Feb. 21st and after the young shoots had reached about 15 cm., they were reduced to 5 equally large ones.

While one pot served as control, the main pot received 0.001 g. potassium iodid on each of the following dates: March 11, and 25, April 14, 21, 28, and May 6. The total amount of potassium iodid was therefore

¹ This interesting difference between the cress on the one hand and buckwheat and maize on the other might be explained by the assumption that the iodine, when liberated from the compound, is absorbed by the allyl compounds produced in the cress before it can seriously injure the dying protoplasm.

² He found that in a culture solution containing 0.2 per mille sodium iodid, young buckwheat plants could not grow at all (*Ein natürliches System der Giftwirkungen* p. 108).

³ J. Roy. Agr. Soc. Engl. [III], 11, 566-591, Abstract in the Journ. Chem. Soc. May, 1901.

only 0.006 gr. Still, this exerted a stimulant action as the further development revealed. The formations of flowers commenced on April 22, and had ended on the 16th of May.¹ One day afterwards a photograph was taken (see Plate XX), which shows that under the influence of potassium iodid the plant reached a greater height than the control plant. Up to the flowering period the pots received almost every day 300 c.c. water, later on 500 c.c. The fruits had ripened on the 2nd of June, and were weighed in the fresh state, while the straw was weighed in the air dry state.

The results were as follows :

	Potassium iodid.	Control.
Weight of the fresh fruits	72.4 gr.	60.5 gr.
Weight of air dry seeds	26.3 ..	23.2 ..
Weight of air dry straw	15.5 ..	10.7 ..

This result doubtless shows that a stimulating action of this small quantity of iodid had taken place.

¹ At that time aphides made their appearance on the leaves, they were easily killed by touching them with a fine hair brush moistened with a carbolic acid solution of 1%.





I.

II.

Plate showing the influence of soil treatment on growth. I. I. 100 gms. of soil.
II. Control plant. To page 201.



On the Poisonous Action of Potassium Ferrocyanid on Plants.

BY

S. Susuki.

The observation of *Knop* that chlorotic plants turn green not only by ferric salts but also by potassium ferrocyanid, led me to try whether the iron in this last named form could not be used with advantage in water cultures, since the iron would retain its solubility in presence of phosphates, whereby its absorption would be facilitated. *Knop*^{*} observed, however, not only a useful but also an injurious action consisting in the stoppage of growth when he applied the potassium ferrocyanid in a dilution of 0.1 per mille.†

Since, however, it seemed probable that this injurious action might be avoided by a higher dilution of that compound, I have applied a dilution of 0.01 per mille. The solution had the following composition:—

Calcium nitrate (anhydrous).....	3.	per mille.
Potassium nitrate.	1.	„ „
Magnesium sulphate (cryst.)	1.5	„ „
Monopotassium phosphate.....	1.	„ „

This solution received in the control case (a) 0.02 per mille ferric phosphate in fine suspension while in two other cases, (b) and (c), 0.01 per mille potassium ferrocyanid. The culture liquid (c) contained further 0.6 per mille ammonium sulphate.

Young barley shoots were placed in these solutions on Dec. 14th, and kept in a room near the window, at a temperature ranging from 4° to 15°C. After a few weeks a very marked difference was noticed. The

* J. B. f. Agr. Chem. 1869 p. 268. Also, *ibid*, 1884, p. 140.

† Algeæ are not injured by this dilution, but by one of 0.5% (*cf. Zinn, System of poisons and actions* p. 55.)

roots of those plants which had received potassium ferrocyanid showed much less development than the roots of the control plants—they had fewer lateral roots and less root hairs. Gradually also a yellowing of the leaves set in, leading to death. On Feb. 3rd the still living leaves were counted, the length of the longest leaves measured and further the total weight of the still living parts of the leaves and the roots determined. The results were as follows:—

	Number of the living leaves.	Length of the longest leaves.	Total fresh weight.
a.	8	21 c.m.	4.4 gm.
a.	10	21	3.6 „
b.	6	18.5	1.8 „
b.	5	17.0	1.9 „
c.	0*	12.0	1.5 „
c.	0**	13.0	1.0 „

It seems therefore that the ammonium ferrocyanid probably formed in the solution (c) is still more poisonous than the potassium ferrocyanid. My result shows that the ferrocyanid compounds are exceedingly injurious even in the high dilution of 0.01 per mille. I also have noticed like *Knop* the formation of some prussian blue on the surface of the roots. It may be asked, in what this poisonous action of potassium ferrocyanid consists. The supposition suggests itself that this salt is split in the plant with the production of hydrocyanic acid and that this is really the poisonous principle.† This is rendered still more probable by the observation of *Knop* that the potassium ferrocyanid as such is changed very soon in the juices of the plant. But on the other hand there exist cases in which hydrocyanic acid appears to act less poisonously. Some plants in Java produce free hydrocyanic acid in the course of a metabolism peculiar of their own. Thus the leaves of *Pangium edule* are said to contain as much as 1% of hydrocyanic acid for the dry matter.

* and **: All leaves were dying.

† *Loew* and *Tsukamoto* (This Bulletin, Vol. II, No. 1) observed a poisonous action on germinating seeds of hydrocyanic acid in a dilution of 0.2 per mille. A solution of 1 per mille killed young radish plants in 15 hours.

Further there exist several glucosides in plants, such as amygdalin in the bitter almonds, in *Pygium parviflorum* and *Gymnema latifolium*, and linamarin in the hairs and seedlings of *Linum usitatissimum* which glucosides yield on decomposition by enzymes also hydrocyanic acid. *Soave* has shown that bitter almonds produce hydrocyanic acid from amygdalin during germination.¹ *Lutz*² proved the formation of hydrocyanic acid in the seedling of a Japanese *Viscum*. It also has been found in the roots of *Manihot utilissima* and *Vicia* and in still other plants, as *Passiflora quadrangulata* and *Colocasia gigantea*.

An exposure to very dilute gaseous hydrocyanic acid for a short time will not injure plants, but it will suffice to kill noxious insects investing the plants. *A. F. Woods* and others recommend therefore the treatment of invested plants in greenhouses with hydrocyanic acid gas.

As the main results of my tests follows:—Potassium ferrocyanid is—even in high dilution—not suited as a source of iron for the chlorophyll-bearing plants, since it will gradually injure the plants and even the chlorophyll itself.

¹ It may serve there as a protection against depredations by insects. If it is decomposed in formic acid and ammonia in the seedling, the latter may again be utilized for building up proteins.

² Bull. Soc bot. de France, 1897.



On Oxidizing Enzymes in the Vegetable Body.

BY

K. Aso.

Introductory Remarks.

Although various investigations on the oxidizing actions by enzymes have been published during the past ten years, certain questions are still unsolved, especially in regard to the identity of the enzymes which cause various color reactions. The following observations in regard to this point may therefore be not be without some value.

The fact that the juices of many fresh vegetable and animal objects can produce a blue color with guaiac tincture on addition of hydrogen peroxid was known long ago but the true cause was recognized but recently. *Yoshida*¹ was the first author who pointed out that an especial enzyme with oxidizing actions is contained in the sap of the lac tree,² to which is due the blackening of this sap in contact with air by the oxidation of urushic acid, the most important constituent of the sap, to oxy-urushic acid. *Bertrand*³ who continued the study of that sap called this enzyme, laccase. He and *Bourquelot* demonstrated further the wide distribution of this laccase in the vegetable kingdom and that it can produce a blue reaction with guaiac even in absence of hydrogen peroxid. *Ray-Pailhade*⁴ ascertained the presence of laccase in germinating seeds. While laccase does not act on tyrosin, a special enzyme called by *Bertrand* tyrosinase,⁵ changes

¹ *Yoshida*, Journ. Chem. Soc. XLIII, 1883.

² This juice is called 'Urushi' in Japan.

³ Bull. Soc. Chim. 11, p. 717, 1894.

⁴ Compt. rend. 121, p. 1162, 1895.

⁵ Bull. Soc. Chim. 13, p. 793, 1896.

tyrosin to a red and ultimately to a black substance. This enzym is present in the root of *Dahlia*, the tubers of potato and some fungi, as *Russula*.

*Martinaud*¹ observed a laccase-like enzym, œnoxydase, in grapes and other fruits, and ascribed the cause of discoloration of red wines to this enzym. Recently *Tolomei*² showed that a similar oxidase is present in several yeasts and has close relation to the production of the bouquet of certain wines. *Bréaudat*³ found an oxidase in the leaves of *Isatis*, that causes the oxidation of indican to indigo; *Tolomei*,⁴ an oxidase, called olease, in ripe olives that causes an oxidation of the olive oil; *Sarthou*⁵ observed an oxidizing enzym called by him, shinoxidase, in the latex of *Schinus molle*. *Dubois*⁶ ascribed the cause of the production of light by certain animals and plants to an oxidizing enzym, which he called luciferase. *Lepinois* studied especially the occurrence of the oxidizing enzym, which gives a blue reaction with guaiac and hydrogen peroxid and called it *peroxidase*. Recently, *Raciborski*⁷ studied the distribution of the same enzym in certain plants. The name leptomin given by him to this enzym is however not justified, since *Lepinois* had named it already *peroxidase*. Similar investigations were made by *Grüss*. *Loew*⁸ who observed this enzym and laccase in the leaves of tobacco, attributes the changes of these in the curing and fermentation process to their actions.⁹ *Woods*¹⁰ observed an increase of the oxidizing enzymes in plants pathologically affected. *U. Suzuki*¹¹ investigated the relation of oxidizing enzymes

¹ Compt. Rend, 124, p. 512, 1897.

² Real. Acad. Linc. 1896, 5, 1, p. 52.

³ Comp. Rend, 127, p. 769, 1898.

⁴ Real. Acad. Linc. 1896.

⁵ Journ. Pharm. Chim, 6 ser. 12, p. 104 and 11, p. 482, 1900.

⁶ C. R., 123, p. 653, 1896. (Carl Oppenheimer: Die Fermente u. ihre Wirkungen, p. 301).

⁷ Ber. d. d. Botan. Ges. XVI, 119, 1898.

⁸ Report, No. 65, U. S. Department of Agric. 1900, p. 18.

⁹ This so-called fermentation is not due to bacterial action as *Loew* has abundantly proved.

¹⁰ C. Bakt, II. Abt, 1899, p. 745.

¹¹ Bull. College of Agric. Tokyo. Vol. IV, No. 1.

in the case of the mulberry dwarf disease. Lately, *Hunger*¹ observed the presence of oxidase and peroxidase in the milk of the cocoa nut, *Newton* and the writer in the tea leaf. Recently a particular enzyme, called spermase, was found to exist in resting barley² and yeast³ by *Grüss*. A new enzyme, catalase, which is of general occurrence was discovered and fully studied by *Loew*.⁴

As to the physiological rôle of Oxydase (laccase) and peroxidase, *Loew* has pointed out that it very probably consists in changing by partial oxidation injurious by-products of the benzene series, generated in the course of metabolism. Catalase further can perform the highly important function of destroying hydrogen peroxid which may be produced as a by-product in the course of cellular respiration. Many investigations have indeed shown that, whenever the molecular oxygen of the air serves for oxidations at the common temperature, hydrogen peroxid is produced as a by-product.

The Tests for Oxidizing Enzymes Applied in this Investigation.

Although there are known quite a number of color tests upon oxidizing enzymes, only the following served here for comparison.

1. Test with guaiac tincture.

According to *Bertrand*,⁵ an alcoholic solution of guaiacum resin turns blue even in absence of hydrogen peroxid, when a trace of laccase acts on it in presence of air. When the laccase is present in a larger quantity, the blue color will turn to green and then to yellow. It is well known that this blue reaction may be obtained also with various oxidizing agents, such as ferric chlorid, chlorine, nitrous acid, potassium ferrocyanid and

¹ Bull. de l'Institut Botanique de Buitenzorg No. VIII.

² Wochenschr. f. Brauerei, 1899, No. 40; Bot. Centr. Blt., 1901, No. 1, p. 8.

³ Brewer's Journal, Vol. XXV, No. 10, 11, 12, 1901.

⁴ Report, No. 68, U. S. Department of Agric., 1901.

⁵ Compt. Rend., 120, p. 166, (1895).

some salts of the heavy metals, also with quinone. *Schönbein* also mentioned that pure mercury¹ and the noble metals produce this blue reaction. Traces of alkali are not favorable for the development of the blue guaiac color by laccase; neutral or slightly acid solutions are best suited. Certain organic substances will also interfere with this reaction, as mentioned farther below.

For my tests I applied a frequently renewed guaiac tincture of 2%, kept in small flasks of colored glass, protected against direct sunlight.

2. Test with guaiac tincture and hydrogen peroxid.

The blue reaction which is obtained with guaiac tincture and hydrogen peroxid was observed long ago by *Thénard*, and *Blanche* and *Taddei*. *Schönbein* ascribed this reaction to all kinds of enzymes and for a long time it was also applied as a test for the common diastase. It was but recently that *Schönbein's* view was found incorrect by *Bertrand*. *Schönbein* further had entertained the view that the power of fresh animal and vegetable juices of decomposing hydrogen peroxid with development of oxygen, was a property of all enzymes, but also this conclusion was recently proved to be erroneous.

*Spitzer*² ascribed the decomposition of hydrogen peroxid to the same enzyme which produces a blue color with guaiac tincture and hydrogen peroxid, what did not agree with *Lepinois*³ observation that there is no proportional relation between the intensity of oxygen development and the intensity of color reactions such as with guaiac tincture, guaiacol etc. Finally, *Loew* proved that the property of catalysing hydrogen peroxid is due to a special enzyme, called by him, catalase,⁴ which frequently is present as an impurity in other enzyme preparations. In order to test for peroxidase, I prepared a solution of hydrogen peroxid by dissolving

¹ I have observed that this blue coloration did not increase upon addition of hydrogen peroxid, hence its production can not be due to a supposed formation of hydrogen peroxid by mercury.

² Pflüger's Archiv, Vol. 67, 1897.

³ Compt. Rend, May 26, 1899.

⁴ Report of U. S. Department of Agriculture, No. 68, 1901.

5 grms. of sodium peroxid in 200 c.c. of water containing a little more than the calculated amount of sulphuric acid, in order to provide for a faint acid reaction, indispensable for the preservation of the hydrogen peroxid. Tincture of guaiac was prepared by dissolving 2 grms. of good transparent guaiacum resin in 100 c.c. of absolute alcohol.

In testing for peroxidase with guaiac, the solution must be always neutral or of a slight acid reaction as already pointed out. Special precaution must be paid to have the solution of guaiac frequently renewed, since an old one will cause a blue reaction with hydrogen peroxid alone. Since much hydrogen peroxid will injure the oxidizing enzymes, care has to be taken to apply only very little.

3. *Test with guaiacol and hydrogen peroxid.*

The color reaction with guaiacol was first applied by *Bourquelot*.¹ Recently *Dupouy*² observed an oxidase in the saliva which produces a red color with guaiacol and hydrogen peroxid. Like him, I also made use of a 1% aqueous solution of guaiacol.³ In cases of mere traces of the corresponding enzym, a red coloration appears after 5-10 minutes; otherwise, at once.

4. *Test with Paraphenyldiamine and hydrogen peroxid.*

A color reaction of vegetable objects with paraphenyldiamine had first been observed by *Bourquelot*.⁴ That base was applied by *Storch*⁵ for a test upon fresh milk which yields a deep violet color on addition of a salt of that base and hydrogen peroxid, while boiled milk fails to give this reaction. The writer tested the behavior of this reagent with many

¹ C. R. Soc. biol. 46, p. 896. 1896.

² Jahresbericht f. Agric. Chem. 1899, II, p. 449.

³ This solution must be slightly acid. Other oxidizing enzymes as well as certain compounds (kresols) require a weak alkaline medium. Schinioxidase acts best in a neutral medium (Sartheu).

⁴ C. R. Soc. biol. 46, p. 496. 1896.

⁵ Jahresber. f. Thier-Chem. 28, p. 256.

vegetable objects and found that, when the reaction is successful, a green color is first produced, which turns to dark violet; in only a few cases it was other wise. In my tests I have always applied a 2% aqueous solution of hydrochlorid of paraphenyldiamine freshly mixed with an equal volume of a 0.8% of sodium acetate solution. The acetate gives the reaction better than the hydrochlorid (In absence of an oxidizing enzym the acetate produces itself but very slowly a reddish brown color. Control tests are required).

5. *Test with Tetramethylparaphenyldiamine.*

*Grüss*¹ applied at first paper which had been soaked in an alcoholic solution of tetramethylparaphenyldiamine. In his recent work² on yeast, the reagent was employed in the following manner: 'a granule of tetramethylparaphenyldiamine of the size of a pin head was dissolved in four c.c. of water. The solution was allowed to flow on to a piece of filter paper, 5 × 5 cm., so that the paper, which can be most conveniently placed in a Petri dish, is thoroughly saturated,' immediately before the objects were tested.³ In my case, the chlorid of tetramethylparaphenyldiamine was used instead of the free base, and the tests were carried on according to *Grüss's* method.⁴ I observed this color reaction with several vegetable juices.⁵ Upon addition of a few drops of a 0.1% solution of the said salt to an extract of malt, or the root of radish, a violet coloration set in immediately, but not in the control case.

¹ Journ. Chem. Soc. 1901. p. 33.

² Brewer's Journ. Vol. XXV. 1901. He called this paper tetra paper, and the paper moistened with solution of the chlorid of tetramethylparaphenyldiamine and soda, tetra soda paper.

³ He wrote in his latter article, that chlorid of tetramethylparaphenyldiamine gave an exceedingly strong reaction in the case of resting barley.

⁴ I did not use tetra soda paper.

⁵ Tetramethylparaphenyldiamine is easily colored violet by the air alone, so that a control test has to be carried on in every instance.

6. *Test with tetramethylparaphenyldiamine and hydrogen peroxid.*

This test had not been in use previously. During my search however, for spermase in seeds and other vegetable objects, I noticed that a section of potato failed to give a reaction with an alcoholic solution of tetramethylparaphenyldiamine alone, but a beautiful violet color upon addition of a drop of peroxid. This phenomenon led me to repeat this test with milk; and indeed I observed that fresh milk behaves exactly like the potato in this regard. This color sets in at once while in absence of the enzym the coloration appears but very slowly.

Boiled milk fails to give this test. Hence, I propose this reaction as a useful test for distinguishing fresh milk from boiled milk¹

Behavior of Various Objects.

The first series of tests were made with slices of vegetable objects and the second with the juices of plants.

¹ There is the so-called indophenol reaction for oxidases consisting in the production of a blue color with a mixture of α -naphthol with paraphenyldiamine and sodium carbonate. This reaction is however not very delicate and appears slowly also in absence of oxidases.

Slice of	a. Guaiac tincture alone.	b. Guaiac tincture + H ₂ O ₂ .	c. Guaiacol + H ₂ O ₂ .	d. Parapheny- lendiamine + H ₂ O ₂ .	e. Tetramethyl- paraphenyl- endiamine + H ₂ O ₂ .	Remarks.
Potato.	+	+	+	+	+	Each reaction was intense.
Root of Ipomaea Batatas.	+	+	+	+	+	(d) was weaker than the other reactions.
Root of Raphanus sativus, (radish.)	+	+	+	+	+	(a) gave the weakest reaction.
Root of Arctium Lappa.	+	+	+	+	+	(d) gave the weakest reaction.
Apple.	+	+	+	-	+	(c) was weakest.
Fruit of Diospyros Kaki.	+	+	+	-	-	(a) gave the weakest reaction.
Bamboo-shoot.	+	+	+	+	o	All reactions were intense.
Rhizome of Balanophora ¹ Sp.	+	+	+	+	+	(a) and (b) were stronger than the other reactions.

In these tables + means a positive, and -, a negative result and o, that no test was made. In the comparison of these tests, it must be kept in mind, however, that the acidity of the juices and the presence of certain compounds influence the intensity of the coloration.

¹ This is a phanerogamic parasite.

Longitudinal section of	a. Guaiac tincture alone.	b. Guaiac tincture + H ₂ O ₂ .	c. Guaiacol + H ₂ O ₂ .	d. Paraphenylenediamine + H ₂ O ₂ .	f. Tetramethylparaphenylenediamine alone.	Remarks.
Resting barley.	+	+	+	+	+	The first reaction weaker; all reactions appeared only in the embryo.
Resting wheat.	+	+	+	+	+	..
Resting rice.	+	+	+	+	+	..
Resting soy-bean.	-	+	+	+	+	..
Germinated barley.	+	+	+	+	+	The first reaction (a) was stronger and the fifth (f) weaker than the rest.
Germinated wheat.	+	+	+	+	+	..
Germinated rice.	+	+	+	+	+	..
Germinated soy-bean.	+	+	+	+	+	..
Aspergillus oryzae on boiled rice.	-	-	-	+	-	The violet color with (f) appeared along the rim, where the fungus developed.

The plumules of the germinated plants were about 1 cm. long and the tests obtained showed the distribution of the oxidizing enzymes to the whole extent of the sections.

The tests obtained leave no doubt that the oxidase which gives a blue reaction with guaiac tincture (laccase?) increases during germination while spermase decreases, which is in accordance with the observations of *Grüss*, that the reaction for spermase which at first increases, decreases later on, until it fails altogether.

The second series of tests were made with various vegetable juices. The objects were crushed in a mortar with addition of some quartz sand and extracted with some water. The filtrates behaved as follows :

Juice of	Reaction of Juice. ¹	a. Guaiac tincture alone.	b. Guaiac tincture + H ₂ O ₂ .	c. Guaiacol + H ₂ O ₂ .	d. paraphenylen-diamine, + H ₂ O ₂ .	e. Tetramethyl-paraphenylen-diamine, + H ₂ O ₂ .	Remarks.
Potato.	Very slightly acid.	+	+	+	+	+	With the base (e) alone a violet color appeared.
Root of Batata.	Almost neutral.	+	+	+	+	+	All reactions were intense.
Root of Radish.	Faintly acid.	+	+	+	+	+	No reaction with the base (e) alone.
Ripened fruit of Kaki.	"	+	+	+	+	+	"
Bamboo-shoots.	Almost neutral.	+	+	+	+	o	All reactions were intense.
Leaves of Radish.	Slightly acid.	+	+	+	+	+	In testing with (a), a few drops of guaiac tincture are not sufficient to produce a blue color.
Leaves of Barley.	"	+	+	+	+	+	"

Leaves of Tea.	"	+	+	+	+	+	+	With the alcoholic ² precipitate.
Leaves of Tobacco, (fresh.)	"	+	+	+	+	+	+	All reactions were intense.
Cured Japanese tobacco.	Almost neutral.	+	+	+	+	+	+	"
Cured tobacco from Italy.	"	-	-	-	o	o	o	Catalase ⁴ in moderate quantity.
Berry-yeast.	Faintly acid.	-	-	-	-	-	trace	Catalase gave intense reaction.
Koji. ³	"	-	trace	trace	-	-	trace	Catalase in moderate quantity.
Malt.	Almost neutral.	+	+	+	+	+	+	Slight reaction appeared with the base (c) alone.

¹ The reaction was tested with litmus paper.

² The fresh juice did not give any reaction except for catalase; since tannin interferes with all these reactions. Hence the alcoholic precipitate, dissolved in a little water, served for the tests.

³ The aqueous extract was precipitated with alcohol, and the precipitate, dissolved in a little water, served for these tests.

⁴ Catalase was present in all vegetable objects tested.

Also an animal secretion, saliva, was compared in regard to these tests with the following results. The samples came from different persons.¹

	a. guaiac tincture.	b. guaiac tincture. + H ₂ O ₂	c. guaiacol. + H ₂ O ₂	d. paraphenyldiamine. + H ₂ O ₂	e. tetramethyl- paraphenyldiamine + H ₂ O ₂
I.	—	trace.	+	trace.	trace.
II.	—	—	+	o	o
III.	—	—	+	o	o
IV.	trace.	—	+	—	o
V.	trace.	—	+	trace.	trace.

While the guaiacol reaction (c) was intense in every instance, the fourth (d) and the fifth reaction (e) appeared merely in traces. The guaiac reaction for oxidase and peroxidase was obtained only exceptionally in certain samples of saliva. An aqueous extract of cow's liver and horse kidney did not yield the guaiacol reaction, while extract of cow's pancreas gave it, although weaker than saliva. The pancreas also yielded a blue reaction with guaiac tincture and hydrogen peroxid, while liver and kidney in this case failed to produce it.

Exist there several peroxidases?

Although I was unable to find any vegetable object which would give the guaiacol reaction in absence of peroxidase, the behavior of saliva shows clearly that the guaiacol reaction is—at least in this case—not due to the common peroxidase, recognized by the blue reaction with guaiac resin and hydrogen peroxid. We have therefore to distinguish two

¹ Control tests were made with boiled saliva, and in no case a coloration sets in.

kinds of peroxidases, one that gives the red reaction with guaiacol and hydrogen peroxid, the other that gives a blue with guaiac resin and hydrogen peroxid. It seems further probable that there exists a third peroxidase¹ which gives a violet coloration with tetramethylparaphenylendiamine in the presence of hydrogen peroxid; the reactions mentioned in the above tables are in favor of this view. Though a certain parallelism between the intensities of these color reactions was observed in some cases, it was not recognized in others, hence the view that each reaction might be caused by a separate enzym, is justified. On the other hand, one might object that while there exists a separate peroxidase that gives the red guaiacol test in saliva, there is no proof that the common peroxidase does not give both reactions at the same time, since thus far no object was observed that would give the blue guaiac—H₂O₂ test without giving also the red guaiacol test.

In order to test this objection I have made the following comparisons as to the killing temperature of oxidizing enzymes.

The fact that the acidity of the plant juice, the degree of dilution, the duration of heating and the presence of certain salts have a modifying influence on the height of the temperature at which the change of an enzym to the inactive modification takes place, was pointed out by *Loew*² in his 'Physiological Studies of Connecticut Leaf Tobacco.' Since these influences have not always been paid careful attention to, the existing discrepancies between the data obtained can hardly surmise us. The following table shows the various data obtained thus far :

¹ It seems to me very probable that the reaction with paraphenylendiamine and hydrogen peroxide, and that with tetramethylparaphenylendiamine and hydrogen peroxid might be caused by the same enzym.

² Report of U. S. Department of Agric., No. 65. 1900. p. 21.

Name of the enzym.	Temperatur which kills the enzym.	Duration of Heating.	Remarks.	Name of the author.
An oxidase in Urushi.	63°C.	—	—	<i>Yoshida.</i>
An oxidase in Urushi (Laccase).	above 70°C.	—	—	<i>Bertrand.</i>
Tyrosinase.	below 70°C.	—	Injured at 50°C.	"
Oenoxidase.	72°C.	4 min.	—	<i>Martinand.</i>
"	55°C.	1½ hours.	—	"
Oxidase in the stalks of the sugar cane.	60°C.	—	—	<i>Raciborski.</i>
Oxidase in a tobacco leaf.	66--67°C.	3 min.	1 part dry leaf was extracted with 20 parts H ₂ O	<i>Loew.</i>
Oxidase in tea-leaves.	76--77°C.	5 min.	The solution was neutral.	The writer.
Peroxidase in the stalks of the sugar cane.	95°C.	—	—	<i>Raciborski.</i>
Peroxidase in tobacco leaf.	87°C.	3 min.	The reaction of the solution was neutral.	<i>Loew.</i>
Peroxidase in 33% alcohol solution.	70°C.	a few seconds.		"
Peroxidase in 10% (NH ₄) ₂ SO ₄	93°C.	after a short time.		"

Name of the enzym.	Temperature which kills the enzym.	Duration of Heating.	Remarks.	Name of the author.
Peroxidase in cocoa milk.	on boiling.	—	Still active.	<i>Hung. v.</i>
Salive oxidase which produces guaiacol reaction.	92°C.	—	Trace of the reaction.	<i>Dufour.</i>
β -catalase in a tobacco leaf.	72°C.	more than 15 min.	—	<i>Lev.</i>
„	74°C.	very soon.	—	„
„	75°C.	1 sec.	The greater part was injured.	„
α -catalase in a tobacco leaf.	75°C.	5 min.	Faint trace of the reaction.	„
„	80°C.	1 min.	—	„
An oxidase in milk which gives Storch's reaction.	80°C.	—	—	„

In order to decide whether the reactions caused by hydrogen peroxid with guaiac resin, guaiacol, paraphenylendiamine, and tetramethylparaphenylendiamine disappear at one and the same degree of temperature, the following tests were carried out.

Potato-, bambooshoot-, barley leaves-, and radish root juice were heated for a short time to 80°C or over with the following results:

(+) This forms a noticeable exception from the general rule that enzymes are killed below the boiling heat of water.

According to *Limossier* peroxidase of pus is not destroyed at 120° when dissolved in a weak acid medium. (*La semaine med.* vol. 18). A similar observation was made by *Szitzler* with peroxidase from animal organs (*Pflüg. Arch.* vol. 67, p. 615).

	Potato juice, weak acid.	Juice of bamboo-shoots, almost neutral.	Juice of young barley- leaves, weak acid.	Juice of radish root, very slightly acid reaction.				
Degree and duration of Heating.	80°C, 5 Min.	85°C, 5 Min.	80°C, 5 Min.	85°C, 5 Min.	80°C, 3 Min.	80°C, 5 Min.	82-83°C, 5 Min.	85°C, 3 Min.
Oxidase.	none.	slight trace.	none.	none.	slight trace.	slight trace.	none.	none.
Peroxidase.	trace.	strong.	moderate.	trace.	strong.	strong.	none.	none.
Green reaction, ¹	trace.	none.	none.	none.	strong.	strong.	none.	none.
Violet "	none.	—	none.	none.	strong.	strong.	none.	none.
Red "	moderate.	weak, but gradually increasing.	moderate.	moderate.	strong.	strong.	trace.	none.

¹ In order to abbreviate these reactions are named according to the color produced; thus the red reaction means the reaction with guaiacol and hydrogen peroxid; the green reaction that with paraphenylenediamine and hydrogen peroxid; the violet reaction that with tetramethylparaphenylenediamine and hydrogen peroxid.

The influence of concentration upon the killing temperature was observed with the enzymes of cured tobacco-leaves.¹ A leaf of cured tobacco (5 g.) was extracted with 100 c.c. water, and tested with the following results :

Degree and duration of heating.	80°C, 5 Min.	84°C, 5 Min.	85°C, 5 Min.	86—87°C, 5 Min.	88°C, 5 Min.
Oxidase.	trace.	none.	none.	none.	none.
Peroxidase.	strong.	weaker.	weaker.	weaker.	trace.
Green reaction.	strong.	weaker.	weaker.	weaker.	none.
Violet reaction.	strong.	weaker.	weaker.	weaker.	none.
Red reaction.	strong.	weaker.	weaker.	weaker.	trace.

A portion of that extract was diluted with an equal volume of water (a), and another with twice its volume of water (b).

Upon heating to 87°C for 2 minutes, the following result was obtained :

	Original solution.	(a)	(b)
Oxidase.	none.	none.	none.
Peroxidase.	trace.	none.	none.
Green reaction.	none.	none.	none.
Violet reaction.	none.	none.	none.
Red reaction.	strong.	moderate.	trace.

Though the killing temperature is not a constant magnitude under all conditions, nevertheless these comparisons show very clearly that the red reaction caused by guaiacol and hydrogen peroxid is due to a separate

¹ The reaction of the extract of the cured leaves was neutral.

enzym which has more resistance-power towards heat than the other related oxidizing enzymes. The enzymes which cause the green and the violet reactions have an intermediate resistance-power between that of oxidase and that of peroxidase. That there exist also quite a number of different oxidizing enzymes acting in absence of hydrogen peroxid, can be inferred from observations of *Bertrand* and of *Bourquelot*. The latter has observed among other things that when the extract of the fungus *Russula delicata* was kept with some chloroform, at first will be lost the oxidizing action upon tyrosin, later that upon guaiacol, and finally after eight weeks also that upon guaiac.¹

Influence of Foreign Substances upon the Color Reactions.

The investigations on the behavior of oxidizing enzymes towards foreign substances are very incomplete. The power of the oxidizing enzymes of an animal organ is not injured by certain poisons or by freezing. Only hydrocyanic acid and hydroxylamine hinder the action. 80% alcohol does not kill oxidizing enzymes, but a stronger one will injure them slowly. Acids and alkalies act injuriously. While chloroform may promote the action of certain oxidizing enzymes, a prolonged contact will act injuriously. Of some interest is the comparison of the behavior of catalase² with that of other oxidizing enzymes :

Salts of a strong acid or alkaline reaction injure this enzym while salts of a neutral reaction do not. A remarkable fact is the depression of the *activity* of the soluble or β -catalase by nitrates, while the *enzym* itself is not injured by them. In general, the retarding influence of a salt is increased with the amount of the salt. Sodium carbonate attacks β -catalase slowly ; 2% sodium fluorid and 5% dipotassium oxalate solutions cause no injury. 5% potassium sulphocyanid and thiourea interfere with the catalysing action, but have no direct injurious influence upon the enzym. Mercuric chlorid acts very injuriously. While highly dilute acids

¹ *Bourquelot* : J. B. f. Thierchem. 26. p. 886. Cf. also his observations on the potato (ibid).

² *Loew* : Catalase, an enzym of general occurrence. U. S. Dept. of Agric. Report No. 86.

retard the action of catalase, dilute alkaline solutions promote it. When the amount of a mineral acid in the solution reaches more than 0.5%, catalase is soon killed. At the ordinary temperature, 0.1% acetic acid does not injure catalase in one hour, but gradually at 55°C. 2% sulphuric acid destroys the power of catalase in fifteen minutes. Saturated baryta solution injures α -catalase slowly, and kills β -catalase in two days. While 0.1% caustic soda causes no injury, 1% of it kills catalase instantly. Alcohol above 30% has a retarding influence while more dilute alcohol or absolute alcohol does not injure catalase within twenty hours. Little chloroform and ether have no direct influence on the action of catalase: 1% phenol retards the action of the enzym; 5% formaldehyde destroys its action in a very short time; 0.4% nitrous acid injures the enzym considerably in one day. α -catalase shows considerable resistance to the action of hydrocyanic acid of 2%, while β -catalase is gradually killed by it. After the evaporation of hydrocyanic acid, regeneration of the action of β -catalase is only observed, when the amount of this acid has been very small. Hydrogen sulfid and also phenylhydrazine injure the insoluble or α -catalase but slowly at the ordinary temperature, also a 5% hydroxylamine solution does so. In view of the interest connected with the chemical behavior of enzymes I undertook a series of analogous experiments with the oxidizing enzymes mentioned above.

My observations were as follows.

1. *Influence of salts upon the color reactions mentioned.*

5 c.c. of a dilute juice¹ of radish root were mixed with various salts and left with some drops of ether for 48 hours before testing.

In carrying out the oxidase reaction 1 c.c. of guaiac tincture was added; for the green reaction, 1 drop of sodium acetate, 1 drop of paraphenyldiamine and 2 drops of hydrogen peroxid; for the red reaction, an equal volume of guaiacol solution and 2 drops of hydrogen peroxid; for the violet reaction, 2 drops of an alcoholic solution of 1% tetramethylpara-

¹ It is necessary to dilute the juice moderately to obtain the color reaction of a degree suitable for comparison.

phenyldiamine and 2 drops of hydrogen peroxid. In each case the intensity of the color produced was compared with that of the control solution without salts.

Salt.	Concentration.	Oxydase.	Red reaction.	Green reaction.	Violet reaction.
Sodium chlorid.	5%	weaker.	weaker.	weaker.	weaker.
Potassium nitrate.	5%	weaker.	weaker.	weaker.	strong.
Magnesium nitrate.	5%	slightly weaker.	strong.	strong.	strong.
Calcium nitrate.	5%	"	"	"	"
Magnesium sulphate.	5%	strong.	strong.	strong.	"
Ammonium sulphate.	5%	"	"	"	"
Sodium sulphate.	5%	"	"	"	"
Dipotassium phosphate.	5%	weaker.	weaker.	a violet color appeared instantly.	weaker.
Monopotassium phosphate.	5%	strong.	strong.	strong.	strong.
Sodium carbonate.	2%	weaker.	weaker.	turned quickly to violet.	slightly weaker.
Potassium oxalate.	1%	weaker.	weaker.	not green, but violet.	almost none.
Ammonium oxalate.	2%	strong.	strong.	strong.	weaker.
Sodium fluorid.	2%	none	moderately strong.	moderately strong.	moderately strong.
Sodium silicofluorid.	0.3%	none.	none.	none.	none.

It will be seen that sodium chlorid, potassium nitrate, calcium nitrate, magnesium nitrate and dipotassium phosphate in a concentration of 5% injure these enzymes, or weaken at least the color reactions caused by them; of special interest is here also the influence of potassium nitrate, since this depresses the action also of catalase, without injuring the enzym itself.¹ Sodium carbonate (2%) and potassium oxalate (1%) weaken also these color reactions. The influence of sodium chlorid (5%) and potassium nitrate (5%) was tested once more, this time after 24 hours, with a more concentrated juice of radish and also with milk, and a decisive depression observed, especially in the case of chlorid of sodium and in regard to the red reaction.

The striking influence of sodium fluorid and of sodium silicofluorid on the appearance of the color reactions led me, not only to repeat the tests with radish juice, but to make also some further tests: 3.5 grms. of air dry tobacco leaves were crushed in a mortar and extracted with 300 c.c. water.

The filtrate served for the following tests:

	Concentration.	Oxydase.	Peroxydase.	Red reaction.	Green reaction.	Violet reaction.	Time of testing.
Sodium fluorid.	2%	trace.	weaker.	weaker.	weaker.	weaker.	Immediately.
"	2%	none.	weaker.	weaker.	weaker.	weaker.	After about one hour.
"	5%	none.	"	"	"	"	Immediately.
Sodium silicofluorid.	2%	none.	weaker than in the case of NaF.	weaker than in the case of NaF.	weaker than in the case of NaF.	weaker than in the case of NaF.	"
"	2%	none.	"	"	"	"	After about one hour.
"	5%	none.	"	"	"	"	Immediately.

¹ I. c. *Leete*: Catalase, an enzym of general occurrence, U.S. Dept. of Agric., Report No. 68.

In the next test, 50 grms. of malt were crushed and extracted with 300 c.c. water and a similar effect observed with this filtrate.

	Oxydase.	Peroxydase.	Time of testing.
Sodium fluorid 5%	none.	weaker than control.	tested, immediately.
Sodium silicofluorid 0.3%	none.	slight.	

It follows therefore that sodium fluorid and silicofluorid have a injurious influence on oxidizing enzymes, especially on oxidase proper.¹ The sodium silicofluorid acts further more energetically than the fluorid.

2. *Influence of dilute acids and alkalis on the color reactions.*

The juice of radish root rendered slightly alkaline with caustic soda, yielded no blue reaction for oxidase or peroxidase. The greenish coloration produced appeared also in the absence of the enzymes. The red reaction set in, but slightly, while the green and violet reactions failed to appear. Upon acidifying these solutions, however, with acetic acid, the oxidase and peroxydase reactions as well as the other reactions mentioned appeared with great intensity. Moreover, when these solutions were made again weak alkaline with caustic soda, these colors disappeared again, and reappeared on adding some acetic acid.

100 grms. of a fresh radish root were crushed and extracted with 300 c.c. water. The filtrate served for the following tests, which always were made after neutralization of the juice.

¹ Other enzymes seem to have more resistance power toward sodium fluorid. Cf. *Arthur* and *Huber*, Jahresb. Thierchem. 1893, p. 640.

Acids or alkalis,	Concentration,	Time after mixing,	Oxydase,	Peroxydase,	Red reaction,	Green reaction,	Violet reaction,
Hydrochloric acid,	1%	5 hours.	none,	none,	trace,	trace,	trace,
Nitric acid,	1%	"	none,	none,	none,	none,	none,
Sulphuric acid,	1%	"	"	"	"	"	"
Acetic acid,	2%	"	"	"	"	"	"
Oxalic acid,	2%	"	"	"	"	"	"
Caustic soda,	1%	"	"	"	"	"	"
Tartaric acid,	2%	24 hours.	"	"	"	"	"

This was repeated with the water extract from a cured tobacco leaf with the difference that the tests were made 2—3 minutes after mixing :

Acids or alkalis,	Concentration,	Oxydase,	Peroxydase,	Red reaction,	Green reaction,	Violet reaction,
Hydrochloric acid,	2%	none,	trace,	trace,	none,	none,
"	1%	trace,	trace,	weaker,	trace,	weaker,
Tartaric acid,	2%	weaker,	strong,	strong,	strong,	strong,
Caustic soda,	2%	none,	none,	none,	none,	none,
"	1%	weaker,	weaker,	weaker,	weaker,	weaker,

3. *Influence of Poisons.*

Influence of hydrocyanic acid: Two leaves of cured tobacco (about 10 grms.) were extracted with 200 c.c. water to which 2% hydrocyanic acid was added. Hereby all the color reactions mentioned were prevented, but after removing the hydrocyanic acid by a current of air, they could be reproduced,¹ except in the case of oxidase.

Influence of hydrogen sulphid: Radish juice and an aqueous extract of cured tobacco were saturated with hydrogen sulphid. Upon replacing this substance by air immediately afterwards, all reactions were still obtained. But, when after standing for 48 hours the hydrogen sulphid was replaced by air, these reactions appeared very much weaker, and that upon oxidase not at all.

Behavior to phenylhydrazine: 10 grms. of cured tobacco leaves were extracted with 200 c.c. of water. To 100 c.c. of this extract, some hydrochlorid of phenylhydrazine and sodium acetate were added, whereby some yellowish flocculent precipitate was formed. After 24 hours, much absolute alcohol was added to precipitate the enzymes and separate them from phenylhydrazine, since this might have interfered with the color-reactions. The precipitate was collected in a filter, washed thoroughly with alcohol and dissolved in a little water. All color reactions failed in this case.

Do Sugars Prevent the Color Reactions?

It is a well known fact that tannin interferes with the guaiac blue reactions and also with the actions of myrosin and emulsin. Recently, *Hunger*² observed that a reducing sugar present in the milk of the cocoa-

¹ Cf. also *Epstein*, *Archiv. f. Hygiene*, vol. 36 p. 140. Prussic acid prevents the coloration of beet juice exposed to air, while after expulsion of that acid by a current of air this oxidative process sets in again.

² *Hunger* expressed in a recent article (*Ber. Deutsch. Bot. Ges.* vol. 19, 1901), the supposition that in my tests for oxidase and peroxidase in ripened and unripe kaki fruits, it was the sugar and not the tannin which interfered with the reaction. This is however not so.

nut prevents guaiac blue reactions of oxidase and peroxidase. Moreover he observed that the more intense the guaiac-blue reaction is obtained with the sugar cane, the less sugar is present.¹ I observed, however, that the guaiac reaction for oxidase is not interfered with by the addition of 10% glucose, fructose or cane sugar, but it requires a little increase of the guaiac tincture.

Just as little as these sugars, albumin and pepton prevent the guaiac reaction. In 100 c.c. of a dilute radish juice, 10 grams of egg albumin or pepton were dissolved and this mixture tested after 24 hours. The above-mentioned reactions were still obtained.

Do zymogens of oxidizing enzymes exist?

The "regeneration" of enzymes depends upon the presence of zymogen. The zymogens of the vegetable enzymes have been studied but little. It is already known that there exist zymogens of pepsin, trypsin, rennet and diastase in the animal body. As for vegetable enzymes, zymogens of a proteolytic enzyme in *Nepenthes* and lupin-seeds, of inulase in the resting tuber of artichoke, of lipase and rennet of the castor oil seed and of a diastasic enzyme in barley and wheat have been observed.² Thus far, however, zymogens of oxidizing enzymes have never been investigated, although they must exist, as the following observations will show.

1. Juice of barley was heated to 80°C. for 5 minutes, whereupon the oxidase reaction failed to appear, the oxidase having been changed. After standing for 24 hours, the oxidase reaction was again obtained. Another portion of the same juice was heated to 85°C. for 5 minutes, whereupon no oxidase reaction, but a trace of peroxidase and the red reaction was obtained. After 24 hours standing, a weak oxidase and a strong peroxidase reaction were again observed. The reaction of the barley

¹ *Hunger*, Het optreden der oxydasereactie in verband met de localisatie der glyucose in het suikerriet. *Archief voor de Java-Suikerindustrie*, 1901.

² Cf. *R. Green*: *The Soluble Ferments and Fermentation*, p. 389-391.

was very faintly acid. Similar observations were made by *Albert F. Woods*.¹

2. Prof. *Loew* has observed that the juice of bamboo shoots which on boiling lost every trace of active enzymes, showed again a reaction for peroxidase and the red guaiacol reaction after 24 hours standing. But when, after the boiling, 1% acetic acid was added, no "regeneration" was observed.

3. Juice of batata diluted with a moderate quantity of water and of perfectly neutral reaction was boiled for a minute, whereby all power of reactions disappeared. After 24 hours, the power of giving the reactions was regenerated. Prof. *Loew* observed that the oxidase reaction could thus be three times "regenerated."

4. A moderately diluted radish juice was boiled for a few minutes :

After 24 hours :no regeneration.

„ 48 „peroxidase, red and violet
reactions appeared slightly.

„ 4 days.....the same.

5. The same tests were carried on with milk which was boiled for a few minutes :

After 24 hours :no regeneration.

„ 48 „all original reactions
reappeared, but weaker.

„ 4 days.the same.

6. The water extract of cured tobacco leaves boiled for a few minutes was tested as follows :

After 24 hours : no regeneration.

„ 48 „ trace of peroxidase and red reaction
reappeared, the latter stronger than
the former.

¹ Also this author inferred the existence of corresponding zymogens. These facts as well as the observations of *Slatzoff* (*Z. physiol. Chem.* 31.) and *Epstein* (*Arch. Hyg.* 30.) seem to fully establish the enzym nature of the oxidases. Recently, however, *Kastle* and *Loewenhart* (*Amer. Chem. Journ.* 24) have tried to prove them to be organic peroxides and supported their view by showing that these also are very sensitive towards such poisons as kill enz

After 3 days: Peroxidase and red reactions reappeared weak.

„ 5 days: The two reactions mentioned appeared; also a faint trace of oxidase and the violet reaction.

7. For the “regeneration” of catalase, the following experiment was made: 100 c.c. of beer yeast paste was heated to 85°C. to kill the catalase. After 3 days standing in presence of some ether the result upon addition of hydrogen peroxid was as follows:

	Oxygen developed.	
	After 5 min.	After 30 min.
Control.....	29.1 c.c.	30.9 c.c.
Heated	4 c.c.	4.8 c.c.

“Regeneration” of Catalase seems to have taken place in a small degree but further experiments are necessary to draw a safe conclusion.

In general, the existence of zymogens of oxidizing enzymes appears to be highly probable. The duration of the heating will of course much influence the result, since zymogens also will gradually thus be destroyed.

Separation of the Oxydizing Enzymes from each other.

Since the nature of the oxidizing enzymes is not quite fully established, isolation meets with some difficulty. Prof. *Loew* has already shown that oxidase and peroxidase are not nucleoproteids, but behave like albumoses. All oxidizing enzymes may be precipitated with alcohol, hence the fractional precipitation with alcohol might be of some value, as the following experiments will show:

One volume of the juice of radish root was mixed with two volumes of absolute alcohol, whereby a white precipitate was produced. With the filtrate all reactions were obtained with the exception of that of oxidase. The precipitate, however, dissolved in a little water, yielded an intense oxidase reaction, but the other four reactions above-mentioned also were obtained.

Upon addition of three volumes of absolute alcohol to the radish juice, the filtrate failed to show the reactions, while the precipitate yielded all the reactions very strong.

Therefore, a separation of the peroxidase from oxidase is possible only in the first case mentioned. A similar observation was made by *Behrens* with the juice of tobacco-leaves. Upon mixing with double the volume of strong alcohol a filtrate was obtained, which gave only the peroxidase reaction.

All oxidizing enzymes tested are precipitated by saturation with ammonium sulphate. The juice of radish was saturated with ammonium sulphate. The filtrate did not yield any color reactions while the residue contained the substances which produce all color reactions caused by the oxidizing enzymes. On addition of 1% acetic acid to the radish juice, a small quantity of a white precipitate was obtained, but all reactions could be obtained with the filtrate. Hence the substances which cause such color reactions belong in all probability to kinds of albumoses and not to the nucleoproteids.

Another method of separating peroxidase from oxidase may be based on the behavior towards sodium fluorid and sodium silicofluorid, but much caution is here necessary, since after the early destruction of oxidase, the peroxidase is also gradually attacked by sodium fluorid and sodium silicofluorid.¹

General Conclusions.

1. Various vegetable objects which yield the well known guaiac reactions for oxidase and peroxidase, also yield a red reaction with guaiacol and hydrogen peroxid.
2. *Storch's* reaction on milk with paraphenylendiamine and hydrogen peroxid is also obtained with many vegetable objects. Generally a green color appears first, but in certain cases it changes soon to violet, while in most cases, this change is very slow.

¹ I used 5% solution of sodium fluorid and a saturated solution of sodium silicofluorid and tested after well shaking the mixed solution.

3. A new reaction for an oxidizing enzyme was found, which consists in the production of a deep violet color on the addition of tetramethylparaphenyldiamine and hydrogen peroxid. This reaction is obtained with various vegetable objects.
4. This new reaction may be used to distinguish fresh milk from Loiled one.
5. The spermase reaction found by *Grüss* is also obtained with several seeds, resting as well as germinated ones. In the case of resting seeds, only the embryo yields this reaction.
6. The red guaiacol reaction is caused by a separate enzyme which is more stable than even the peroxidase.¹
7. Sodium fluorid and sodium silicofluorid interfere with all the color-reactions. Oxidase is killed sooner than the other oxidizing enzymes.
8. The green and violet reactions might be caused by enzymes different from oxidase and peroxidase, since their killing temperatures lies between that of oxidase and peroxidase. This influence becomes also probable by the comparison of the resistance power to injurious compounds.
9. Sugars do not interfere with the color reactions caused by oxidizing enzymes in any notable degree. Neither interfere soluble egg albumin and pepton. However tannin interferes very seriously.
10. The presence of zymogens of the oxidizing enzymes is very probable.
11. The separation of peroxidase from oxidase may be accomplished by adding two volumes of absolute alcohol to one volume of a plant juice. Hereby oxidase is precipitated while the main part of the other oxidizing enzymes is present in the filtrate.
12. The oxidizing enzymes which produce these color reactions resemble albumoses.

¹ There seems to exist a related oxidase which produces the same color with tetramethylparaphenyldiamine as *Bombycidol* has observed with *Nasutin*.



On the Curing of the Kaki Fruit.

BY

S. Sawamura.

The fruit of *Diospyros Kaki* L. is an article of food extensively consumed in Japan. The flesh of this fruit contains glyucose and fructose, while the reserve carbohydrate in the seeds is mannan.¹ The fruit was found to consist of:²

	Sweet variety.	Astringent variety.
Water	82.03	83.65
Nitrogenous matters	0.61	0.58
Fat.....	0.02	0.02
Sugar and other N free extract ..	13.62	12.56
Fiber and kernels	3.29	2.76
Ashes	0.43	0.43

There exist sweet and astringent varieties of this fruit. In the unripe state both contain much tannin, but only with the former variety this tannin, and consequently the unpleasant taste, disappears in the ripening process.³ This change is brought on by oxidizing enzymes which act on the tannin as *Aso*⁴ has shown. As to the astringent variety artificial means are resorted to, to remove the unpleasant taste. These means are:

1. Keeping the fruits for 12 hours in a barrel containing vapors of

¹ *Ishii*. These Bulletins, Vol. II, No. 2, 1896.

² According to the analyses of the Tokyo Sanitary Exp. Station.

³ *Gerber* found that when this fruit is allowed to ripen in a confined atmosphere, it yields 10% of ethyl alcohol, mixed with other alcohols, among which amyl alcohol was copious, and further that the aromatic principle was a mixture of amyl and ethyl acetates with traces of oenanthyates and pelargonates. (*Green: Fermentation* p. 351.)

⁴ *Botanical Magazine*, Tokyo, 1900.

alcohol. Generally *Sake*-barrels just emptied are used for this purpose.

II. Keeping the fruits for 12 hours in warm water.¹

III. Subjecting the peeled fruits to a dessicating process in the sun.

It will be seen at once that the methods intend to kill the cells. Hence there can be no doubt that the disappearance of the tannin taste can not be due to the action of the protoplasm. I have kept fruits for control in a flask containing vapors of chloroform, and also in this case the tannin taste, which had disguised the sweetness of the fruits, disappeared. My quantitative tests further showed that the change did not consist in the transformation of tannin into sugar.² There remains, therefore, only one conclusion in regard to the effect of the curing process, and this is that the tannin is changed to a tasteless substance by partial oxydation brought on by the oxidizing enzymes present in the fruits. These enzymes are confined to the cytoplasm, while the tannin to the vacuole. By killing the cells the osmotic properties of the cytoplasm are changed, and the oxidases can now pass into the vacuole, where they can mix with the cell sap and exert their action upon the tannin.

¹ 36°-40° C. are sufficient to effect the change.

² For this purpose a ripe fruit, which had still an astringent taste, was divided into two equal parts, one of which was cured by exposing to vapors of chloroform, while the other left without any treatment. In the former 55.91% of sugar but no tannin was found, while in the latter 56.18% of sugar and 8.23% of tannin were contained.

On the Different Forms of Lime in Plants.

BY

K. Aso.

In the quantitative determination of mineral constituents of plants no attention was thus far paid to the different forms in which these compounds occur in the plants. The usual way to incinerate the plants before the analysis of the mineral constituents was carried on, did not permit any distinction. But, since the occurrence of different forms may be of considerable interest, I made some determinations of lime and magnesia in this direction. Lime may occur in plants 1) as salts easily soluble in water, 2) as salts difficult soluble in water, but easily soluble in dilute acetic acid and further 3) as salts insoluble in water and dilute acetic acid, but soluble in hydrochloric acid. In this last mentioned case, only calcium oxalate comes into consideration. Finally 4) compounds of lime with organized matter may occur, from which the lime also can be extracted by dilute acetic acid but not by boiling water.

Since the juices of plants have generally a more or less acid reaction and since I applied a relatively very large amount of water in the first extraction, the second form of lime salts will probably be almost entirely removed by the first treatment with a relatively very large quantity of boiling water.

The plants serving for my investigations were collected in the morning while poor in starch and kept in darkness for a few days until the iodine test showed that the last portion of the starch was consumed. This was done to reach comparable results, since the amount of starch varies so greatly in different periods of the day that the results of the analysis would be too much influenced by it.

As objects were selected :

1. Potato. (Leaves and stems, collected before flowering, on Nov. 9. 1900).
2. Buckwheat. (Leaves and stems, collected after ripening, on Nov. 8. 1900).
3. Wild clover. (Leaves and stems, collected before flowering, on Apr. 26. 1901).
4. Barley. (Leaves and stems, collected before flowering, on Apr. 26. 1901).

20 grams of air-dried finely powdered substance were extracted twice with one liter of boiling water,¹ and the residue thoroughly washed with hot water until every trace of soluble lime was removed. This residue was extracted with one liter of 5% acetic acid in the cold for 24 hours, frequently shaking the mixture, filtering and washing the residue with distilled water until the filtrate had lost every trace of acid reaction. The residue thus obtained, was treated with one liter of 5% hydrochloric acid for 24 hours with frequent stirring.² In each of these extracts the lime and magnesia content was determined separately.

The results obtained were as follows :

Objects.	In 100 parts of dry matter,			Total.
	CaO, soluble in :			
	Water	Acetic acid	Hydrochloric acid.	
Potato	0.332	0.875	1.586	2.793
Buckwheat	0.056	0.367	1.524	1.947 ³
Clover	0.858	0.742	0.489	2.089
Barley	0.438	0.259	trace	0.697

¹ The aqueous solution had a slight acid reaction in each case.

² In the final residue, lime and magnesia were absent or present only in very minute traces.

³ The lime factor $\frac{\text{CaO}}{\text{MgO}}$ for buckwheat before flowering is 3, while in the time of fruiting it is 1.3, which shows that magnesia plays also here a very important rôle in the fruiting process.

Objects.	In 100 parts of dry matter.			Total.
	MgO, soluble in :			
	Water	Acetic acid	Hydrochloric acid.	
Potato	1.617 ¹	0.550	0.217	2.384
Buckwheat	1.050 ¹	0.417	trace	1.467
Clover	0.491	0.162	trace	0.653
Barley	0.307	0.094	trace	0.401

We see from this table, that the quantities of the different forms of lime vary considerably. In the case of potato and buckwheat, only a small quantity of lime compounds soluble in water are present; more lime is found in the acetic extract and still more in the hydrochloric acid extract. Much calcium oxalate is produced in these leaves, while those of barley² contain only a trace of it.

As to the magnesium compounds, they are either soluble in water or in acetic acid. Only a trace of magnesia remained in the residue.

Church's investigation³ with albino leaves have demonstrated that lime is more abundant in the green leaves than in the white. In order to see whether this pathogenic albinism shows a chemical analogy to the normal albinism, I have determined the lime also in the white and green parts of the leaves of *Arundo Donax* separately.⁴

¹ Probably present as secondary phosphate, in which form it is not poisonous for the nuclei.

I have made also analogous determinations of lime in maize-stalks with the following result :

In 100 parts of dry matter; soluble in

CaO	Water, 0.130	Acetic acid, 0.128	Hydrochloric acid, trace
MgO	0.241	0.120	trace

But, as these stalks had been dried and were exposed to the rain on the field after harvesting, the result is not a normal one.

² Calcium oxalate is absent in most *Gramineæ*.

³ Journ. Chem. Soc. 1878 and 1886.

⁴ This separation by scissors was made as carefully as possible, nevertheless it was not absolutely complete.

The analysis was carried out in the way above mentioned.

In 100 parts of dry matter, total ash :

White parts 11.83

Green parts 14.47

	CaO soluble in			Total.
	Water	Acetic acid	Hydrochloric acid.	
White parts	0.213	0.216	trace	0.429
Green parts	0.313	0.226	trace	0.539

	MgO soluble in			Total.
	Water	Acetic acid	Hydrochloric acid.	
White parts	0.387	0.068	—	0.455
Green parts	0.444	0.069	—	0.513

White parts.
Green parts.
 Lime-factor : 0.9 1.1

From this table, it is clearly seen that the total ash and lime-content of the green parts exceed those of the white parts, and also that the lime-factor $\frac{\text{CaO}}{\text{MgO}}$ in the green parts is larger than 1, while that in the white smaller than 1. Hence the inference that the amount of lime increases with that of the chlorophyll bodies, other things being equal, has again found confirmation.

On the Alcohol Production in Phænogams.

BY

T. Takahashi.

Since the interesting discovery of *E. Buchner* that the expressed juice of yeast can cause the alcoholic fermentation of glyucose and fructose, the question has been raised, whether zymase is also produced in phænogams, and whether the alcohol production in the process of intramolecular respiration of cells of phænogams is due to zymase or to the action of the protoplasm itself. Former observations made by *Brefeld*¹ were not in favor of the assumption of zymase. He had observed that the expressed *juice* of grapes the surface of which had previously been sterilized, did not show any alcoholic fermentation while the sterilised *intact grapes* themselves formed some alcohol by intramolecular respiration, like many fruits rich in sugar do.

Recently *Godlewski* published an exhaustive investigation on the intramolecular respiration of the pea.² He stated the interesting fact that peas and other seeds kept under water can form considerable quantities of alcohol.³ In order to decide whether the living protoplasm itself or zymase causes this alcohol formation, *Godlewski* ground the peas to a fine powder, whereby the protoplasm would be killed, but zymase can remain intact. In this condition hardly a trace of carbonic acid (and consequently also no alcohol) was formed during two days,⁴ while the same weight of

¹ Landw. Jahrb. 5. (1876).

² Bulletin de l'Académie des Sciences de Cracovie, 1901.

³ The pea seeds seem to produce more alcohol than many other seeds. *Godlewski* mentions: „Die Menge des Alkohols, welche bei der intramolekularen Athmung der in reinem Wasser liegenden Erbsensamen sich bildet, kann bis zu 22% der ursprünglichen Trockensubstanz der Samen erreichen.“

⁴ Later on bacterial action was noticed.

entire seeds produced 5 cc. carbon dioxid in 24 hours. But although this result apparently proves the absence of zymase, *Godlewski* hesitated to draw this inference, and declares among other things: „Es wäre möglich, dass durch das Zerreiben der Pflanzenmasse irgend welche Substanzen aus gewissen Zellen frei gemacht werden, welche, sei es durch Niederschlagen der Zymase, sei es auf andere Weise, die Wirkung derselben aufheben.“

I have made the following experiments in regard to the intramolecular respiration of the pea.

One hundred seeds, weighing 33,3795 g. in the air dry state were left for one hour in a 1 per mille solution of mercuric chlorid in order to destroy all adhering germs, then washed with sterilized water and transferred to a sterilized Erlenmeyer flask nearly filled with sterilized water and connected with a small flask containing baryta water. The room in which this flask was now observed for 38 days showed mostly on average a temperature of 16°, sometimes however only 9°. Four days after the start of the experiment little bubbles were observed rising from the peas and this slow development was continuous with the exception of those days, on which the temperature had sunk to 9°. During the 38 days of observation, the water above the peas had remained perfectly clear, proving that the original sterilization was perfect. A careful microscopical examination of the surface of the peas at the close of the experiment also proved the absence of mucor, yeast and bacteria.

A determination of alcohol by distillation yielded 2 grams, calculated from the spec. grav. of the distillate at 17,5°=0,99646. The iodoform test proved further that the alcohol was indeed ethyl alcohol. This result fully confirms *Godlewski's* observation. The quantities of alcohol, however, were larger in *Godlewski's* experiment, what can be accounted for by the higher temperature (17,4-24,7°) in the latter case.

A further test proved that a number of the peas from the flask had *still retained their germinating power*. The water, further, in which the peas had remained and from which the alcohol had been distilled off served for the determination of the solid matter it had extracted from the peas. The residue weighed 1,146 g.=4,01% of the dry matter of the

peas.¹ Of this nearly one fourth consisted of mineral matter.

In order to decide whether zymase was the cause of the alcohol² production, the skin of the peas was removed and kernel and skins separately placed in a sterilized 10% solution of glucose and kept at 31°C. Even the smallest quantities of zymase would thus have been betrayed by some development of carbon dioxide, but *not a single bubble was noticed even after one day*. I must infer, therefore, that *zymase is absent and cannot be the cause of the alcohol production in the intramolecular respiration of the pea*.³ Protoplasm itself is the producer, but it works very much slower than does zymase, and at a temperature of 31°C. seems to stop that action.

A few words may here be permitted in regard to the relation between the intramolecular and the normal respiration. *Gottewski* arrives at the view: „Die intramolekulare Athmung im Sinne der alkoholischen Gärung bildet unter normalen Bedingungen aller Wahrscheinlichkeit nach das erste Stadium der normalen Athmung in allen denjenigen Fällen, wo sich dieselbe auf Kosten der hydrolysierten Kohlenhydrate vollzieht.“ This view seems to me not justified. In the first place we would have to assume two different ways of respiration one for fat, another for sugar; the fat would be capable to be burnt up directly, the sugar not directly but only after transformation into alcohol. This would contradict all our conceptions in regard to the chemical character of fat and sugar. The latter is much easier oxidized than the fat, why should the protoplasm not be capable to oxidise sugar directly, but the fat? To transform the sugar into alcohol before the combustion takes place would not only be entirely superfluous but would render the respiration more difficult; it is, e.g., well known that sugar is oxidized much quicker in the animal organism than alcohol is. In the second place it would follow from *Gottewski's* view, that those seeds that can produce more alcohol by intramolecular

¹ The fresh peas contained 28.55% of dry matter.

² It may be mentioned here that *Marc* has found some alcohol in pea sections that had germinated under normal conditions at 24°C. for 48 hours.

The view of *Eiffrent* that zymase exists in the pea (quoted by *Green*, *Fermentation*) finds no support by my experiments.

respiration should also show a more energetic normal respiration, the pea should excell therefore many other seeds, what is not the case as far as our knowledge goes. There may be other products formed in other seeds from sugar, when they are forced to intramolecular respiration, as fat, lactic acid, etc.—

There exists certainly a connection between the normal and the intramolecular respiration but this relation is different from that entertained generally. Doubtless a high degree of chemical energy exists in the living protoplasm. When this is transferred upon the imbedded molecules of sugar, a certain lability is produced in them which leads to direct combustion when free oxygen is present, but to various other decompositions when free oxygen is absent. This is in a few words the theory of *O. Loew*.¹

¹ Cf. *O. Loew*: Die chemische Energie der lebenden Zellen (Chapter XII), München 1899.

Can Alcohols of the Methane Series be Utilized as Nutrients by the Green Plants?

BY

S. Sawa.

While alcohols in moderate concentration act poisonously on the higher plants, as *Tsukamoto* has shown,¹ it seemed to me probable that in proper dilution various alcohols might be a nutrient for them. It is known that methyl and ethyl alcohol occur in small quantities in certain green plants. Methyl alcohol was observed by *Gutzeit* as well as by *Maquenne* in the distillate obtained from juices of *Evonymus*, *Hedera*, *Lolium*, *Urtica*, *Galium*, *Helianthus*, *Syringa*, *Dahlia*, *Acorus*, *Heracleum*, and *Pastinaca*.

Ethyl alcohol has been found in *Heracleum*, *Pastinaca* and *Anthriscus*. *P. Mazé* has observed that ethyl alcohol is a normal product of vegetation in the germination of seeds. He found alcohol, for instance, in germinated peas kept for 48 hours at 24° C.² He thinks that this alcohol was formed from glucose by a kind of fermentation process in the cells. It has been known long ago further that alcohol may be formed in the interior of sweet fruits. It has also been shown by *Bokorny* that plant-cells can form starch from methylic alcohol under the influence of light.³ This fact renders it probable that the starting point for the preparation of starch in the leaves is the formic aldehyde, the next

¹ Journal of the College of Science Tokyo, 1895.

² Compare also *Gollwieski*, Jahresber. f. Tierchemie 1897, p. 700.

³ In darkness this process does not take place.

oxidation-product of methyl alcohol.¹ It seemed to me of interest to compare, therefore, the action of methyl alcohol on the growth of the plant with that of some other higher alcohols. For the following experiments served young onion plants grown from the seed which were kept at first in 0.1% solutions of methyl, ethyl, butyl, and isobutyl alcohols in order to observe, whether there were any poisonous actions in that dilution, and since after 10 days no injurious action was observed, they were now placed in the following nutrient solution which was obtained by mixing 10% solutions of the nutrients in the following proportions :

45 cc. Calcium nitrate,
15 ,, Magnesium sulphate,
24 ,, Potassium nitrate,
6 ,, Mono-potassium phosphate,
trace of ferrous sulphate.

5 cc. of this mixture were added to 100 cc. of the 0.1% solutions of the alcohols on the 26th of March. The length of the leaves was measured at the same time and the solutions renewed as often as a turbidity due to the development of yeast and bacteria was observed. There was soon a considerable difference, as seen from the following table containing the results. The experiment lasted for 29 days. The letter "o" in the table signifies the leaves present at the starting of the experiment while the letter "n" signifies the new leaves formed. The temperature of the room varied between 12-22°C. The flasks containing the plants were placed on a table well exposed to the diffused day-light, but not to the direct sun-light.² Some of the leaves dried off gradually at the tips and these dried-off parts were not considered in the measurement.

¹ *Kinoshita* has further found that methylic alcohol can be used by green plants for the formation protein (Bul. College of Agriculture, Tokyo 1895 vol. II, No. 4.) *Loew* had observed before, that it can be used by bacteria as food, even in absence of other organic material.

² Perhaps the nutrient effect of assimilation under very bright light would have obscured the nutritive effects of methyl alcohol.

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Alcohol,	Length on March 16th.		Length on March 26th.		Increase in absence of mineral nutrients.		
	Individual leaves cm.	Summed up.	Individual leaves cm.	Summed up	Absolute cm.	Relative %	
Methyl alcohol	A	o 21.5	} 32.7	o 23.0	} 51.0	18.3	55.9
		o' 11.2		o' 18.0			
		n 10.0					
	B	o 19.5	} 38.7	o 26.5	} 53.3	14.6	37.7
o' 19.2	o' 14.3						
n 12.5							
Ethyl alcohol	A	o 21.7 (1)	} 43.2	o 7.0	} 47.7	4.5	10.4
		o' 21.5		o' 28.2			
		n 12.5					
	B	o 24.8	} 50.2	o 26.0	} 55.0	4.8	0.8
		o' 13.8 (2)		o' dead			
		o'' 11.6		o'' 29.0			
Butyl alcohol	A	o 16.5	} 30.0	o 24.0	} 35.0	5.0	16.7
		o' 13.5		o' 11.0			
		n 10.5					
	B	o 18.2	} 28.4	o 22.2	} 35.4	7.0	24.6
o' 10.2	o' 13.2						
n 11.0							
Isobutyl alcohol	A	o 20.1	} 30.0	o 21.3	} 41.3	11.3	37.7
		o' 9.9 (3)		o' dead			
		n 20.0					
	B	o 18.2	} 35.4	o 24.5	} 42.5	7.1	20.1
o' 17.2	o' 18.0						
n 10.2							
Control	A	o 14.9 (4)	} 28.4	o dead	} 38.8	10.4	36.6
		o' 13.5		o' 26.8			
		n 12.0					
	B	o 20.5	} 31.7	o 24.5	} 40.5	8.8	27.7
o' 11.2	o' 16.0						
n 10.0							

Alcohols.	Length on April 24th.		Increase after mineral nutrients were given.		Remarks.
	Individual leaves cm.	Summed up	Absolute cm.	Relative %	
Methyl alcohol	o dead	} 121.7	70.7	138.6	(5) A bud was formed on April 17th.
	o' 30.2				
n 29.0					
n' 25.0					
n'' 20.5					
n''' 11.5 (5)					
	n'''' 5.5				
	o dead	} 124.0	70.7	133.6	
	o' 26.0				
	n 44.5				
	n' 32.0				
	n'' 21.5				
Ethyl alcohol	o 25.5	} 104.5	56.8	119.1	(1) 1.7 cm on the tip had dried off.
	o' 45.0				
	n 34.0				
	o 20.0	} 93.0	38.0	69.1	(2) 3.0 cm on the tip had dried off. (6) A bud was formed on April 18th.
	o' dead				
	o'' 37.5				
	n 35.6 (6)				
Butyl alcohol	o dead	} 63.2	28.2	80.6	
	o' 23.5				
	n 29.7				
	n' 16.0				
	o dead	} 52.6	17.2	48.6	
	o' 24.0				
	n 28.6				
Isobutyl alcohol	o 21.5	} 80.7	30.4	95.4	(3) 5.0 cm on the tip had withered.
	o' dead				
	n 35.7				
	n' 23.5				
	o dead	} 60.5	18.0	42.6	
	o' dead				
	n 33.0				
	n' 27.5				
Control	o dead	} 70.2	31.4	80.9	(4) 3.3 cm on the tip had died off. (7) A bud was formed on April 20th.
	o' 29.0				
	n 36.2				
	n' 5.0 (7)				
	o dead	} 76.5	36.0	88.9	(8) A bud was formed on April 19th.
	o' 31.5				
	n 37.0				
	n' 8.0 (8)				

The result is therefore that methyl alcohol in 0.1% solution has acted as a nutrient and this was even plainly visible during the first period of the 10 days when no mineral nutrients were added so that the mineral nutrients previously absorbed had come alone into play. Ethyl alcohol had less nutritive value while butyl and isobutyl alcohols had a retarding effect on the growth.¹

¹ The higher alcohols are also more poisonous for plants than the lower as *Tsubamoto* had already observed.



On the Occurrence of Mannan.

BY

C. Kimoto.

The peculiar horn-like consistency of the seeds of *Trachycarpus excelsa*, a palm tree, frequently serving in Japan as an ornamental tree, led me to the supposition that it might be rich in mannan, especially since the test with iodine showed the absence of starch. Although small quantities of mannan have been observed in many seeds, there exist on the other hand not many cases in which it forms the only or the principal reserve carbohydrate in seeds. Instances of this kind are the nuts of *Phytelephas* and the seeds of *Diospyros Kaki*.¹

Recently *Bourquelot* and *H. Hérissey* observed a considerable amount of mannan in the seeds of *Phoenix Canariensis*. Of considerable interest is further the observation of *Tsujii* that the root of *Conophallus Conyaku* (*Amorphophallus Rivieri*), used in Japan as an article of food, is exceedingly rich in mannan.²

Tsukamoto has shown further that this plant contains also mannan in the leaves and stalk.³ Of special interest is the fact discovered by *Gabriel Bertrand*⁴ that while xylan is present in the wood of *Angiosperms* it is replaced in the *Gymnosperms* by mannocellulose. The wood of *Abies pectinata* yielded 9.6% mannose, on being boiled five hours with hydrochloric acid of 5%. *Gnetaceæ* which form the transition between the *Gymnosperms* and the *Angiosperms* gave thus no or very little mannose.

In order to test the seeds of *Trachycarpus excelsa* for mannan, the seeds were finely cut up after removing the shells, and 2.07 g. (\pm 1.49 g.

¹ *Ishii*. Bul. vol. II, No. 2 of Imp. College of Agriculture, University of Tokyo.

² *Ibid* page 103 and *Kimoshita*, *Ibid* No. 4.

³ *Ibid*, vol. III.

⁴ *C. r.*, vol. 129, page 1023.

dry matter) boiled for three hours with sulphuric acid of 3%, replacing the water lost by evaporation. The filtrate was neutralized with barium carbonate, the liquid evaporated to moderate concentration and acetate of phenylhydrazine added, which produced a voluminous crystalline precipitate. After standing one day it was collected on a filter and recrystallised; it melted at 190°C. and weighed 0.822 g., corresponding to 0.528 g. mannose and or 0.475 g. mannan, and to 31,36% of the dry seed.

The observation of *Bertrand* mentioned above on the occurrence of mannan in coniferous trees led me to look for mannan also in the wood of *Cryptomeria*. I boiled small chips of the wood (dry matter=91g.) with dilute sulphuric acid of 3% for three hours, and proceeded as above, whereby I obtained indeed a hydrazon which had all the properties of mannosephenylhydrazon. From the quantity obtained—10.2 g.—it follows that the wood of *Cryptomeria* contains 6,35% mannan.

The seeds of *Rhodea Japonica* were also examined for mannan, since starch is absent in them. After removing the shells and pulverising the seeds, the treatment was essentially the same as above mentioned.

The analytical data are as follows :

Original substance	11.2444 g.
Dry matter	8.7141 g.
Mannose phenylhydrazon	2.1252 g.

corresponding to 1.3645 g. mannose.

Hence this result shows that the dry matter of the seed of *Rhodea Japonica* contains 14.28 % mannan.

While I was occupied with the investigation of the reserve carbohydrates in the seeds of *Aucuba Japonica*, I noticed in a recent number of *Comptes Rendus de l'Académie des Sciences*, November 25, 1901., that *Mr. Champenois* had made an investigation on the same object and found in these seeds galactan, mannan and pentosan. A similar investigation was published in the first December number by *G. Dubat*, who found mannan also in the seeds of some *Liliaceae*.

On the General Occurrence of *Bacillus* *Methylicus* in the Soil.

BY

T. Katayama.

A very important function of bacteria in the soil is the oxidation of organic matter, whereby carbonic acid is produced which may partly be absorbed by the root and carried to the leaves for assimilation, and partly serve to dissolve carbonates and phosphates of lime and magnesia, and thus facilitate the absorption of certain mineral nutrients by the roots.

Many kinds of bacteria have thus far been observed to occur in soils, but one kind of bacterium which is present in the air of Japan as well as in Europe has not yet been looked for in soils, it is *Bacillus methylicus* which is obligate aerobic.¹

This microbe has the characteristic faculty to assimilate salts of formic acid and certain related compounds, as oxymethyl-sulfonate and methyl-sulfate of sodium and methyl alcohol, all containing only one atom of carbon in the molecule.

The isolation, therefore, of this bacillus is rather simple, since the other microbes thus far known cannot subsist on formates.

Bacillus methylicus occurs frequently in putrefying liquids, as can easily be ascertained by infecting from such liquids, a culture solution containing $\frac{1}{2}\%$ of sodium formate as the only organic nutrient. *Bacillus methylicus*² alone will thus develop, forming reddish films. Since it appeared of some interest, to ascertain whether this exquisite aerobic

¹ This microbe was first observed by O. Loew, Central Blatt, f. Bakteriologie, 1892.

² According to the nomenclature of K. B. Lehmann this name would have to be changed to *Bacterium methylicum*, since it forms no spores.

microbe is of general occurrence in soils I have examined soils from different parts of Japan in this direction.

The samples of soils were well shaken with water (50 gr. with 100 c.c.) and 10 c.c. of this liquid was added to the following solution, that had previously been heated to 100° C,

Sodium formate	0.5%
Dipotassium phosphate	0.2,,
Diammonium phosphate.....	0.1,,
Magnesium sulphate	0.01,,

In some cases a thin growing film of red color made gradually its appearance, especially along the rim of the solutions, while in other cases the film was very pale or white.

Of this a second infection was then made into other flasks containing also this solution, in order to exclude the other soil bacteria, that had been suspended in the 10 c.c. soil extract applied in the first solution.

A plate culture on gelatin was finally prepared and from a colony thus obtained the following tests for identification were made.

1). On potato; slight red elevated colonies, giving a wormlike appearance above the streak.

2). In bouillon it grows in the form of a coherent skin which on shaking sinks to the bottom; the bouillon liquid itself remains clear.

3). In stab culture, it grows only on the upper surface of the canal formed, not in the depth, liquefying the gelatin on the surface gradually.

4). On a gelatin plate, the colonies grow in elevated round forms and of a very slight reddish color, and begin to liquefy the gelatin after a few days.

5). In the formate solution, the cell has the form of a short straight rod, generally 1 μ . thick and 2-3 μ . long, but in bouillon and on gelatin, it grows longer and shows sometimes the form of the comma bacillus.

In a number of cases, I have observed, however, in making the gelatin plate culture from the second infection of the formate solution that besides red colonies more or less white colonies of the same character also appeared.

On inoculating from these white colonies in bouillon and making potato-cultures, I observed that this white bacillus resembled in every respect, except the color, to the red *Bacillus methylicus*, and I think it highly probable, therefore, that this is a variety of it.

I examined altogether 20 soils from the depth of 3 cm. and found the *Bacillus methylicus* in every instance.

The results are seen from the following table :

Locality.	Kind of soil.	Date, (were inoculated in the formate solution.)	Color of film, (after 2 months.)
This College, Komaba.	light loam { 1*	14 January	red
	(mulberry plantation) † 2	..	slightly reddish (few samples) white (many samples)
" " "	light loam { 1	..	white
	(experimental field, without manure for 14 years) † 2	..	
" " "	light loam { 1	..	slightly reddish
	(forest) † 2†	..	white
Noda, Shimosa province.	fertile loam { 1	18 January	white
	(farm) † 2
Near the river Tone, Musashi province.	sandy soil { 1
	(rice field) † 2
Kumagai, Musashi province.	fertile, clayey loam { 1
	(farm) † 2
Kumagai, Musashi province.	un-manured, clayey { 1
	loam (near rail road) † 2

* This sample was taken from the depth of 1 cm.

† This sample was taken from the depth of 5 cm.

Locality.	Kind of soil.	Date. (were inoculated in the formate solution)	Clor of film, (after 2 months)
Ueda, Shinano province.	fertile loam. { 1	19 January	white
	(mulberry plantation) { 2
" " "	un-manured gravelly soil { 1
	(mulberry plantation) { 2
Nagano, Shinano province.	fertile, clayey loam. { 1
	(mulberry plantation) { 2
Nagano, Shinano province.	fertile, clayey loam. { 1
	(forest) { 2
Nagano, Shinano province.	clayey loam, { 1
	(experimental field, without manuring for many years) { 2
Kawasaki, Musashi province.	fertile, sandy loam. { 1	5 February	..
	(orchard) { 2
Kyoto, Yamashiro province.	fertile, sandy loam. (forest of oak trees)	11 March	red

It was noticed very clearly that manured fertile soils yielded also the *Bacillus methylicus* in a greater number than sterile poor soils, to judge from the great difference in the time of film developement in the formate solution.

Since this bacillus occurs in putrefying manure, in the air and in every one of the examined soils, it can be concluded that it is of general occurrence.

I intend to make further investigations in regard to this microbe and to its colorless variety.

On the Liquefaction of Mannan by Microbes.

BY

S. Sawamura.

Since I had repeatedly occasion to observe the loss of viscosity of a certain mucilage used in the manufacture of Japanese paper, I was led to examine the action of microbes on mannan. The mucilage in question was derived from *Hydrangea paniculata* Sieb. var. *minor*, and consisted to a considerable extent of mannan containing also some araban and galactan. The paper manufacturer, being not acquainted with bacterial action could not explain the rapid loss of viscosity when the diluted mucilage was kept for some time.¹ My examination of such spoiled mucilage soon revealed the presence of numerous micro-organisms to which no doubt also was due the observed production of acidity.

Since the mucilage in question contained chiefly mannan and since we know that galactan is not liquefied by bacteria as the experience with agar cultures has demonstrated long ago, it seemed to me of considerable importance to test various kinds of microbes upon the power of liquefying mannan jelly. In Japan occurs in commerce a food called "*Kōnyaku*," prepared from the root of *Conophallus Konyaku* by treating with dilute milk of lime, and resembling starch paste, which consists almost exclusively of mannan.² For my experiments I prepared a much more diluted product dissolving 3% of the refined dry product of the said root in a hot solution containing 1% of pepton and 0.1% of magnesium sulphate

¹ This calamity was easily avoided by antiseptic means, which the writer proposed.

² *Tsuji*. Mannan as an Article of Human Food. Bulletin of this College. Vol. II. No. 2. p. 103.

Kinoshita. On the Occurrence of Two Kinds of Mannan in the Root of *Conophallus Konyaku*. *ibid.* Vol. II. No. 4. p. 205.

Tsukamoto, *ibid.* Vol. II. No. 7. p. 406.

and dipotassiumphosphate. This solution gave on cooling a transparent jelly resembling the agar jelly, used for bacteriological cultures.

After the mixture was sterilised in the usual way it was infected with various kinds of bacteria and yeasts, and kept at 36°C. for two days. The results are seen from the following table.

Names of the Microbes infected,	Liquefaction of Mannan Jelly.
Saccharomyces cerevisiae,	-
„ apiculatus,	-
„ from the mucilage,	-
Micrococcus from Koji,	-
Streptococcus from silk-worm,	-
Bacillus capsulatus,	-
„ cyanogenus,	-
„ Hueppe,	-
„ megatherium,	-
„ mesentericus ruber,	-
„ „ vulgatus,	+
„ pyocyaneus,	-
„ prodigiosus,	-
„ typhi murium	-
„ Zenkerei	-
„ subtilis,	-

It was only *Bacillus mesentericus vulgatus* of the sixteen species tested that liquefied mannan in two days, the same microbe which also can saccharify starch. But according to my observation it does not saccharify araban. The above named microbe cultures were observed for several weeks further, but it was only *Bacillus prodigiosus* that showed a weak action on mannan within that time. The careful examination of the spoiled mucilage mentioned above convinced the writer, that the loss of viscosity was caused by *Bacillus mesentericus vulgatus*.

But this phenomenon is considerably accelerated when a certain wild yeast propagates luxuriantly in the mucilage.¹ This influence is difficult to explain, since this yeast in itself has no action on mannan and further does not ferment the mannose formed by the bacterial action.²

I infected sterilised mannan jelly with *Bacillus mesentericus vulgatus* alone, and in a second case with this bacillus and that wild yeast together, and in a third case with that bacillus and beer yeast. After keeping the jelly at 36°C. for some days the sugar formed was determined with Fehling's solution. The results were as follows:—

Microbes.	Length of Culture.	Strength of Solution.	Sugar found in % of mannan.	Surplus.
Vulgatus.	4 days.	10%	13.782%	—
Vulgatus + the Wild Yeast.	„	„	17.675%	28.23%
Vulgatus.	2 days.	5%	5.742%	—
Vulgatus + the Wild Yeast.	„	„	8.614%	50.02%
Vulgatus.	1 day.	5%	3.676%	—
Vulgatus + Beer Yeast.	„	„	4.023%	38.95%

¹ The accelerating action of yeast on diastase was observed by *Moritz*, Central-Blatt für Agriculturchemie 1902, p. 286.

² 0.04 vol. % of alcohol was formed by cultivating it in 10% glucose solution at 36°C. for 12 days.

The amount of alcohol formed from mannan by beer yeast alone was so minute that it could not be quantitatively estimated. Further investigations are necessary to explain satisfactorily the accelerating action of the yeasts on the liquefying action of *Bacillus mesentericus vulgatus*.

Summary.

While thus far no microbe was observed liquifying galactan, there exists an exception as regards mannan, since *Bacillus mesentericus vulgatus* can easily liquefy mannan jelly. *Bacillus prodigiosus* appears to contain also traces of this enzym.

I must here express my thanks to Prof. Dr. O. Loew for his useful suggestions made to me during this and other investigations, and to Prof. Dr. Y. Kozai who kindly provided me with the pure cultures of bacteria used in this experiment, and also to Mr. T. Yamasaki, Assistant of this College.



Chemical Note on a Singular Phænogamic Parasite.

BY

T. Suda.

In the province of Tosa and in the southern part of Kiushiu in Japan a phænogamic parasite frequently develops on the roots of *Symplocos* and allied plants. Sometimes it occurs also in the Idsu province and around Nikko. Of this interesting phænogam, however, only female plants have thus far been found in Japan. In Tosa and Kiushiu people prepare from it a sticky mass called 'Torimochi' resembling bird lime, by steeping the rhizom in water and crushing it well. This mass is of black color.

This singular plant is a kind of *Balanophora* but not identical with *Balanophora dioica* occurring in India and Java where people dry the plant and use it directly like candles for illuminating purposes, since it contains a resinous substance in considerable quantities.

*J. D. Hooker*¹ states that *Balanophora dioica* in India has not the long branching rhizomes and not so much resin as the related *Balanophora elongata* growing in Java.

Th. Poleck published some studies on this latter species and found the melting point of the resin (Balanophorin) 90-95° C.

Several points in regard to the Japanese species of *Balanophora* seemed to me of some interest, it was the amount of resin present and further, the amount of lime and magnesia, since phænogamic parasites require less lime than green plants.

The material furnished to me contained 20.95% dry matter and this yielded 7.81% of ash. The amount of the resinous compound present, extracted by ether, was 15.87% of the dry matter. For determination

¹ Transactions of the Linnean Society. Vol. XX. Part I. (1863).

of lime and magnesia in the upper part 10 grams of dry matter were incinerated and the analysis carried out as usual. I obtained :

CaO	0.129%
MgO	0.244%

This shows that also this phænogamic parasite like the well known *Cuscuta* is poor in lime compared with green plants and that the amount of magnesia is larger than that of lime, while in the leaves of green plants the reverse is observed.



On the Action of Formaldehyd on Pepsin.

BY

S. Sawamura.

Formaldehyd exerts an injurious action on enzymes, as was first observed by *O. Loew*.¹ Pepsin and diastase are killed in one day when left in a neutral 5% solution of formaldehyd. *Pottevin*² observed an injurious action on rennet and sucrase, the latter being injured in already one hour at 54°C. by a formaldehyd solution of even less than 5%. At low temperature, however, sucrase resists formaldehyd more than other enzymes do, as *Bokorny*³ observed. This author⁴ also stated that a formaldehyd solution of 1% kills maltase in 24 hours, one of 5% in half an hour, and that one of 0.5% prevents the action of rennet. Catalase⁵ is killed in one hour by a solution of about 4% of formaldehyd.

Bliss and *Novy*⁶ stated on the other hand that pepsin and diastase are not injured even after weeks by diluted solutions of formaldehyd, and this statement as far as it relates to pepsin was corroborated by *Pekel-haring*⁷ who writes: „Mit Formal zu einem Gehalt von 2-3% versetzte Loesungen von Pepsin in Salzsäure können ohne merklichen Verlust an verdauender Wirkung Tage lang aufbewahrt werden.“ „Beim Prüfen muss der Gehalt an CH₂O mittelst Verdünnung oder Dialyse herabegesetzt werden, damit nicht das Fibrin selbst für die Verdauung ungeeignet gemacht werde.“

¹ Journ. f. prakt. Chem. 37, p. 104, (1888).

² Annales de l' Institut Pasteur 8, p. 796 (1894).

³ Pflüg. Arch. 85, p. 267.

⁴ Ibid.

⁵ *Loew*; Report No. 68. U. S. Dept. of Agriculture (1901).

⁶ Journ. of Exper. Med. 4, p. 47 (1898).

⁷ Z. physiol. Chem. 35 p. 29 (1902).

These statements, contradicting apparently the observations of others, induced me to make some experiments with pepsin. Five grams of the commercial pepsin were dissolved in 50 c.c. of a 10% solution of formaldehyd, while another 5 g. were dissolved in distilled water containing a little thymol. After 24 hours standing both solutions were precipitated with strong alcohol and the precipitates after well washing with alcohol dissolved in water containing 0.2% hydrochloric acid. These solutions were kept with some fibrin¹ at 36°C. for 24 hours with the result :

Normal pepsin : Fibrin dissolved completely.

Pepsin treated with }
formaldehyd } : Fibrin not attacked at all.

In a second experiment the mucous membrane of a hog's stomach was digested with four times its weight of 0.2% HCl for a week. To one part of the filtrate was now added 20% formalin (8% formaldehyd) while to another the same amount of water with some thymol. After one day these solutions were tested as above with the same result. The result of my first experiment is more decisive than that of the second, since the adhering formaldehyd had been carefully separated from the pepsin before the fibrin served for the experiment. Two causes may be responsible for the discrepancy between my results and those of *Pekelharing*. In the first place, my formaldehyd solution was of higher concentration, and in the second place, that author tested a solution of pepsin in 0.2% hydrochloric acid, while I had a perfectly neutral solution. It may be that the hydrochloric acid protects just those labile groups which otherwise enter in combination with formaldehyd.

¹ This fibrin after being freshly prepared was kept in glycerol. Before application it was kept for some time in very dilute hydrochloric acid and washed.

Ueber die Einwirkung des *Hara*-Brennens.

VON

O. Shishido, *Ringakushi*.

Einleitung

In alter Zeit war das japanische Inselland vermuthlich vollkommen mit Wald bedeckt, und die Bewohner suchten ihren Lebensunterhalt im Walde; Früchte müssen ihnen ganz unentbehrlich gewesen sein. Aber mit der Zunahme der Bevölkerung kam zuerst die Frage der Ernährung derselben; man musste also seine Lebensmittel künstlich gewinnen, und so entstand unsere Landwirthschaft; daraus folgte dass die Wald-Bestände, welche sich in den Ebenen und Gebirgen befanden, mit der Zeit nach und nach abnehmen mussten; ja die Bestände wurden als ein Hinderniss für die Landwirthschaft sogar vielfach angesehen. Da diese Abholzung grosser Arbeit und Zeit bedarfte, so fing man an, die Bestände durch das Feuer zu vernichten; Verbrennung des Waldes und Fällung der Bestände dauerten bis in spätere Zeitalter fort.

Auf diese Weise entstanden die heutigen kahlen Gebirge und bestandslosen Ebenen, welche wir "*Hara*" nennen. Diese Fläche an *Hara* ist in Japan ausserordentlich gross, die folgende Zusammenstellung gibt uns eine ungefähres Bild davon: (December 33 Meiji, 1900)

Staatswaldungen	13072002 <i>chō</i>	
Kronwaldungen	2091784 <i>chō</i>	
{ Privatwaldungen,	} 7430091 <i>chō</i>
{ Gemeindewaldungen,		
{ andere Waldungen }		
Summe	22591177 <i>chō</i>	

Staats- <i>Hara</i>	1434666 <i>chō</i>
Kron- <i>Hara</i>	154174 <i>chō</i>
{ Privat- <i>Hara</i> Gemeinde- <i>Hara</i> und andere <i>Hara</i> }	1053462 <i>chō</i>
Summe	2645302 <i>chō</i>
(1 <i>chō</i> = 9917. □. m)	

demnach beträgt die Flächen-Summe der *Hara* in Japan 2645302 *chō*.

In Japan herrscht die Sitte, jährlich das *Hara*-Gras zu brennen; es gehört somit zu den wichtigsten Fragen, die Erfolge des Brennens zu untersuchen, besonders interessirt dieses Thema den Forstmann, der vielfach unter dieser Brennsitte zu leiden hat. Aus diesem Grunde untersuchte ich die Vegetation, die Boden- und die Wuchs-Verhältnisse auf verschiedenen *Hara*. Für meine Arbeit fand ich hinreichenden Stoff in der Gegend der Kiyosumi-Schulwäldungen.

I. Abschnitt.

Die Geschichte der "Kiyosumi-Hara."

Wie erwähnt, haben ausgedehnte Wald-Bestände in alten Zeiten das ganze Land bedeckt; diese Wälder wurden aber vielfach abgeholzt oder abgebrannt. Solche Zerstörungen des Waldes dauerten bis in die neuesten Zeiten herein; Ebene und Gebirge sind solcherart in grossem Massstabe in Kahlflächen (*Hara*) umgewandelt worden. Die *Hara*, welche nun auf diese Weise entstanden ist, benutzt man hauptsächlich zu landwirthschaftlichen Zwecken, nämlich zur Gewinnung von Grasdung, oder gebraucht sie als Wiesen; der letzte Fall kommt aber in Japan sehr selten vor.

Die genannte Kiyosumi-*Hara* ist getheilt in zwei Gemeinde-*Hara*, d. h. jene von Amatsu und von Tōjō und einen Theil, welcher zu den Schulwäldungen gehört. Von diesen Gemeinde-*Hara*'s existiren keine urkundlichen Nachweise. Niemand kennt deren Entstehungs-Geschichte; nach der Ansicht der Gemeindevorsteher in Amatsu und Tōjō sind diese *Hara* nicht in derselben Weise entstanden wie die meisten *Hara* in Japan

sich bildeten. In der Zeit, als die Schōgun aus dem Hause Tokugawa über das ganze Land herrschten, bestand in den meisten Gegenden die Sitte, kahle Gebirge dem Privatbesitz zu überweisen; aber in den Provinzen Awa und Kazusa (wozu die Kiyosumi-*Hara* gehört) existirte diese Sitte nicht.

Diese Gemeinde-*Hara* sind in den ältesten Zeiten noch teilweise mit Beständen bedeckt zu denken und verblieben der Gemeinde als Freigüter. Man benutzte die *Hara* hauptsächlich, um eine Grasart, sogenanntes "Kaya" (*Miscanthus sinensis* Anders) zu gewinnen; um ein möglichst gutes Wachsthum desselben zu erzielen, brannte man diese *Hara*, jährlich.

Nur über die Geschichte der *Hara*, welche zu den Schulwäldungen gehört, hat man einige Kenntniss; der Wald an Sannodai und derselbe an der Nordseite von Suzuriischi, also die jetzige *Hara*, sind im Jahre 2 Tempo gänzlich abgeholzt worden, seitdem sind sie *zweimal* gebrannt worden, einmal im 24 Jahre Meiji (1891) und das zweitemal in Meiji 32 (1899).

Die Gemeindevorsteher in Amatsu und Tōjō sagten mir, dass man sich jetzt wiederum entschlossen hat zur Aufförstung dieser *Hara*.

II. Abschnitt.

Zustand der Kiyosumi-Hara.

I. KAPITEL: *Bodenzustände.*

A) *Lage und Fläche.*

Die Kiyosumi-*Hara* umfasst drei *Hara*, nämlich jene im Schulwald, dann jene von Amatsu, Uchiura, und endlich die *Hara* von Tōjō, Seijō, Hiroba und Hamaogi; die ganze Fläche der zwei letzteren Gemeinde-*Hara* hat 391,0325 *ha*; die Fläche der in den Schulwäldungen gelegenen *Hara* beträgt ca 20 *ha*.

Diese *Hara* befindet sich an der Nordostseite von Awa, angrenzend an die Provinz Kazusa; das Bergland, auf welchem diese *Hara* liegen, erstreckt sich von dem Platze der Schulwäldungen gegen das Meer zu. Der Lage nach gehört diese *Hara* wie Prof. Honda bestimmte, der subtropischen

Waldzone an. Diese *Hara* ist auf Gebirgsausläufern steiler Ausformung, welche bis zum Meere reichen, ausgebreitet und hat keine nennenswerthe ebene Fläche aufzuweisen.

B) *Boden.*

(Grundgestein)

Der Boden, aus welchen die Halbinsel Awa gebildet ist, besteht hauptsächlich aus drei Gesteinsarten: Tuff, Schieferthon und Sandstein. Diese Gesteine sind im Allgemeinen rau und weich, leicht verwitterbar und bilden viele Bodenmodificationen.

II. KAPITEL: *Das Klima.*

Das Klima übt natürlich auf den Pflanzenwuchs einen grossen Einfluss aus.

1. *Die Temperatur.*

Der "Kuroshio" oder warme Meeres-strom, welcher an die Südost-Küste der japanischen Insel, von Südwest nach Nordost bespült, übt auf die Lufttemperatur einen grossen Einfluss aus; namentlich wirkt er auf das Klima der Halbinsel Awa in höchstem Grade ein, weil sie sich in dem Ocean hineinstreckt; es ist also kühl im Sommer, warm im Winter, d. h. es ist das Klima ein sogenanntes Seeklima mit abgestumpften Extremen.

2. *Feuchtigkeit*

Die Halbinsel Awa hat eine grosse Luft-Feuchtigkeit, wodurch die Regenmenge ausserordentlich anwächst, im August erreicht die relative Luftfeuchtigkeit ihr Maximum und im Februar ihr Minimum.

3. *Der Nebel.*

Wo warme und kühle Strömungen des Meeres sich mischen, wie an der Ostseite der Halbinsel, werden häufig dichte Nebel erzeugt; diese treten meist in den Monaten April bis September auf.

4. *Frost.*

Er kommt in Kiyosumi sehr selten vor, Frosttage sind in Kiyosumi in einem Jahre nur 20 und zwar meist in der Zeit von Ende Oktober bis Anfang März.

5. *Der Wind.*

Wind hat auf den Pflanzenwuchs einen grossen Einfluss., deshalb muss sein Einfluss beachtet werden.

a) *Hauptwind.*

Von April bis September (5 Monate) herrscht Westwind, während in den 7 anderen Monaten sich hauptsächlich Nordost oder Nordwestwind einstellen; im Allgemeinen sind Nordwinde die Hauptluftströmungen.

b) *Sturm.*

Es gibt in dieser Gegend oftmals Stürme; nach den Untersuchungen in Chōshi und Fura (32, Meiji, 1899) herrschen die "*heftigen* Winde" meist in Winter, während in Februar, März, Spätwinter und Anfangs des Frühjahres "*Stürme*" auftreten. Das Klima ist in Kiyosumi für Menschen und Pflanzen günstig zu nennen, da die Temperaturdifferenzen zwischen Sommer und Winter sehr gering und die Feuchtigkeitsmengen, welche zum Pflanzenwuchse nötig sind, reichlich vorhanden sind.

III. Abschnitt.*Cultur der Gegend bei Kiyosumi.*

Auf der Halbinsel Awa und Kazusa befinden sich zahlreiche niedrige Höhenzüge, zwischen denen in den Thälern eine recht mässige Fläche an Ackerboden vorhanden ist.

In der Kiyosumi Gegend, wo das Hügelland seine grösste Höhe erreicht, findet man Ackerland nur an der Küste in geringer Ausdehnung; es ist eben kein Land vorhanden, welches vortheilhaft landwirthschaftlich

benutzbar wäre, wenn auch hier und da die Landwirtschaft bis zum Berghange sich ausdehnt, wo dessen Gefälle minder steil ist. Die Flächen der *Hara* des forst- und landwirtschaftlich benutzten Bodens beziffern wie folgt sich:

Namen.	Ackerfläche.	Waldfläche.	<i>Hara</i> fläche.	Summa.	<i>Hara</i> Procent.
Kamogawa	198.31 ha.	10.84 ha.	8.36 ha.	217.51 ha.	3.8 %
Amatsu	149.33 „	981.20 „	318.19 „	1448.72 „	21.9 „
Kominato	108.35 „	751.75 „	192.37 „	1052.47 „	18.3 „
Tōjō	395.88 „	1180.45 „	228.71 „	1805.04 „	12.7 „
Seijō	300.40 „	507.40 „	102.35 „	910.15 „	11.2 „
Kameyama	544.19 „	1152.50 „	391.62 „	2088.31 „	18.7 „
Kururi	275.08 „	1527.64 „	30.40 „	1833.12 „	1.6 „
Ōmi	140.15 „	247.91 „	69.48 „	457.55 „	15.1 „
Ōikawa	262.46 „	1756.36 „	317.45 „	2336.27 „	13.5 „
Nischihata	657.92 „	904.99 „	1992.01 „	3554.92 „	56.0 „
Ōtaki	333.83 „	313.70 „	312.10 „	959.63 „	32.5 „
Fusano	817.71 „	1033.55 „	476.84 „	2328.10 „	20.5 „
Fusamoto	447.81 „	291.34 „	192.46 „	931.61 „	20.7 „
Katsuura	204.53 „	191.33 „	159.90 „	555.71 „	28.7 „
Seikai	246.16 „	536.78 „	140.03 „	922.97 „	15.8 „
Ueno	569.34 „	769.59 „	49.00 „	1387.93 „	3.6 „

Man sieht, dass der Waldgrund im Allgemeinen die Ackerkulturfläche an Grösse überwiegt, namentlich hat Amatsu eine Waldfläche von 67,7%, daher ist auch die Hauptbeschäftigung der Leute dortselbst die Verkohlung des Holzes, Holzschlag, Holztransport u. s. w. ferner bemerkt man die grosse Ausdehnung der *Hara*; diese *Hara* werden zwar theils zur Futter- und Kayage Gewinnung benutzt, aber thatsächlich geben die „meisten“ Theile der *Hara* in diesen Gegenden nahezu keinen Ertrag.

IV. Abschnitt.

*Die Hara und ihre Besitzverhältnisse.*I. KAPITEL: *Besitzarten:*(A) *Der Universität gehörende Hara.*

Ein Theil derselben befindet sich in Kiyosumi und anderer liegt in Kiwada, an der Nordseite der Schulwaldungen; die ganze Fläche der Univ. *Hara* beziffert ca 20 ha; sie soll jedoch baldigst in Wald umgewandelt werden. Unter diesen *Hara* gibt es einen Theil, die *Hara* Musadogadai, deren Benutzungsrecht den Leuten von Kiyosumi vergünstigungsweise zur Gewinnung der *Kaya* (*Miscanthus sinensis* Anders) überlassen wird, dafür müssen sie dann der Universität gewisse Gegenleistungen stellen.

(B) *Die Gemeinde-Hara.*

Den Haupt-Theil der sogenannten Kiyosumi-*Hara* nimmt die *Gemeinde-Hara* ein, von der zwei Arten unterschieden werden: *erstens* die *Hara* von Amatsu und Uchiura deren totale Fläche 318 ha. beträgt, *zweitens* jene von Tōjō, Seijō, Hiroba und Hamaogi mit einer Fläche von 88.9 ha. Die erstere bildet die sogenannte Mukōmine-*Hara* welche nördlich an die Schulwaldungen grenzt, und sich nach den Küsten Amatsu und Uchiura erstreckt; die zweite *Hara* reicht von den Schulwaldungen gegen Tōjō hin.

II. KAPITEL: *Gewinnung der Haraprodukte.*

Auf der *Gemeinde-Hara* können die Berechtigten nach ihren Bedürfnissen, den Anwuchs der *Hara* ernten, es gibt *keinerlei* Beschränkung darin, sei es dass Jemand seinen Ernteantheil an Gras verkaufen oder einem Andern unentgeltlich überlassen will; nur die Gewinnungszeit der Produkte ist etwas beschränkt; es muss nämlich die Gewinnung der *Kaya* im December, und jene der anderen Gräser, von Juli bis August stattfinden; in der der Universität gehörenden *Hara* ist die Gewinnung dieser

Gräser principiell *nicht* gestattet, nur in Musadogadai, welches ebenfalls zu den Schulwaldungen gehört, können die Leute in Kiyosumi, nach Erforderniss Kaya bekommen, gegen gewisse Gegenleistungen.

V. Abschnitt.

Das Brennen der Hara.

I. KAPITEL: *Zweck des Brennens.*

(A.) *Entstehung aus alter Sitte.*

Die Entstehung der Hara ist im Allgemeinen dem Niederbrennen des Waldes zuzuschreiben, man wollte diese Fläche eben nicht wieder Wald werden lassen und brannte sie deshalb von Jahr zu Jahr, um Gras als Futter und Düngungsmittel zu bekommen, namentlich war Kaya das Hauptprodukt. Die Sitte des *Harabrennens* dauert bis Heutzutage, so werden z. B. die meisten Theile der Kiyosumi-*Hara* noch jetzt jährlich gebrannt.

Der Vorsteher in Amatsu sagte mir, dass die Leute in diesen Gegenden der Ansicht huldigen, dass die Pflanzen der *Hara* durch das Brennen kräftiger werden.

(B.) *Landwirthschaftliche Benutzung der Hara.*

Da wie erwähnt in diessen Gegenden nur gering Menge an Ackerfläche sich vorfindet, so bedarf es hier wenigen Grases als Düngmittel; auch erhalten die Leute grosse Mengen Fischdünger, welcher für den Ackerboden wirksamer ist.

(C.) *Werth des Harabrennens zur Gewinnung von Futtergräsern.*

In Kiyosumi u. Umgegend wird eine grosse Zahl Rinder gehalten welche für den Transport des Holzes nicht entbehrt werden können; man bedarf für den Unterhalt dieses Viehes viel Futtergras, welches allein auf der *Hara* gewonnen wird. Um nun Gräser für Futterzwecke in möglichst grosser Menge zu erhalten, brennen die Bauern die *Hara* alljährlich.

II. KAPITEL: *Verfahren des Harabrennens.*

Wer *Haragras* brennen will, hat dies bei dem Gemeindevorsteher anzumelden, welcher dann Nachricht davon an die Polizeibehörde gibt. Wenn die Erlaubniss ertheilt ist, beginnt man mit dem Brennen des *Haragrasses*, nachdem die Absicht u. den Zeitpunkt des Brennens den benachbarten Bodeneigenthümern mitgetheilt wurde.

III. KAPITEL: *Zeit des Harabrennens.*

Die Ernte des *Kaya* (*Miscanthus sinensis*) erfolgt in jener Jahreszeit, wo das *Kayagras* seine maximale Höhe erreicht hat, das ist in der Regel Mitte December der Fall; die übrig bleibenden Gräser auf der *Hara* brennt man dann im Monate Februar; bald nach dem Brennen begrünt sich die *Hara* wieder, und schon Ende April ist die *Hara* mit neuem Graskleide bedeckt.

IV. KAPITEL: *Die Methode des Harabrennens.*

Sobald die Polizeibehörde dem Eigenthümer einer *Hara* die Erlaubniss zum *Harabrennen* gegeben hat, beginnt derselbe mit dem Brennen der *Hara* an einem stillen Tage, wobei ein Polizei- und ein Gemeinde-Beamter sowie der benachbarte Bodeneigenthümer gegenwärtig sein sollen. Auf den Grenzlinien stellen sich Leuten an um benachbarte *Hara* oder Wald vor dem überlaufenden Feuer schützen zu können.

VI. Abschnitt.

*Physikalische Eigenschaften des Bodens.*I. KAPITEL: *In Kiyosumi.*A) *Tiefe.*

Die Tiefe des verwitterten Bodens ist nach Ortsverhältnissen verschieden, nämlich in den Thälern ist er tiefer als auf den Bergen, weil der Boden am Abhänge nach und nach thalwärts herabgeführt wird durch Regen und andere atmosphärische Niederschläge.

Die Tiefe der Verwitterungsschicht welche ich auf verschiedenen Probeflächen untersucht habe ist folgende :

Namen der Probestfläche.	Fläche.	Neigung	Standort.	durchschnittliche Tiefe.
Kiridōschi (Nordseite)	20 □ m.	35°	am Mittelhang	0.29 m.
.. (Südseite)	„	33°	„	0.35 „
Kajisaka (Nordseite)	„	39°	„	0.41 „
.. (Südseite)	„	32°	„	0.28 „
Ōmiyama (Nordseite)	„	34°	„	0.27 „
.. 1. (Südseite)	„	34°	„	0.50 „
.. 2. (Südseite)	„	36°	„	0.47 „
Samodai (Nordseite)	„	35°	„	0.21 „
.. (Südseite)	„	31°	„	0.61 „
Sazurūischi (Nordseite)	„	32°	„	0.33 „
.. (Südseite)	„	33°	„	0.29 „
Musadogadai (Nordseite)	„	32°	„	0.53 „
.. (Südseite)	„	29°	„	0.44 „
Nanamagari (Nordseite)	„	35°	„	0.45 „
Sengen { Urawaki	(Nordseite)	„	„	0.82 „
	(Südseite)	„	„	0.71 „
			durchschnittlich.	mitteltiefgründig 0.434 m.

Immer wird man auf oft gebrannter *Hara* einen *schichten* Boden finden, während der *selten gebrannte Haraboden* oder Waldboden *mitteltief- bis tiefgründig* ist.

Auf Grund meiner Untersuchungen komme ich zu dem Schlusse dass der *Haraboden* in Kiyosumi im Allgemeinen mitteltief-gründig ist.

B) Bindigkeit.

Die Bindigkeit des Bodens ist in den einzelnen Theilen der *Hara* etwas verschieden, je nachdem gewisse Parthien der *Hara jährlich* oder *periodisch* gebrannt wurden; die *jährlich* gebrannten Theile haben *lockeren* oder *grob* gekrümelten Boden, während auf *selten gebrannter Hara* die Struktur feiner und gekrümelter ist.

Im Allgemeinen aber kann man den Boden auf der Kiyosumi-*Hara* wegen der Luftfeuchtigkeit als ziemlich mild oder locker ansprechen.

C) *Feuchtigkeit.*

Die *Hara*, deren Bodenüberzug jährlich gebrannt wird, müsste eigentlich stärker ausgetrocknet sein, weil der Boden öfter und länger direkt der Luft ausgesetzt ist; aber er ist dennoch durchweg ziemlich feucht, der Grund davon ist ebenfalls darin zu suchen, dass die Luftströmungen auf dieser Halbinsel an sich sehr feucht sind, so dass dieser Unterschied nicht so fühlbar wird.

II. KAPITEL: *Äussere Zustände der Kiyosumi-Hara.*

Die *Hara* in Kiyosumi wurde frühe schon von Wald-Beständen entblöst, jetzt ist die ganze Fläche derselben hauptsächlich von Kaya Grass (*Miscanthus sinensis* Anders) bedeckt. Aber die Menge der Kaya ist natürlich je nach dem Wiederholungszeitraume des Brennens verschieden; in allen jenen Theilen, welche *weniger* oft gebrannt werden, *wachsen* sich Baumarten (wie hauptsächlich *Quercus glandulifera*, *Quercus serrata*, *Quercus acuta*, *Quercus vibraycana*, *Machilis japonica*, *Evonymus europaeus* var. *hamiltonianus*, *Rhododendron indicum* var. *Kaempferi*, *Salix Sieboldiana*, *Spiraea callosa* n. s. u.) und Halbbäume den *Miscanthus*arten bei. Aus diesem Umstande können wir leicht erkennen dass die *Hara*, wenn man sie für längere Jahre in Ruhe liesse, wieder zum ihrem eigentlichen Urzustande kommen, d. h. in Wald sich umwandeln würde. So kann man z. B. in Nanamagari, wo die *Hara* seit 10 Jahren *nicht* gebrannt wurde, folgende Baum- und Halbbaumarten beobachten:

- Quercus glandulifera*
- Quercus serrata*
- Evonymus alatus* var. *subtriflora*
- Spiraea callosa*
- Eurya Japonica*
- Diervilla glanduliflora*
- Viburnum dilatatum*

Machilus Thunbergii
 Clethra barbinervis
 Smilax china
 Quercus acuta
 Quercus myrsinaefolia
 Rosa multiflora
 Rhododendron indicum var. Kaempferi
 Rubus palmatus
 Litsea hypoleuca
 Salix Sieboldiana
 etc.

Auf "alljährlich" gebrannter *Hara* können nur Grasarten vorkommen, welche ich in einem späteren Abschnitte besonders anführen werde.

III. KAPITEL: *Das Verhältniss zwischen Boden und Klima.*

(Verwitterungsprocess)

Der Boden ist das Verwitterungsprodukt der Grundgesteine, und die Verwitterungsgrösse hängt von dem Klima ab, welches auf dem betrachteten Platze herrscht. Die Verwitterungsschicht bedeckt den Boden in den Gebirgen bei Kiyosumi in sehr verschiedener Dicke. Da der Boden der Kiyosumi *Hara* aus den Gesteinen der Tertiärperiode gebildet ist, so sollte die Verwitterungsschicht eigentlich ziemlich bedeutend sein, aber die Wirklichkeit zeigt uns eine grosse Ungleichheit des *Harabodens*. Er ist in seiner Verwitterungsschicht verschiedentlich *sehr flachgründig*, namentlich ist dies am Kamme und in oberen Hänge zu bemerken; der Grund hiezu liegt wohl in der Steilheit der Gebirge, wodurch bei Regengüssen die verwitterten Bodentheilchen, welche keine genügend schützende Pflanzendecke haben, abgeschwemmt werden. Diese Erscheinung muss natürlich auf der *Hara* intensiver als im Walde sein, weil der *Haraboden* direkt frei der Atmosphäre ausgesetzt ist, während im Walde der ganze Boden durch die Kronen der Bäume eine Überschirmung erfährt. Die Verwitterungsschichtendicke wurde in einzelnen *Haratheilen* folgendermassen gefunden:

Namen.	Jahre des <i>Hara</i> brennens.	Dicke der Verwitterungsschicht	Mittel.
Kiridōshi (Nord seite)	alljährlich gebrannt	69.6 m. m.	} 71.3 m. m.
„ (Süd seite)	„ „	73.1 „ „	
Kajisaka (Nord seite)	„ „	52.3 „ „	} 60.0 „ „
„ (Süd seite)	„ „	67.7 „ „	
Ōmiyama (Nord seite)	für drei Jahre nicht gebrannt	84.2 „ „	} 89.8 „ „
„ (Süd seite)	für 3 Jahre nicht gebrannt	95.3 „ „	
Musadogadai (Süd seite)	für 6 Jahre nicht gebrannt	113.3 „ „	113.3 „ „
Ōmiyama (Süd seite)	„ „ „	72.1 „ „	72.1 „ „
Suzuriischi (Süd seite)	über 8 Jahre nicht gebrannt	100.5 „ „	100.5 „ „
Sanmodai (Nord seite)	bis 24 Meiji alljährlich und später im Jahre 33 Meiji gebrannt	66.9 „ „	} 102.4 „ „
„ (Süd seite)	gleich	133.8 „ „	
Suzuriischi (Nord seite)	„	121.2 „ „	121.2 „ „
Nanamagari (Nord seite)	für 10 Jahre nicht gebrannt	110.7 „ „	110.7 „ „
Musadogadai (Nord seite)	über 10 Jahre nicht gebrannt	98.1 „ „	98.1 „ „
Sengen (Nord seite)	Urwald	88.4 „ „	} 92.8 „ „
„ (Süd seite)	„	97.2 „ „	

(Die Neigung der einzelnen *Hara* ist in der vorgegangenen Tabelle bereits bezeichnet worden.)

Die Verwitterungsschichten u. damit der produktive Boden werden auf der ungeschützten *Hara* nach und nach immer seichter durch die Abschwemmung der feinen Theile thalwärts. Die Nützlichkeit u. Notwendigkeit der Verwitterungsschicht für den Pflanzenwuchs brauche ich kaum weiter zu besprechen.

VII. Abschnitt.

Vergleich der Effekte des Brennens der Hara in verschiedenem Brenn-Terminus.

Um den Einfluss des brennens *Hara* besser zu beobachten ist nöthig, dass man ausführliche Untersuchungen auf verschiedenen *Hara*'s macht. Die meisten Theile der Gemeinde-*Hara* in Kiyosumi sind, wie ich schon

erwähnte, *alljährlich* gebrannt, während die der Schulwaldung gehörende *Hara* nur sehr selten gebrannt wurde; die *Hara* in Kiwada, welches auch der Universität gehört, wird ebenso alljährlich gebrannt. Ich untersuchte nun folgende Theile:

1. Kiridōshi und Kajisaka. alljährlich gebrannt.
2. Ōmiyama. seit 3 Jahre nicht gebrannt.
3. Ōmiyama (Südseite) und Musadogadai (Südseite). seit 6 Jahre nicht gebrannt.
4. Suzuriishi (Südseite). über 8 Jahre nicht gebrannt.
5. Sannodai und Suzuriishi (Nordseite). bis 24 Meiji (1891) alljährlich und später im Jahre 33 Meiji (1900) gebrannt.
6. Nanamagari. seit 10 Jahren nicht gebrannt.
7. Musadogadai (Nordseite). über 10 Jahre nicht gebrannt.

ausserdem habe ich den Urwald *Sengen* zum *Vergleich* herangezogen.

I. KAPITEL: Prüfung mittels Probeflächen.

Um den Antheil der verschiedenen Grasarten an jeder *Hara* festzustellen, habe ich Probeflächen von 2 □. m. genommen, und gebe nun die Mittel aus je drei Proben.

Kiridōshi (Nordseite).

(alljährlich gebrannt)

(Neigung = 35°)

(Datum 10/4 u. 25/4)

lateinischer Name.	japanischer Name.	Zahl der Gräser auf 2 □. m.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	318	38.6
<i>Sanguisorba officinalis</i> L.	Waremokō	80	9.7
<i>Senecio krameri</i> Fr. et Sav.	Yaburegasa	74	9.0
<i>Thalietrum minus</i> L. var. <i>elatum</i> Lecoy.	Akikaramatsu	67	8.1
<i>Arundinella anomala</i> Steud.	Todashiba	51	6.2
<i>Plectanthus glaucocalyx</i> Max. var. <i>japonicus</i> Max.	Hikiokoshi	37	4.7

<i>Lespedeza bicolor</i> Turcz.	[Hagi]	34	4.1
<i>Saussurea Tanakae</i> Fr. et Sar. var. <i>phyllolepis</i> Max.	Tohiren	26	3.2
<i>Pteris aquilina</i> L.	Warabi	16	1.9
<i>Potentilla fragarioides</i> L. var. <i>ternata</i> Max.	Mitsubatsuchiguri	16	1.9
<i>Lysimachia clethroides</i> Duby.	Okatoranoo	15	1.8
<i>Viola silvestris</i> Kit. var. <i>gryoseras</i> A. Gr.	Tachitsubosumire	13	1.5
<i>Serratula atriplicifolia</i> B. et H.	Kumatoribokuchi	12	1.4
<i>Dioscorea Tokoro</i> Makino.	Onidokoro	11	1.3
<i>Polygonum cuspidatum</i> S. et Z.	Itadori	10	1.2
<i>Cirsium japonicum</i> DC.	Noazami	8	5.4
<i>Atractylodes lyrata</i> S. et Z.	Okera	6	
<i>Carex breviculmis</i> R. Br.	Aosuge	5	
<i>Poa trivialis</i> L.	Himeichigotsunagi	3	
<i>Seseli libanostis</i> Koch. var. <i>daucifolia</i> DC.	Ibukibōfu	3	
<i>Cirsium spicatum</i> (Max).	Yamaazami	3	
<i>Euphorbia Esula</i> L.	Hagikusō	2	
<i>Maottia ignita</i> (Vell).	Kwaensō	2	
<i>Heteropappus hispidus</i> Less. var. <i>isochaetus</i> Fr. et Sav.	Yamajinogiku	2	
<i>Viola japonica</i> Langsd.	Ko sumire	2	
<i>Allium japonicum</i> Rgl.	Yama rakkyō	2	
<i>Aster indicus</i> L.	Yomena	2	
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Max.	Yomogi	1	
<i>Disporum sessile</i> Don.	Hōchakusō	1	
		Σ22	

Kiridōshi (Südseite).

(alljährlich gebrannt)

(Neigung = 33°)

(Datum 10/4 u. 25/4)

lateinischer Name.	japanischer Name.	Zahl der Gräser in 2 □ m.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	344	31.0
<i>Imperata arundinacea</i> Cyr. var. <i>Koenigii</i> (Penth).	Chigaya	173	14.0

Brachypodium silvaticum R. et S.	Yama kamojigusa	140	12.1
Inula salicina L.	Kasensō	64	5.5
Miscanthus sacchariflorus Hack.	Ogi	62	5.4
Sanguisorba officinalis L.	Waremokō	54	4.7
Lysimachia clethroides Duby.	Okatoranoo	40	3.5
Viola Patrinii DC. var. chinensis Ging.	Sumire	36	3.1
Serratula atriplicifolia B. et H.	Kumatoribokuchi	35	3.0
Pteris aquilina L.	Warabi	33	2.8
Smilax china L.	[Sankirai]	25	2.1
Thalictrum minus L. var. elatum Lecoy.	Akikaramatsu	24	2.0
Athyrium nipponicum Bak.	Inuwarabi	18	1.6
Senecio Krameri Fr. et Sav.	Yaburegasa	16	1.4
Plectranthus inconspicuus Miq.	Yamahakka	16	1.4
Euphorbia Sieboldiana Mor. et Dec	Natsutodai	15	1.3
Lespedeza Sieboldi Miq.	[Hagi]	8	} 3.6
Cirsium spicatum Max.	Yama azami	7	
Aster indicus L.	Yomena	6	
Polygala japonica Houtt.	[Hime Hagi]	6	
Patrinia scabiosaefolia Link.	Ominaeschi	6	
Discocorea Tokoro Makino.	Onidokoro	5	
Potentilla fragarioides L.	Kijimuschiro	5	
Polygonum cuspidatum S. et Z.	Itadori	5	
Vitis Thunbergii S. et Z.	Ebizuru	4	
Seseli Libanostis Koch. var. daucifolia DC.	Ibukibōfu	4	
Gymnadenia conopea R. Br.	Chidorisō	1	

Aus den Tabellen, ersicht man "*dass auf jährlich gebrannter Hara*" hauptsächlich die folgende Grasarten auftreten :

- Miscanthus sinensis Anders.
- Sanguisorba officinalis L.
- Serratula atriplicifolia B. et H.
- Senecio Krameri Fr. et Sav.
- Pteris aquilina L.

- Thalictrum minus L. var. elatum Leroy.
 Polygonum cuspidatum S. et Z.
 Imperata arundinacea var. Koenigii (Benth.)
 Potentilla fragarioides var. ternata Maxim.
 Brachypodium silvaticum R. et S.
 etc.

Diese Grasarten sind vorzugsweise *Lichtgräser*, d. h. sie können meist nur auf nacktem Boden gedeihen, oder kommen wenigstens öfter u. vorzugsweise in nahrungsarmem Boden vor. Auf der *Hara*, welche seit *drei* Jahren *nicht* gebrannt ist, sind dagegen die folgende Grasarten anzutreffen gewesen.

Ōmiyama (Nordseite).

(für 3 Jahre nicht gebrannt.)

(Neigung = 34°).

(Datum 11/4 u. 26/4).

lateinischer Name,	japanischer Name,	Zahl der Gr. in 2 1/2 m.	%
Miscanthus sinensis Anders.	Susuki	25	25,2%
Smilacina japonica A. Gr.	Yukizasa	81	8,0
Aster japonicus Miq.	Yamashirogiku	65	5,9
Carex breviculmis R. Br.	Aosuge	53	4,5
Artemisia vulgaris L. var. indica Max.	Yomogi	41	3,5
Potentilla fragarioides L. var. ternata Max.	Mitsubatsuchiguri	30	2,3
Cypripedium japonicum Thunb.	Kumagaiso	37	3,2
Plectranthus inconspicuus Miq.	Yamahakka	34	2,9
Cirsium japonicum DC.	Nozami	32	2,7
Petasites japonicus Miq.	Fuki	31	2,9
Carex conica Boott.	Himekansuge	25	2,2
Viola Patrinii DC. var. chinensis Ging.	Sumire	20	1,7
Krauhia floribunda (Willd.) Taub.	Fuji	18	1,5
Viola silvestris Kit. var. grypoceras A. Gr.	Tachisu (ostanre)	17	1,4
Polygonum cuspidatum S. et Z.	Rudori	17	1,4

<i>Lysimachia clethroides</i> Duby.	Okatoranoo	16	1.4
<i>Euphorbia sieboldiana</i> Mor. et Dec.	Natsutōdai	16	1.4
<i>Thalictrum minus</i> L. var. <i>elatum</i> Lecoy.	Akikaramatsu	16	1.4
<i>Crepis japonica</i> Benth.	Onitabirako	16	1.4
<i>Dioscorea</i> Tokoro Makino.	Onidokoro	15	1.4
<i>Poa trivialis</i> L.	Hime ichigotsunagi	15	1.3
<i>Lactuca squarrosa</i> Miq. forma <i>indivisa</i> Max.	Honba akinonogeshi	15	1.3
<i>Polygala japonica</i> Houtt.	[Himehagi]	15	1.3
<i>Erigeron annuus</i> Pers.	Himejoon	15	1.3
<i>Aspidium dissectum</i> Mett.	Hoshida	15	1.3
<i>Disporum sessile</i> Don.	Hōchakusō	14	1.2
<i>Disporum pullum</i> Salist.	Tōchikuran	14	1.2
<i>Cirsium spicatum</i> Maxim.	Yamaazami	14	1.2
<i>Clematis recta</i> L. var. <i>paniculata</i> Thunb.	Sennusō	13	1.1
<i>Carpesium abrotanoides</i> L.	Yabutabako	13	1.1
<i>Eupatorium japonicum</i> Thunb.	Sawa hiyodori	12	1.0
<i>Acanthopanax pivaricatum</i> S. et Z.	Ukogi	12	1.0
<i>Arundinella anomala</i> Steud.	Todashiba	12	1.0
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Max.	Rindō	12	1.0
<i>Senecio krameri</i> Fr. et Sav.	Yaburegasa	12	1.0
<i>Pteris aquilina</i> L.	Warabi	12	1.0
<i>Sanguisorba officinalis</i> L.	Warumokō	12	1.0
<i>Plectranthus graucocalyx</i> Max. var. <i>japonicus</i> Max.	Hikiokoshi	10	0.9
<i>Saussurea Tanakae</i> Fv. et. Sav. var. <i>phyllolepis</i> Max.	Tōhiren	8	} 4.2
<i>Serratula atriplicifolia</i> B. et H.	Kumotorihokuchi	8	
<i>Manettia iginta</i> (Vell.)	Kwaensō	8	
<i>Atractylodes lyrata</i> S. et Z.	Okera	7	
<i>Aster indicus</i> L.	Yomena	6	
<i>Patrinia villosa</i> Juss.	Otokoeshi	5	
<i>Brachypodium silvaticum</i> R. et S.	Yamakamoji gusa	3	
<i>Osmunda regalis</i> L. var. <i>japonica</i> Milde.	Zenmai	3	

Omiyama (Südseite).

(für 3 Jahre nicht gebrannt.

(Ncigung = 34')

(Datum 11/4 u. 26/4.)

lateinischer Name.	japanischer Name.	Zahl der Gr. in 2. u. 3. M.	Zahl der Gr. in 4. M.
<i>Miscanthus sinensis</i> Anders.	Sesuki	271	271
<i>Chrysanthemum sinense</i> var. <i>japonicum</i> Max.	Ryunōgilu	75	75
<i>Disporum sesile</i> Don.	Hōchōjō	52	52
<i>Carex breviculmis</i> R. Br.	Aosuge	51	51
<i>Agrimonia viscidula</i> Bge. var. <i>japonica</i> Miq.	Kimmizuluki	42	42
<i>Houttuynia cordata</i> Thunb.	Dokudami	37	37
<i>Plectranthus inconspicuus</i> Miq.	Yamahakka	35	35
<i>Cirsium spicatum</i> Maxim.	Yamaazami	31	26
<i>Thalictrum minus</i> L. var. <i>elatum</i> Lecoy.	Akikaramatsu	27	25
<i>Senecio Krameri</i> Fr. et Sav.	Yaburegasa	27	24
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Max.	Yomogi	27	24
<i>Athyrium nipponicum</i> Bak.	Inuwarai	27	21
<i>Carex lanceolata</i> Boott.	Hikasuge	25	21
<i>Oxalis corniculata</i> L.	Katabami	25	21
<i>Poa trivialis</i> L.	Hime ichigōsuragi	25	21
<i>Dioscoria Tokoro</i> Makino.	Onidokoro	22	22
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Max.	Rindō	21	17
<i>Paederia tomentosa</i> Bl.	Hekusokaruma	21	17
<i>Andropogon micranthus</i> Kth.	Hime aburasusuki	21	17
<i>Dianthus superbus</i> L.	Kawaradeshiko	21	17
<i>Crematis recta</i> L. var. <i>peniculata</i> Thunb.	Sem'nsō	21	17
<i>Astilbe thunbergii</i> Miq.	Torishishōma	21	17
<i>Crepis japonica</i> Benth.	Onitōrako	21	17
<i>Lysimachia clethroides</i> Duby.	Oltatoranoo	18	15
<i>Lygodium japonicum</i> Sw.	Furusshōjō	17	15
<i>Lactuca thunbergiana</i> (A. Gr.) Maxim.	Nigama	17	15

Aster japonicus Miq.	Yamashirogiku	17	1.4
Aster scaber Thunb.	Shirayamagiku	16	1.3
Pteris aquilina L.	Warabi	16	1.3
Vitis thunbergii S. et Z.	Ebizuru	15	1.3
Tricyrtis japonica Miq.	Hototegisisō	15	1.3
Rubus incisus Thunb.	[Nigaichigo]	15	1.2
Lespedeza pilosa S. et Z.	Nekohagi	15	1.2
Brachypodium sylvaticum R. et S.	Yamakaomōjigusa	15	1.2
Potentilla fragarioides L.	Kizimushiro	15	1.2
Smilacina japonica A. Gr.	Yukizasa	14	1.2
Carpesium abrotanoides L.	Yabutabako	14	1.2
Sanguisorba officinalis L.	Waremokō	13	1.1
Atractylodes lyrata S. et Z.	Okera	11	0.9
Patrinia scabiosaefolia Link	Ominaeschi	8	} 2.1
Carex conica Boott.	Hime kansuge	8	
Eupatorium japonicum Thunb.	Sawa hiyodori	6	
Saussurea Tanakae Fr. et Sav. var. phyllolepis Max.	Tō hiren	3	

1212

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Es wird daraus klar, dass diese *Hara* (Ōmiyama) verhältnissmässig *viele* Grasspecies enthält; es sind hier die erwähnten Lichtgräser seltener, während andere Arten in grösserer Zahl gefunden werden. Aus dem Aufwuchse kann die Bodenbeschaffenheit beurtheilt werden; es gedeihen auf *schlechtem* Boden eben schlechte Grasarten und auf *gutem* Boden die besseren Arten.

Auch die grössere Zahl der Gewächsorten deutet an, dass ein Boden nahrungsreicher ist; in nahrungsarmem Boden können nur wenige Species fortkommen bei sonst gleichen Verhältnissen. Man kann daher schliessen, dass die *zuerst* genannte *Hara* (Kiridōshi) nahrungsrärmer ist als die *zweite* (Ōmiyama).

II. KAPITEL. Folgen des Brennens einer *Hara*.

(A) Veränderung der Gewächsorten durch das Brennen.

Die Veränderung der Gewächsspecies lässt sich aus Untersuchungen in Kiyosumi erkennen. Diese *Hara* wurde, wie schon bemerkt, auf

verschiedenen Theilen in verschiedenen Zwischenräumen gebrannt. Die folgenden Tabellen beleuchten in ihren Ergebnissen diese Frage (die einzelne Probefläche = 20 [] m. gross.)

Kajisaka (Nordseite),

(*alljährlich* gebrannt)

(Neigung = 39°)

(Probefläche = 20 [] m.)

(Datum 10¹/₄ u. 26¹/₄)

lateinischer Name,	japanischer Name,	№
<i>Miscanthus sinensis</i> Anders.	Su-uki	28
<i>Sanguisorba officinalis</i> L.	Waremokō	8
<i>Carex Duvaliana</i> Fr. et Sav.	Kesuge	8
<i>Brachypodium sylvaticum</i> R. et S.	Yamakanaŷegusa	7
<i>Pteris aquilina</i> L.	Warabi	5
<i>Gerbera anandria</i> Sch. Bip.	Senbonjari	5
<i>Thalictrum minus</i> L. var. <i>elatum</i> Lecoy.	Akikaramatsu	5
<i>Phegopteris Totta</i> Mett.	Mioshida	5
<i>Astilbe Thunbergii</i> Miq.	Torifashishōma	4
<i>Artemisia japonica</i> Thunb.	Otoloyonagi	4
<i>Patrinia villosa</i> Juss.	Otokoeshi	3
<i>Polygonum cuspidatum</i> S. et Z.	Itadori	3
<i>Carex lanceolata</i> Boott.	Hikagesuge	2
<i>Atractylodes lyrata</i> S. et Z.	Okera	2
<i>Centaurea atriplicifolia</i> (DC.)	Yamabikuchō	2
<i>Senecio krameri</i> Fr. et Sav.	Yatategasa	1
<i>Aster scaber</i> Thunb.	Shirayamaŷaku	1
<i>Euphorbia sieboldiana</i> Mor. et Dec.	Natsutōchi	1
<i>Aster trinervius</i> Roxb. var. <i>japonica</i> Max.	Konkiku	
<i>Osmunda regalis</i> L. var. <i>japonica</i> Mille.	Zemmaŷ	
<i>Angelica decursiva</i> Miq.	Nōtake	
<i>Serratula coronata</i> L.	Tamaŷuki	
<i>Cirsium spicatum</i> Maxim.	Yamaŷama	

Sanicula elata Miq.	Umanomitsuba	}	6.
Aconitum sinense S. et Z.	Torikabuto		
Sedum kamtschaticum Fisch.	Kirinsō		
Disporium pullum Salisb.	Tōchikuran		
Cypripedium japonicum Thunb.	Kumagaisō		
Saussurea ussuriensis Max.	Kikuazami		
Coelopleurum Gmelini Ledeb.	Shishiudo		
Adenophora verticillata Fisch. var. verticillata (Fr. et Sav.)	Tsurigane ninjin		
Saussurea affinis Spr.	Kitsuneazami		
Petasites japonicus Miq.	Fuki		
Hosta Sieboldiana Engl.	Tō gibōshi		
Solidago Virga-aurea L.	Awadachisō		
Patrinia scabiosaefolia Link.	Ominaeshi		
Leonurus macranthus Maxim.	Kisewata		
Smilax china L.	[Sankirai]		

100

Kajisaka (Südseite).

(alljährlich gebrannt)

(Neigung = 32°)

(Probefläche = 20 □. m.)

(Datum 10/4 u. 26/4)

lateinischer Name.	japanischer Name.	%
Miscanthus sinensis Anders.	Susuki	34
Brachypodium selvaticum R. et S.	Yamakamojigusa	6
Arundinella anomala Steud.	Todashiba	5
Thalictrum minus L. var. elatum Locoy	Akikaramatsu	5
Sanguisorba officinalis L.	Waremokō	4
Potentilla fragarioides L. var. ternata Maxim.	Mitsubatsuchiguri	4
Plectlanthus glaucocalyx Max. var. japonicus Max.	Hikiokoshi	3
Phegopteris Totta Mett.	Mizoshida	3
Artemisia japonica Thunb.	Otoko yomegi	3
Poa trivialis L.	Hime ichigotsunagi	3

<i>Aster scaber</i> Thunb.	Shirayamagiku	3
<i>Senecio krameri</i> Fr. et Sav.	Yaburegasa	2
<i>Euphorbia Sieboldiana</i> Mor. et Dec.	Natsutōdai	2
<i>Viola Patrini</i> DC. var. <i>chinensis</i> Ging.	Sumire	2
<i>Taraxacum officinale</i> Wigg. var. <i>glaucescens</i> Koch.	Tanpojo	2
<i>Pteris aquilina</i> L.	Warabi	2
<i>Lysimachia clethroides</i> Duby.	Okatorano	2
<i>Aster japonicus</i> Miq.	Yamashirogiku	1
<i>Disporum sesile</i> Don.	Hōchaku-ō	1
<i>Cirsium spicatum</i> (Maxim.)	Yama azami	1
<i>Saussurea ussuriensis</i> Maxim.	Kiku ² azami	1
<i>Polygonum cuspidatum</i> S. et Z.	Itadori	1
<i>Coclopleurum Gmelini</i> Ledeb.	Shishiudo	
<i>Seseli Libanostis</i> Koch. var. <i>daucifolia</i> DC.	Ibukibōfu	
<i>Ixeris Thunbergii</i> A. Gr.	Nigana	
<i>Pieris hircacoides</i> L. var. <i>japonica</i> Rgl.	Kōzorina	
<i>Viola japonica</i> Longsd.	Kosumire	
<i>Dioscorea Tokoro</i> Makino.	Onidokoro	
<i>Melandryum firmum</i> Rohrb.	Fushiguro	
<i>Senecio campestris</i> DC.	Sawaoguruma	
<i>Calystegia sepium</i> R. Br.	Hirugao	
<i>Allium japonicum</i> Rgl.	Yamarakkyō	
<i>Serratula coronata</i> L.	Tamura-ō	
<i>Agrostis perennans</i> Tuck.	Nukabo	
<i>Lilium Maximowiczii</i> Rgl.	Kōniyuri	
<i>Rumex acetosa</i> L.	Suiba	
<i>Lithospermum Zollingeri</i> A. DC.	Hotarukazura	
<i>Cryptogramme japonica</i> Prantl.	Tachishinobu	
<i>Polygonatum giganteum</i> Dietr. var. <i>Thunbergii</i> Max.	Narukoyuri	
<i>Inula salicina</i> L.	Kasensō	
<i>Cimicifuga foetida</i> L. var. <i>simplex</i> Huth.	Sarashinashōma	
<i>Vicia unijuga</i> Al. Br.	[Nantenhagi]	
<i>Rubus parvifolius</i> L.	[Nawashiro ichigo]	
<i>Graminea</i> Sp.	Graminea Sp.	

Ōmiyama (Nordseite)

(seit 3 Jahre nicht gebrannt)

(Neigung = 34°)

(Probefläche = 2 □. m.)

(Datum 12/4 u. 26/4)

lateinischer Name.	japanischer Name.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	30
<i>Aster japonicus</i> Miq.	Yamashirogiku	5
<i>Carex breviculmis</i> R. Br.	Aosuge	5
<i>Carex Duvariana</i> Fr. et Sav.	Kesuge	5
<i>Arundinella anomala</i> Steud.	Todashiba	5
<i>Disporum sesile</i> Don.	Hōchakusō	4
<i>Serratula coronata</i> L.	Tamabōki	4
<i>Potentilla fragarioides</i> L. var. <i>Ternata</i> Max.	Mitsubatsuchiguri	3
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Max.	Yomogi	3
<i>Krauhnia floribunda</i> (Willd.) Taub.	Fuji	3
<i>Pieris hieracioides</i> L. var. <i>japonica</i> Rgl.	Kōzorina	3
<i>Adenophora verticillata</i> Fisch. var. <i>verticillata</i> (Fr. et Sav.)	Tsuriganeninjin	2
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Max.	Rindō	2
<i>Artemisia japonica</i> Thunb.	Otokoyomogi	2
<i>Agrimonia viscidula</i> Bg. var. <i>japonica</i> Miq.	Kimmizubiki	2
<i>Euphorbia Sieboldiana</i> Mor. et Dec.	Natsutōdai	2
<i>Pteris aquilina</i> L.	Warabi	2
<i>Senecio Krameri</i> Fr. et Sav.	Yaburegasa	2
<i>Chrysanthemum sinense</i> Sab. var. <i>japonicum</i> Max.	Ryūnōgiku	1
<i>Aster scaber</i> Thunb.	Shirayamagiku	1
<i>Angelica decursiva</i> Miq.	Nodake	1
<i>Clematis recta</i> L. var. <i>paniculata</i> (Thunb.)	Seminsō	1
<i>Brachypodium silvaticum</i> R. et S.	Yamakamojigusa	1
<i>Patrinia villosa</i> Juss.	Otokoeshi	1
<i>Rumex acetosa</i> L.	Suiba	1

<i>Brachypodium japonicum</i> Miq.	Kamojigusa	
<i>Solidago virga-aurea</i> L.	Awadachisō	
<i>Cirsium spicatum</i> Maxim.	Yamaazami	
<i>Calanthe discolor</i> Lindl.	Ebine	
<i>Saussurea ussuriensis</i> Maxim.	Kikuazami	
<i>Petasites japonicus</i> Miq.	Fuki	
<i>Coclopleurum gmelini</i> Ledeb.	Shishiudo	
<i>Phytoloea acinosa</i> Roxb. var. <i>esculenta</i> Max.	Yamagobō	
<i>Penthorum sedoides</i> L. var. <i>chinense</i> Max.	Sawa shion	
<i>Viola silvestris</i> Kit. var. <i>gryoceras</i> A. Gr.	Tachitsubosumire	
<i>Astilbe Thunbergii</i> Miq.	Toriashishōma	10
<i>Osmunda regalis</i> L. var. <i>japonica</i> Milde.	Zemmai	
<i>Dioscorea tokoro</i> Makino.	Onidokoro	
<i>Lysimachia clethroides</i> Duby.	Okatoranoo	
<i>Polygonum cuspidatum</i> S. et Z.	Itadori	
<i>Viburnum dilatatum</i> Thunb.	[Gamazumi]	
<i>Quercus glandulifera</i> Bl.	[Konara]	
<i>Quercus serrata</i> Thunb.	[Kunugi]	
<i>Rhododendron indicum</i> Sw. var. <i>Kaempferi</i> Max.	[Yamatsutsuji]	
<i>Rubus parvifolius</i> L.	[Nawashiroichigo]	
<i>Smilax china</i> L.	[Sankirai]	
<i>Lespedeza bicolor</i> Turcz.	[Hagi]	
<i>Spiraea japonica</i> L. f.	[Shimo:suke]	

Ōmiyama (Südseite).

(seit 3 Jahren nicht gebrannt)

(Neigung = 34°)

(Probefläche = 2 □. m.)

(Datum 10/4 u. 26/4)

lateinischer Name.	japanischer Name.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	29
<i>Phegopteris totta</i> Mett.	Mizoshida	5

<i>Aspidium aristatus</i> Sw.	Hosoba kamawarabi	3
<i>Carex conica</i> Boott.	Iimekansuge	3
<i>Serratula coronata</i> L.	Tamabōki	3
<i>Disporum sessile</i> Don.	Hōchakusō	3
<i>Agrimonia pilosa</i> Ledeb.	Kinmizuhiki	3
<i>Chrysanthemum sinense</i> Sab. var. <i>japonicum</i> Max.	Ryunōgiku	2
<i>Carex Morrowi</i> Boott.	Kansuge	2
<i>Houttuynia cordata</i> Thunb.	Dokudami	2
<i>Potentilla fragarioides</i> L. var. <i>ternata</i> Max.	Mitsubatsuchiguri	2
<i>Picris hieracioides</i> L. var. <i>japonica</i> Rgl.	Kōzorina	2
<i>Viola silvestris</i> Kit. var. <i>grypoceras</i> A. Gr.	Tachitsubosumire	2
<i>Artemisia japonica</i> Thunb.	Otokoyomogi	2
<i>Angelica decursiva</i> Miq.	Nodake	2
<i>Arundinella anomata</i> Steud.	Todashiba	2
<i>Sodium kantschoticum</i> Fisch.	Kirinsō	2
<i>Carex duvariana</i> Fr. et Sav.	Kesuge	2
<i>Aspidium lepidocaulon</i> Hook.	Orizurushida	1
<i>Eupatorium Kirilowii</i> Turcz.	Sawahiyodori	1
<i>Brachypodium silvaticum</i> R. et S.	Yamakamojigusa	1
<i>Aster scaber</i> Thunb.	Shirayamagiku	1
<i>Angelica polymorpha</i> Maxim.	Shiranesenkiu	1
<i>Euphorbia sieboldiana</i> Mor. et Dec.	Natsutodai	1
<i>Lactuca Thunbergiana</i> (A. Gr.) Maxim.	Nigana	1
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Max.	Rindō	1
<i>Pteris aquilina</i> L.	Warabi	1
<i>Senecio crameri</i> Fr. et Sav.	Yaburegasa	1
<i>Osmunda regalis</i> L. var. <i>japonica</i> Mild.	Zemmai	1
<i>Athyrium filix femina</i> Roth.	Meshida	1
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Maxim.	Yomogi	
<i>Cryptogramme japonica</i> Prantl.	Tachishinobu	
<i>Patrinia scabiosaeifolia</i> Link.	Ominaeshi	
<i>Patrinia villosa</i> Juss.	Otokoeshi	
<i>Ophiopogon japonicus</i> Ker.	Yomohige	
<i>Dianthus superbus</i> L.	Kawaranadeschiko	

Saussurea ussuriensis Maxim.	Kikuazami	
Erigeron annuus Pers.	Himejoon	
Lonicera japonica Thunb.	Nindō	
Cypripedium japonicum Thunb.	Kumagaisō	
Plantago major L. var. asiatica Dene.	Obako	
Crematis recta L. var. peniculata Thunb.	Senninsō	
Iris japonica Thunb.	Shoga	
Petasites japonicus Miq.	Fuki	
Lysimachia clethroides Duby.	Okatoranoo	17.
Arisaema japonicum Bl.	Tennanshō	
Vitis Thunbergii S. et Z.	Ebizuru	
Coelopeurum gmelini Ledeb.	Shishiudo	
Clematis japonica Thunb.	Iianshōzuru	
Rubus parvifolius L.	[Nawashiroichigo]	
Akebia quinata Dene.	[Akebi]	
Rosa Wichuraiana Crep.	[Terihanoibara]	
Akebia lobata Dene.	[Mitsubaakebi]	
Polygala japonica Hautt.	[Himehagi]	
Deutzia gracilis S. et Z.	[Himeutsugi]	
Spiraea Thunbergii Sieb.	[Iwayanagi]	
Smilax china L.	[Sankirai]	
Acanthopanax ricinifolium S. et Z.	[Bōdara]	
Quercus glandulifera Bl.	[Konara]	
Quercus serrata Thunb.	[Kunugi]	

100

Ōmiyama (Südseite).

(seit 6 Jahre nicht gebrannt)

(Neigung = 36°)

(Probefläche = 2 □. m.)

(Datum 12/4 u. 26/4)

lateinischer Name.	japanischer Name.	頁
Miscanthus sinensis Anders.	Susuki	20

<i>Carex duvariana</i> Fr. et Sar.	Kesuge	5
<i>Poa trivialis</i> L.	Hiime ichigotsunagi	5
<i>Cryptogramme japonica</i> Prantl.	Tachishinobu	5
<i>Viola silvestris</i> Kit. var. <i>grypoceras</i> A. Gr.	Tachitsubosumire	4
<i>Carex confertiflora</i> Boott.	Shirasuge	4
<i>Trycirtis chirta</i> Hook.	Hototogisusō	4
<i>Polygonatum giganteum</i> Dietr. var. <i>Thunbergii</i> Max.	Narukoyuri	3
<i>Phegopteris</i> Totta Mett.	Mizoshida	3
<i>Eupatorium japonicum</i> Thunb.	Hiyodoribana	3
<i>Thalictrum minus</i> L. var. <i>elatum</i> Lecoy.	Akikaramatsu	3
<i>Gentiana Zollingeri</i> Fawe.	Fuderindō	3
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Max.	Rindō	3
<i>Hypericum sampsoni</i> Hce.	Tsukinukiotogiri	3
<i>Salvia japonica</i> Thunb. var. <i>bipinnata</i> Fr. et Sav.	Akinotamurasō	2
<i>Brachypodium silvaticum</i> R. et S.	Yamakamojigusa	2
<i>Potentilla fragarioides</i> L. var. <i>ternata</i> Max.	Mitsubatsuchiguri	2
<i>Aster japonicus</i> Miq.	Yamashirogiku	1
<i>Rubia cordifolia</i> L. var. <i>mungista</i> Miq.	Akane	1
<i>Gynostemma pedata</i> Bl.	Amachazuru	1
<i>Pteris aquilina</i> L.	Warabi	1
<i>Ophiopogon japonicus</i> Ker.	Janohige	1
<i>Aconitum fischeri</i> Reich.	Torikabuto	1
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Maxim.	Yomogi	1
<i>Patrinia scabiosaeifolia</i> Link.	Ominaeshi	1
<i>Patrinia villosa</i> Juss.	Otokoeshi	1
<i>Polygonum cuspidatum</i> S. et Z.	Itadori	1
<i>Arisaema japonicum</i> Bl.	Tennanshō	1
<i>Poa acroleuca</i> Stena.	Mizo ichigotsunagi	1
<i>Dianthus superbus</i> L.	Kawara nadeshiko	1
<i>Osmorhiza japonica</i> S. et Z.	Yabuninjin	1
<i>Senecio Krameri</i> Fr. et Sav.	Yaburegasa	
<i>Cirsium spicatum</i> Maxim.	Yamaazami	
<i>Viola japonica</i> Langsd.	Kosumire	
<i>Clematis recta</i> L. var. <i>paniculata</i> Thunb.	Senninsō	

<i>Asarum caulescens</i> Miq.	Kan aoi
<i>Aster scaber</i> Thunb.	Shirayamagiku
<i>Plectranthus inflexus</i> Vahl.	Yama hakka
<i>Clematis apiifolia</i> DC.	Botanzuru
<i>Aspidium sculeatum</i> Doell, var. <i>japonicum</i> (Fr. et Sav.)	Inode
<i>Taraxacum officinale</i> Wigg. var. <i>glaucescens</i> Koch.	Tanpopo
<i>Lonicera japonica</i> Thunb.	Nindo
<i>Petasites japonicus</i> Miq.	Fuki
<i>Platanthera chlorantha</i> Cust.	Ginbaisō
<i>Inula salicina</i> L.	Kasensō
<i>Saussurea ussuriensis</i> Maxim.	Kikuazami
<i>Bromus japonicus</i> Thunb.	Suzumenoahiki
<i>Astilbe Thunbergii</i> Miq.	Torishishōma
<i>Calanthe reflexa</i> Maxim.	Natsuebine
<i>Hedera helix</i> L.	[Fuyuzuta]
<i>Rubus Buergeri</i> Miq.	[Fuyuichigo]
<i>Viburnum dilatatum</i> Thunb.	[Gamazumi]
<i>Akebia quinata</i> Dene.	[Akebi]
<i>Akebia lobata</i> Dene.	[Mitsubaakebi]
<i>Euonymus alata</i> K. Koch, var. <i>subtriflora</i> Fr. et Sav.	[Komayumi]
<i>Quercus glandulifera</i> Bl.	[Konara]
<i>Rubus palmatus</i> Thunb.	[Kiichigo]
<i>Aucuba japonica</i> Thunb.	[Aoki]
<i>Torreya nucifera</i> S. et Z.	[Kaya]
<i>Quercus myrsinacfolia</i> Bl.	[Shirakashi]
<i>Spiraea japonica</i> L. f.	[Shimotsuke]
<i>Lespedeza bicolor</i> Turcz.	[Hagi]
<i>Smilax china</i> L.	[Sankirai]
<i>Vaccinium bracteatum</i> Thunb.	[Shashanpo]
<i>Deutzia gracilis</i> S. et Z.	[Himeutsugi]
<i>Litsea glauca</i> Sieb.	[Shirodamo]
<i>Rhododendron indicum</i> Sw. var. <i>Kaempferi</i> Max.	[Yamatsutsuji]
<i>Lindera selicea</i> Bl.	[Kuromoji]
<i>Clethra barbinervis</i> S. et Z.	[Ryōbu]
<i>Diervilla grandiflora</i> S. et Z.	[Hakoneutsugi]

Musadogadai (Südseite).

(seit 6 Jahre nicht gebrannt)

(Neigung = 29°)

(Probefläche = 20 □. m.)

(Datum 11/4 u. 27/4)

lateinischer Name.	japanischer Name.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	18
<i>Carex breviculmis</i> R. Br.	Aosuge	3
<i>Carex duvariana</i> Fr. et Sav.	Kesuge	3
<i>Viola silvestris</i> Kit. var. <i>grypceras</i> A. Gr.	Tachitsubosumire	3
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Maxim.	Yomogi	3
<i>Angelica anomala</i> Pall.	Yoroizasa	3
<i>Aster trinervius</i> Roxb. var. <i>adustus</i> Maxim.	Konkiku	3
<i>Disporum sessile</i> Don.	Hōchakusō	3
<i>Viola patrinii</i> var. <i>chinensis</i> Ging.	Sumire	3
<i>Brachypodium japonicum</i> Miq.	Kamojigusa	3
<i>Aspidium dissectum</i> Mett.	Hoschida	2
<i>Houttuynia cordata</i> Thunb.	Dokudami	2
<i>Thalictrum minus</i> L. var. <i>elatum</i> Lecoy.	Akikaramatsu	2
<i>Patrinia villosa</i> Juss.	Otokoeschi	2
<i>Lysimachia clethroides</i> Duby.	Okutoranoo	2
<i>Aster japonicus</i> Miq.	Yamashirogiku	2
<i>Viola japonica</i> Langsd.	Kosumire	2
<i>Aster scaber</i> Thunb.	Schirayamagiku	2
<i>Pteris aquilina</i> L.	Warabi	2
<i>Arimonia viscidula</i> Bge. var. <i>japonica</i> Miq.	Kinmizuhiki	2
<i>Cirsium spicatum</i> Maxim.	Yamaazami	2
<i>Rubia cordifolia</i> L. var. <i>mungista</i> Miq.	Akane	2
<i>Plectranthus glaucocalyx</i> Max. var. <i>japonicus</i> Max.	Iikiokoschi	1
<i>Galium asprellum</i> Michx.	Ōbayaemugura	1
<i>Senecio Kramerii</i> Fr. et Sav.	Yaburegasa	1

Scrophularia patriniana Wydl.	Hinano usutsubo	r
Euphorbia sieboldiana Mor. et Dic.	Natsutodai	r
Vitis Thunbergii S. et Z.	Ebizuru	r
Serratula coronata L.	Tamurasō	
Plantago major L. var. asiatica Dcne.	Ōbako	
Belamacanda chinensis Lem.	Hiōgi	
Potentilla fragarioides L. var. ternata Maxim.	Mitsubatsuchiguri	
Galanium nepalense Sweet.	Furosō	
Dianthus superbus L.	Kawara nadeshiko	
Lactuca Thunbergiana (A. Gr.) Maxim.	Nigana	
Angelica decurtiva Miq.	Nodake	
Petasites japonicus Miq.	Fuki	
Clematis recta L. var. paniculata Thunb.	Senninsō	
Osmunda regalis L. var. japonica Milde.	Zemmai	
Platanthera mandarinorum Reich. f.	Yamasagisō	
Cirsium japonicum DC.	Noazami	
Akebia quinata Dcne.	[Akebi]	
Clematis apiifolia DC.	Botanzuru	
Rosa multiflora Thunb.	[Noibara]	25
Polygala japonica Haultt.	[Himehagi]	
Rubus parvifolius L.	[Nawashiroichigo]	
Akebia lobata Dcne.	[Mitsubaakebi]	
Lespedeza bicolor Turcz.	[Hagi]	
Deutzia gracilis S. et Z.	[Himeutsugi]	
Quercus glandulifera Bl.	[Konara]	
Smilax china L.	[Sankirai]	
Rubus palmatus Thunb.	[Kiichigo]	
Spiraea japonica L. f.	[Shimotsuke]	
Kraunhia floribunda (Willd) Taub.	[Fuji]	
Viburnum dilatatum Thunb.	[Gamazumi]	
Diervilla grandiflora S. et Z.	[Hakoneutsugi]	
Spiraea Thunbergii Sieb.	[Iwayanagi]	

Suzuriishi (Südseite)

(über 8 Jahre nicht gebrannt)

(Neigung = 33°)

(Probefläche = 20 □. m.)

(Datum 12/4 u. 27/4)

lateinischer Name.	japanischer Name.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	10
<i>Carex lancoolata</i> Boott.	Hikagesuge	4
<i>Phegopteris</i> Totta Mett.	Mizoshida	
<i>Viola silvestris</i> Kit. var. <i>gryoceras</i> A. Gr.	Tachitsubosumire	3
<i>Dianthus superbus</i> L.	Kawaranadeshiko	3
<i>Ixeris Thunbergii</i> A. Gr.	Nigana	3
<i>Aspidium aristatum</i> Sw.	Hosoba kanawarabi	3
<i>Artemisia japonica</i> Thunb.	Otokoyomogi	3
<i>Tricytis hirta</i> Hook.	Hototogisusō	3
<i>Muhlenbergia Huegerii</i> Trin.	Ōnezumigaya	3
<i>Lilium Maximowiczii</i> Regel.	Kōniyuri	3
<i>Sedum Kantschaticum</i> Fisch.	Kirinsō	3
<i>Viola Patrinii</i> DC. var. <i>chinensis</i> Ging.	Sumire	2
<i>Carpesium cernuum</i> L.	Sajigankubisō	2
<i>Brachypodium silvaticum</i> R. et S.	Yamakamojigusa	2
<i>Woodwardia radicans</i> Sm. var. <i>orientalis</i> Lürs.	Komochishida	2
<i>Osmunda regalis</i> L. var. <i>japonica</i> Mild.	Zemmai	2
<i>Potentilla fragarioides</i> L.	Kijimushiro	2
<i>Pteris cretica</i> L.	Ōbainomotosō	2
<i>Rubia cordifolia</i> L. var. <i>mongista</i> Miq.	Akane	2
<i>Euphorbia Sieboldiana</i> Morr. et Dene.	Natsutōdai	2
<i>Cirsium spicatum</i> (Maxim)	Yamaazami	2
<i>Pteris aquilina</i> L.	Warabi	2
<i>Aster japonicus</i> Miq.	Yamashirogiku	2
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Maxim.	Rindō	2

Chrysanthemum sinense Sab. var. japonicum Max.	Ryunōgiku	1
Arimonia cordifolia L. var. mungista Miq.	Kinmizuhiki	1
Artemisia vulgaris L. var. indica Maxim.	Yomogi	1
Solidago Virga-aurea L.	Akinokirinsō	1
Senecio krameri Fr. et Sav.	Yaburegasa	1
Saussurea japonica DC.	Himehigotai	1
Seseli Libanostis Koch. var. daucifolia DC.	Ibukibōfu	1
Serratula coronata L.	Tamura-sō	1
Houttuynia cordata Thunb.	Dokudami	1
Clematis japonica Thunb.	Hanshōzuru	
Astilbe Thunbergii Miq.	Toriashishōma	
Polygonatum giganteum Dietr. var. Thunbergii Max.	Narukoyuri	
Petacites japonicus Miq.	Fuki	
Thalictrum minus L. var. elatum Lecoy.	Akikaramatsu	
Cymbidium virens Lindl.	Shunran	
Lonicera japonica Thunb.	Suikazura	
Inula salicina L.	Kasensō	
Atractylis lancea Thunb.	Okerā	
Saussurea ussuriensis Maxim.	Kikuazami	
Rhodea japonica Rhot.	Omoto	
Ligularia Kämpferi S. et Z.	Tsuwabuki	
Rubus parvifolius L.	[Nawashiroichigo]	
Polygala japonica Hautt.	[Himehagi]	
Rubus incisus Thunb.	[Nigaichigo]	
Hedera helix L. var. colchico C. Koch.	[Fuyūichigo]	
Akebia lobata Dene.	[Mitsuba-akebi]	
Rubus palmatus Thunb.	[Momijūichigo]	
Quercus glandulifera Bl.	[Konara]	
Deutzia gracilis S. et Z.	[Himeutsugi]	
Deutzia scabra Thunb.	[Utsugi]	
Diervilla grandiflora S. et Z.	[Hakoneutsugi]	
Berberis Thunbergii DC.	[Megi]	
Lindera selicea Bl.	[Kuromoji]	
Rhododendron indicum Sw. var. Kämpferi Max.	[Yamatsutsuji]	

<i>Smilax china</i> L.	[Sankirai]
<i>Eurya japonica</i> Thunb.	[Hisakaki]
<i>Lespedeza bicolor</i> Turcz.	[Hagi]
<i>Clethra barbinervis</i> S. et Z.	[Ryōbu]
<i>Litsea glauca</i> Sieb.	[Shirodamo]
<i>Spiraea japonica</i> L. f.	[Shimotsuke]
<i>Aucuba japonica</i> Thunb.	[Aoki]

100

Suzuriishi (Nordseite).

(bis 24 Meiji (1891) alljährlich und später
im Jahre 33 Meiji (1900) gebrannt)

(Neigung = 32°)

(Probefläche = 20 □. m.)

(Datum 13/4 u. 27/4)

lateinischer Name.	japanischer Name.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	12
<i>Phegopteris toita</i> Mett.	Mizoshida	5
<i>Chrysanthemum sinense</i> Sub. var. <i>japonicum</i> Max.	Ryunōgiku	4
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Maxim.	Rindō	4
<i>Carex duvariana</i> Fr. et Sav.	Kesuge	3
<i>Carex confertiflora</i> Boott.	Shirasuge	3
<i>Brachypodium silvaticum</i> R. et S.	Yamakamojigusa	3
<i>Carex brunnea</i> Thunb.	Nakirisuge	3
<i>Cirsium spicatum</i> Maxim.	Yamaazami	3
<i>Kraunhia floribunda</i> (Willd) Taub.	Fuji	3
<i>Disporum sessile</i> Don.	Hōchakusō	3
<i>Trityrtis hirta</i> Hook.	Hototogisusō	2
<i>Carpesium cernuum</i> L.	Sajigankubiso	2
<i>Clematis heracleifolia</i> DC. var. <i>stans</i> S. et Z.	Kusabotan	2
<i>Lactuca denticulata</i> Maxim.	Yakushisō	2
<i>Patrinia villosa</i> Juss.	Otokoeshi	2

<i>Thalictrum minus</i> L. var. <i>elatum</i> Lecoy.	Okikaramatsu	2
<i>Campanula punctata</i> Lam.	Hotarubukuro	2
<i>Solidago Virga-aurea</i> L.	Akinakirinsō	2
<i>Lysimachia clethroides</i> Duby.	Okatoranoo	1
<i>Patrinia scabiosaefolia</i> Link.	Ominaeshi	1
<i>Dianthus superbus</i> L.	Kawaranadeshiko	1
<i>Osmunda regalis</i> L. var. <i>japonica</i> Milde.	Zemmai	1
<i>Ficris hieracioides</i> L. var. <i>japonica</i> Rgl.	Kōzorina	1
<i>Aristolochia Kämpferi</i> Willd.	Ōba umanosuzukusa	1
<i>Serratula coronata</i> L.	Tamurasō	1
<i>Heteropappus hispidus</i> Less.	Yamajinogiku	1
<i>Cryptogramme japonica</i> Prantl.	Tachishinobu	1
<i>Eupatorium japonicum</i> Thunb.	Hiyoderibana	1
<i>Inula salicina</i> L.	Kasensō	1
<i>Woodwardia radicans</i> Sm. var. <i>orientalis</i> Lürs.	Komochishida	1
<i>Asteromæa indica</i> Bl.	Yomena	1
<i>Clematis recta</i> L. var. <i>paniculata</i> Thunb.	Seminsō	
<i>Petasites japonicus</i> Miq.	Fuki	
<i>Aster scaber</i> Thunb.	Shirayamagiku	
<i>Senecio Krameri</i> Fr. et Sav.	Yabaregasa	
<i>Potentilla fragarioides</i> L. var. <i>ternata</i> Maxim.	Mitsubatsuchiguri	
<i>Houttuynia cordata</i> Thunb.	Dokudami	
<i>Coclopleurum gmelini</i> Ledeb.	Shishindo	
<i>Astilbe Thunbergii</i> Miq.	Toriasbishōma	
<i>Euphorbia sieboldiana</i> Mor. et Dic.	Natsutōdai	
<i>Artemisia japonica</i> Thunb.	Otokoyomogi	
<i>Seseli Libanostis</i> Koch. var. <i>daucifolia</i> DC.	Ibukōji	
<i>Plantago major</i> L. var. <i>asiatica</i> Dene.	Ōbako	
<i>Ligularia Kämpferi</i> S. et Z.	Tsuwabuki	
<i>Cymbidium virens</i> Lindl.	Shunran	
<i>Allium japonicum</i> Rgl.	Yamirakky	
<i>Lonicera japonica</i> Thunb.	Nindō	
<i>Rubus Buergeri</i> Miq.	[Fuyūchigo]	
<i>Spiræa japonica</i> L. f.	[Nawashirochigo]	

Rubus parvifolius L.	[Shimotsuke]	} 25
Polygala japonica Haultt.	Himehagi	
Akebia lobata Dene.	Mitsuba-akebi	
Ficus foveolata Wall.	Itabikazura	
Quercus glandulifera Bl.	Konara	
Quercus serrata Thunb.	Kunugi	
Smilax china L.	Sankirai	
Rubus palmatus Thunb.	Kiichigo	
Clethra barbinervis S. et Z.	Ryōbu	
Diervilla grandiflora S. et Z.	Iakoneutsugi	
Deutzia gracilis S. et Z.	Himeutsugi	
Deutzia scabra Thunb.	Utsugi	
Lindera selicera Bl.	Kuromoji	
Torreya nucifera S. et Z.	Kaya	
Aucuba japonica Thunb.	Aoki	
Eurya japonica Thunb.	Iisakaki	
Spiraea Thunbergii Sieb.	Iwayanagi	
Ardisia crenata Sims.	Manryō	
Rhododendron indicum Sw, var. Kämpferi Max.	Yamatsutsuji	
Macleya cordata R. Br.	Chanpagiku	
Poa trivialis L.	Himeichigotsunagi	
Pteris serrulata L. f.	Inomosō	
Pteris aquilina L.	Warabi	

Sannodai (Nordseite).

(bis 24 Meiji (1891) alljährlich und im

Jahre 33 Meiji (1900) gebrannt)

(Neigung = 35°)

(Probefläche = 20 □. m.)

Datum 14/4 u. 27/4

lateinischer Name.	japanischer Name.	%
Miscanthus sinensis Anders.	Susuki	16

Carex breviculmis R. Br.	Aosuge	4
Picris hieracioides L. var. japonica Rgl.	Kōzōrina	4
Carex brunnea Thunb.	Nakirisuge	4
Viola silvestris Kit. var. grypoceras A. Gr.	Tachitsubosumire	3
Tricyrtis hirta Hook.	Hototogisusō	3
Gentiana scabra Bge. var. Buergeri Maxim.	Rindō	3
Salvia japonica Thunb. var. bipinnata Fr. et Sav.	Akinotamurao	3
Carpesium cernuum L.	Sajigankubis	3
Artemisia vulgaris L. var. indisa Maxim.	Yomogi	3
Aster japonicus Miq.	Yamashirogiku	2
Crawfordia pterygocalyx Hemsl.	Tsururindō	2
Carex conica Boott.	Himekansuge	2
Polygonatum lasianthum Maxim.	Miyamanarukoyuri	2
Lactuca denticulata Maxim.	Yakushisō	2
Aspidium lacrum Sw.	Kumawarabi	2
Asarum Blumei Duch.	Kan-aoi	2
Eupatorium japonicum Thunb.	Hiyodorihana	2
Aspidium crythrosarum Eat.	Benishida	2
Aristolochia Kämpferi Willd.	Ōba-umanosuzukusa	2
Osmunda regalis L. var. japonica Mild.	Zemmai	1
Patrinia villosa Juss.	Otokeeshi	1
Astilba Thunbergii Miq.	Toriashishōma	1
Cirsium spicatum Max.	Yamaazami	1
Senecio Krameri Fr. et Sav.	Yaburegasa	1
Cypripedium japonicum Thunb.	Kumagusō	1
Cimbidium virens Lindl.	Shūran	1
Patrinia scabiosaefolia Link.	Omi-aeshi	1
Clematis japonica Thunb.	Hanshōran	1
Lilium auratum Lindl.	Yamayuri	1
Houttuynia cordata Thunb.	Pokudami	1
Pteris aquilina L.	Warabi	
Brachypodium silvaticum R. et S.	Yamakamojigusa	
Ligularia Kämpferi S. et Z.	Tsuwabuki	
Rhodea japonica Rhot.	Omoto	

<i>Clematis recta</i> L. var. <i>paniculata</i> (Thunb).	Senninsō	
<i>Mitchella undulata</i> S. et Z.	Tsuruaridōshi	
<i>Lindera umbellata</i> Thunb.	[Kanakeginoki]	
<i>Quercus glandulifera</i> Bl.	[Konara]	
<i>Rhus succedanea</i> L.	[Tsuraurushi]	
<i>Rosa Wichuraiana</i> Crep.	[Terihanoibara]	
<i>Hedera helix</i> L. var. <i>colchica</i> C. Koch.	[Fuyuzuta]	
<i>Akebia quinata</i> Dene.	[Akebi]	
<i>Spiraea japonica</i> L. f.	[Shimotsuke]	
<i>Trachelospermum jasminoides</i> Lemaire.	[Teikakazura]	
<i>Ficus foveolata</i> Wall.	[Itabikazura]	
<i>Rubus Buergeri</i> Miq.	[Fuyuchigo]	
<i>Akebia lobata</i> Dene.	[Mitsubaakebi]	} 23
<i>Vaccinium bracteatum</i> Thunb.	[Shashanpo]	
<i>Pieris japonica</i> D. Don.	[Asebi]	
<i>Illicium anisotum</i> L.	[Shikimi]	
<i>Thea japonica</i> (L.) Wois.	Tsubaki	
<i>Eurya japonica</i> Thunb.	Hisakaki	
<i>Rubus palmatus</i> Thunb.	Kiichigo	
<i>Rosa multiflora</i> Thunb.	Noibara	
<i>Rhododendron indicum</i> Sw. var. <i>Kämpferi</i> Max.	Yamatsutsuji	
<i>Osmanthus aquifolium</i> B. et H.	Hirragi	
<i>Lindela selicea</i> Bl.	Kuromoji	
<i>Diervilla grandiflora</i> S. et Z.	Hakoneutsugi	
<i>Deutzia gracilis</i> S. et Z.	Himeutsugi	
<i>Rubus parvifolius</i> L.	Shimotsuke	
<i>Smilax china</i> L.	Sankirai	
<i>Litsea glauca</i> Sieb.	Shirodamo	
<i>Zanthoxylum schimnifolium</i> S. et Z.	Inusanshō	

Sannodai (Südseite).

(bis 24 Meiji (1891) alljährlich und im

Jahre 33 Meiji (1900) gebrannt)

(Neigung = 31°)

(Probefläche = 20 □. m.)

(Datum 14/4 u. 27/4)

lateinischer Name.	japanischer Name.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	14
<i>Oxalis corniculata</i> L.	Katabami	4
<i>Carex brevicurmis</i> R. Br.	Aosuge	3
<i>Viola Keiskei</i> Miq.	Marubasumire	3
<i>Carex confertiflora</i> Bott.	Shirasuge	3
<i>Phagopteris totta</i> Mett.	Mizoshida	3
<i>Disporum sessile</i> Don.	Hōchakusō	3
<i>Carex brunnea</i> Thunb.	Nakirisuge	3
<i>Salvia japonica</i> Thunb. var. <i>Buergeri</i> Max.	Akinotamurasō	2
<i>Brachypodium silvaticum</i> R. et S.	Yamakamojigusa	2
<i>Viola silvestris</i> Kit. var. <i>gryoceras</i> A. Gr.	Tachitsubosumire	2
<i>Carpesium abrotanoides</i> L.	Yabutabako	2
<i>Eupatorium japonicum</i> Thunb.	Hiyodoribana	2
<i>Crepis japonica</i> Benth.	Onitabirako	2
<i>Bothriospermum tenellum</i> Fisch et Mey. var. <i>aspergoïdes</i> Max	Hanaibana	2
<i>Aster japonicus</i> Miq.	Yamashirogiku	2
<i>Eupatorium Kirilowii</i> Turcz.	Sawahiyodori	2
<i>Cirsium spicatum</i> Max.	Yamaazami	2
<i>Euphorbia sieboldiana</i> Mor. et Dec.	Natsutodai	2
<i>Aspidium aristatum</i> Sw.	Hosoba-kanawarabi	2
<i>Peracarpa circeoides</i> H. Fee.	Tanigkyō	2
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Maxim.	Yemogi	1
<i>Senecio Krameri</i> Fr. et Sav.	Yaburegasa	1
<i>Lactuca debilis</i> (Thunb.) Maxim.	Jishibari	1

<i>Pteris aquilina</i> L.	Warabi	I
<i>Asplenium liniolatum</i> Thunb.	Hierashida	I
<i>Cyrtopodium japonicum</i> Thunb.	Kumagaiō	I
<i>Gnaphalium multiceps</i> Wall.	Chichikogusa	I
<i>Rubia cordifolia</i> L. var. <i>Mungista</i> Miq.	Akane	I
<i>Thalictrum minus</i> L. var. <i>elatum</i> Lecoy.	Akikaramatsu	
<i>Cryptogramme japonica</i> Prantl.	Tachishinobu	
<i>Cirsium japonicum</i> DC.	Noazami	
<i>Petasites japonicus</i> Miq.	Fuki	
<i>Patrinia villosa</i> Juss.	Otokoeshi	
<i>Astilbe Thunbergii</i> Miq.	Toriashishōma	
<i>Cremastra Wallichiana</i> Lindl.	Saihaïran	
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Maxim.	Rindō	
<i>Lactuca denticulata</i> Maxim.	Yakushisō	
<i>Cymbidium virens</i> Lindl.	Shunran	
<i>Rhodea japonica</i> Rhot.	Omoto	
<i>Clematis arifolia</i> DC.	Botanzuru	
<i>Akebia quinata</i> Dene.	[Akebi]	
<i>Rubus incisus</i> Thunb.	[Nigaichigo]	
<i>Duchesnea indica</i> Fock.	[Hebiichigo]	
<i>Clematis japonica</i> Thunb.	[Hanshōzuru]	
<i>Sambucus racemosa</i> L.	[Niwatoko]	
<i>Myrica rubra</i> S. et Z.	[Yamamomo]	
<i>Eurya japonica</i> Thunb.	[Hisakaki]	
<i>Rhododendron indicum</i> Sw. var. <i>Kempferi</i> Max.	[Yamatsutsuji]	
<i>Torreya nucifera</i> S. et Z.	[Kaya]	
<i>Pieris japonica</i> D. Don.	[Asebi]	
<i>Deutzia gracilis</i> S. et Z.	Himeutsugi	
<i>Lindera selicca</i> Bl.	Kuromoji	
<i>Quercus glandulifera</i> Bl.	Konara	
<i>Thea japonica</i> (L.) Nois.	Tsubaki	
<i>Machilus Thunbergii</i> S. et Z.	Tabu	
<i>Rosa multiflora</i> Thunb.	Noibara	
<i>Acanthopanax ricinifolium</i> S. et Z.	Bōdara	

<i>Litsea glauca</i> Sieb.	Shirodamo
<i>Rubus palmatus</i> Thunb.	Kiichigo
<i>Diervilla grandiflora</i> S. et Z.	Hakoneutsugi
<i>Clematis recta</i> L. var. <i>asiatica</i> Dene.	Senninsō
<i>Lonicera japonica</i> Thunb.	Nindō

Nanamagari.

(seit 10 Jahren nicht gebrannt)

(Neigung = 35°)

(Probefläche = 20 □. m.)

(Datum 15/4 u. 26/4)

lateinischer Name.	japanischer Name.	%
<i>Miscanthus sinensis</i> Anders.	Susuki	11
<i>Artemisia japonica</i> Thunb.	Otokoyomogi	5
<i>Polygonatum officinale</i> All.	Amadokoro	4
<i>Carex incisa</i> Boott.	Kawarasuge	4
<i>Carex duvariana</i> Fr. et Sav.	Kesuge	4
<i>Cryptogramme japonica</i> Prantl.	Tachishinobu	3
<i>Pieris hieratioides</i> L. var. <i>japonica</i> Rgl.	Kōzorina	3
<i>Chrysanthemum sinense</i> Sab. var. <i>japonicum</i> Max.	Ryunōgiku	3
<i>Viola japonica</i> Langsd.	Kosumite	3
<i>Aristolachia Kaempferi</i> Willd.	Ōba-umamosasukusa	3
<i>Disporum sesile</i> Don.	Hōchakusō	2
<i>Carex conica</i> Boott.	Himekansuge	2
<i>Brachypodium silvaticum</i> R. et S.	Yamakamōjigusa	2
<i>Serratula coronata</i> L.	Tamura-sō	2
<i>Aster japonicus</i> Miq.	Yamashirogiku	2
<i>Thalictrum minus</i> L. var. <i>elatatum</i> Lecoy.	Akikaramatsu	2
<i>Potentilla fragarioides</i> L.	Kijimushiro	2
<i>Polygonatum giganteum</i> Dietr. var. <i>Thunbergii</i> Max.	Narukoyuri	2
<i>Sedum kamtschaticum</i> Fisch.	Kirinsō	2

<i>Pteris aquilina</i> L.	Warabi	I
<i>Angelica decursiva</i> Miq.	Nodake	I
<i>Cirsium spicatum</i> Maxim.	Yamaazami	I
<i>Euphorbia Sieboldiana</i> Mor. et Dec.	Natsutōdai	I
<i>Astilba Thunbergii</i> Miq.	Toriashishōma	I
<i>Clematis recta</i> L. var. <i>paniculata</i> Thunb.	Senninso	I
<i>Patrinia villosa</i> Juss.	Otokoeshi	I
<i>Osmunda regalis</i> L. var. <i>japonica</i> Milde.	Zemmai	I
<i>Potentilla fragarioides</i> L. var. <i>ternata</i> Maxim.	Mitsubatsuchiguri	I
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Max.	Yomogi	
<i>Saussurea ussuriensis</i> Max.	Kikuzami	
<i>Senecio Krameri</i> Fr. et Sav.	Yaburegasa	
<i>Arisaema japonicum</i> Bl.	Tennanshō	
<i>Lithospermum zollingeri</i> A. DC.	Hotarukazura	
<i>Asarum Blumei</i> Duch.	Kan-aoi	
<i>Cimicifuga foetida</i> L. var. <i>simplex</i> Huth.	Sarashinashōma	
<i>Lonicera japonica</i> Thunb.	Nindō	
<i>Petasites japonicus</i> Miq.	Fuki	
<i>Salvia japonica</i> Thunb. var. <i>bipinnata</i> Fr. et Sav.	Akinotamurasō	
<i>Dianthus superbus</i> L.	Kawaranadeshiko	
<i>Lilium auratum</i> Lindl.	Yamayuri	
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Max.	Rindō	
<i>Luzula campestris</i> DC. var. <i>capitata</i> Miq.	Suzumenohiye	
<i>Plectranthus glaucocalyx</i> Max. var. <i>japonicus</i> Max.	Yamahakka	
<i>Rubus palmatus</i> Thunb.	[Momijibaichigo]	
<i>Rubus parvifolius</i> L.	[Nawashiroichigo]	
<i>Akebia lobata</i> Dcne.	[Mitsubaakebi]	
<i>Vicia unijuga</i> Al. Br.	[Nantenhagi]	
<i>Ononymus alata</i> K. Koch. var. <i>subtriflora</i> Fr. et Sav.	[Komayumi]	
<i>Spiraea japonica</i> L. f.	[Shimotsuke]	
<i>Quercus glandulifera</i> Bl.	[Konara]	
<i>Eurya japonica</i> Thunb.	[Hisakaki]	
<i>Diervilla grandiflora</i> S. et Z.	[Hakoneutsugi]	
<i>Viburnum dilatatum</i> Thunb.	[Gamazumi]	

<i>Machilus Thunbergii</i> S. et Z.	[Tabu]
<i>Clethra barbinervis</i> S. et Z.	[Ryōbu]
<i>Smilax china</i> L.	[Sankirai]
<i>Quercus acuta</i> Thunb.	[Akagashi]
<i>Quercus myrsinaefolia</i> Bl.	[Urajirogashi]
<i>Rosa multiflora</i> Thunb.	[Noibara]
<i>Rhododendron indicum</i> Sw. var. <i>Kaempferi</i> Max.	[Yamatsutsuji]
<i>Rubus palmatus</i> Thunb.	[Kiichigo]
<i>Lindera selicea</i> Bl.	[Kuromoji]
<i>Spiraea Thunbergii</i> Sieb.	[Iwayanagi]

Musadogadai (Nordseite).

(über 10 Jahre nicht gebrannt)

(Neigung = 32°)

(Probefläche = 20 □. m.)

(Datum 13/4 u. 27/4)

lateinischer Name.	japanischer Name.	%
<i>Miscanthus sinensis</i> Anders.	Sesuki	8
<i>Carex Ringoldiana</i> Boott.	Juzusuge	4
<i>Pertya Scandens</i> Sch. Bip. var. <i>ovata</i> Maxim.	Kōyabōki	4
<i>Brachypodium sylvaticum</i> R. et S.	Yamakamojigusa	4
<i>Phegopteris</i> Totta Mett.	Mizoshida	3
<i>Carex duvariana</i> Fr. et Sav.	Kesuge	3
<i>Viola silvestris</i> Kit. var. <i>grapoceras</i> A. Gr.	Tachitsubosumire	3
<i>Carex brunnea</i> Thunb.	Nokirisuge	3
<i>Chrysanthemum sinense</i> Sab. var. <i>japonicum</i> Max.	Ryunōgiku	3
<i>Aster trinervius</i> Roxb. var. <i>adustus</i> Max.	Kongiku	2
<i>Tricyrtis hirta</i> Hook.	Hotogogisus	2
<i>Saussurea japonica</i> DC.	Himehigotai	2
<i>Viola Patrinii</i> DC. var. <i>chinensis</i> Ging.	Sumire	2
<i>Ixeris Thunbergii</i> A. Gr.	Nigana	2

<i>Arimonia cordifolia</i> L. var. <i>mungista</i> Miq.	Kimmizuhiki	2
<i>Salvia japonica</i> Thunb. var. <i>bipinnata</i> Fr. et Sav.	Akirōtamurasō	2
<i>Aristolochia Kämpferi</i> Willd.	Ōba-umanosuzukusa	2
<i>Gentiana scabra</i> Bge. var. <i>Buergeri</i> Maxim.	Rindō	1
<i>Angelica decursiva</i> Miq.	Nodake	1
<i>Cirsium spicatum</i> Maxim.	Yamaazami	1
<i>Woodwardia radicans</i> Sw. var. <i>japonica</i> I ūrs.	Komochishida	1
<i>Polygonum cuspidatum</i> S. et Z.	Itadori	1
<i>Aster japonicus</i> Miq.	Yamashirogiku	1
<i>Senecio Krameri</i> Fr. et Sav.	Yaburegasa	1
<i>Cirsium japonicum</i> DC.	Nozami	1
<i>Potentilla fragarioides</i> L. var. <i>ternata</i> Maxim.	Mitsubatsuchiguri	1
<i>Astilbe Thunbergii</i> Miq.	Toriashishōma	1
<i>Clematis recta</i> L. var. <i>paniculata</i> Thunb.	Senninsō	1
<i>Thalictrum minus</i> L. var. <i>elatum</i> Lecoy.	Akikaramatsu	1
<i>Artemisia vulgaris</i> L. var. <i>indica</i> Maxim.	Yomogi	
<i>Lonicera japonica</i> Thunb.	Nindō	
<i>Plantago major</i> L. var. <i>asiatica</i> Dene.	Ōbako	
<i>Lysimachia clethroides</i> Duby.	Otokoeshi	
<i>Lilium auratum</i> Lindl.	Yamayuri	
<i>Ligularia Kämpferi</i> S. et Z.	Tsuwabuki	
<i>Belamacanda chinensis</i> Lem.	Ihiōgi	
<i>Calanthe discolor</i> , Lindl.	Ebineran	
<i>Clematis heracleifolia</i> DC.	Tsuriganeso	
<i>Saussurea ussuriensis</i> Maxim.	Kikuazami	
<i>Houttuynia cordata</i> Thunb.	Dokudami	
<i>Clematis heracleifolia</i> DC. var. <i>Stans</i> (S. et Z.)	Kusabotan	
<i>Polygonatum giganteum</i> Dietr. var. <i>Thunbergii</i> Max.	Narukoyuri	
<i>Petasites japonicus</i> Miq.	Fuki	
<i>Taraxacum officinale</i> Wigg. var. <i>glauciscens</i> , Koch.	Tanpopo	
<i>Hedera helix</i> L. var. <i>colchica</i> C. Koch.	Fuyuzuta	
<i>Akebia lobata</i> Dene.	Mitsubaakebi	
<i>Rubus parvifolius</i> L.	Nawashiroichigo	
<i>Rubus Buergeri</i> Miq.	Fuyuchigo	

Clematis japonica Thunb.	Hanshōzuru	} 35
Acanthopanax spinosum Miq.	Ukogi	
Trachelospermum jasminoides Lemeire.	Teikakazura	
Celastrus articulatus Thunb.	Tsurumemodoki	
Eurya japonica Thunb.	Hisakahi	
Quercus myrsinaefolia Bl.	Urajiroga-shi	
Quercus glandulifera Bl.	Konara	
Quercus serrata Thunb.	Kunugi	
Torreya nucifera S. et Z.	Kaya	
Viburnum dilatatum Thunb.	Gamazumi	
Machilus Thunbergii S. et Z.	Tabu	
Lindera selicca Bl.	Kuromoji	
Deutzia gracilis S. et Z.	Himeutsugi	
Diervilla grandiflora S. et Z.	Hakoneutsugi	
Rosa multiflora Thunb.	Noibara	
Rubus palmatus Thunb.	Kiichigo	
Eurya ochraceae Szysz.	Sakaki	
Ardisia crenata Sims.	Manryō	
Spiraea Thunbergii Sieb.	Iwayanagi	
Rhododendron indicum Sw. var. Kämpferi Max.	Yamatsutsuji	
Rubus parvifolius L.	Shimotsuke	
Aukuba japonica Thunb.	Aoki	
Vaccinium bracteatum Thunb.	Shashampo	
Lespedeza bicolor Turcz.	Hagi	

Aus den gegebenen Tabellen ist erkenntlich, dass *Arten und Zahl der Pflanzen* auf den Flächen durch das häufige *Brennen* der *Hara* nach und nach vermindert werden. Auf jeder *Hara* kommt das *Susuki* oder *Kayagrass* im Vorrang vor, weil es ein starkes Anpassungsvermögen hat und überall noch gedeiht, wo der Boden durch Zufall verwüstet und andere Gewächse nicht mehr gut zu wachsen vermögen, es macht eben die geringsten Ansprüche an den Boden.

Auf selten gebrannter *Hara* fällt der Procentsatz an *Kaya* ab, dagegen findet man grosse Mengen von Baum- und Halbbaum-Arten, namentlich

Quercus glandulifera, sowie auch mancherlei Grassarten, welche man auf oft gebrannter *Hara* kaum antreffen wird. *Hara*-Boden welcher längere Zeit nicht gebrannt wird, geht in seine ursprüngliche Vegetationsform (den Wald) wieder über.

Sengen (Nordseite).

(Urwald)

(Neigung = 35°)

(Grassarten der ganzen Nordseite)

(Datum 15/4 u. 28/4)

lateinischer Name.	japanischer Name.	Ordnung.
<i>Viola silvestris</i> Kit. var. <i>gryoceras</i> A. Gr.	Tachitsubosumire	nach der Häufigkeit in absteigender Skala geordnet. ↑ ↓
<i>Saxifraga contusaefolia</i> S. et Z.	Daimonjisō	
<i>Prenanthes acerifolia</i> Maxim.	Fukuwōso	
<i>Cardiandra alternifolia</i> S. et Z.	Kusajisai	
<i>Carex brunea</i> Thunb.	Nakirisuge	
<i>Tricyrtis hirta</i> Hook.	Hototogisusō	
<i>Ligularia japonica</i> Less.	Hankaisō	
<i>Polygonatum lasianthum</i> Maxim.	Miyamanarukoyuri	
<i>Aspidium lacerum</i> Sw.	Kumawarabi	
<i>Heloniopsis japonica</i> Maxim.	Shirohana-shōjōbakama	
<i>Aconitum Fisheri</i> Reich.	Torikabuto	
<i>Ophiopogon japonicus</i> Ker.	Janohige	
<i>Ainsliea acerifolia</i> Sch. Bip.	Momijihaguma	
<i>Carex Morrowi</i> Boott.	Kansuge	
<i>Angerica polymorpha</i> Maxim.	Shiramesenkiu	
<i>Aspidium erythrorum</i> Fat.	Benishida	
<i>Carex duvariana</i> Fr. et Sav.	Kesuge	
<i>Disporum sesile</i> Don.	Hōchakusō	
<i>Hymenophyllum barbatum</i> Bak.	Kōyakokeshinobu	
<i>Senecio Krameri</i> Fr. et Sav.	Yaburegasa	
<i>Astilbe Thunbergii</i> Miq.	Toriashishōma	

Gentiana scabra Bge. var. Buergeri Maxim.	Rindō
Aster japonicus Miq.	Yama-shirog'ku
Anemone hepatica L.	Suhamasō
Cacalia delphiniifolia S. et Z.	Momijigasa
Carex conica Boott.	Himekansuge
Cirsium spicatum Maxim.	Yamaazami
Cypripedium japonicum Thunb.	Kumagaiso
Hosta Caeulea (Andr) Tratt.	Gibōshi
Cimicifuga japonica Spr.	Kikenshōmu
Peris cretica L.	Ōbainomotosō
Clematis japonica Thunb.	Hanshōzuru
Calantha reflexa Maxim.	Natsuebine
Cypripedium debile Rehb. f.	Koatsumorisō
Arisaema japonicum Bl.	Tennanshō
Asarum Blumei Duch.	Kanaoi
Plagiogyria enphlebia Mett.	Kijūnoo
Calanthe discolor, Lindl.	Ebine
Lycopodium serratum Thunb.	Tōgeshiba
Cymbidium virens Lindl.	Shunran

Sengen (Südseite).

(Urwald)

(Neigung = 34°)

(Grassarten in der ganzen Südseite)

(Datum 17/4 u. 28/4)

lateinischer Name.	japanischer Name.	Ordnung.
Ainslia acerifolia Sch. Bip.	Momijihaguma	
Carex conica Boott.	Himekansuge	nach der
Aspidium aristatum Sw.	Hosoba kanawarabi	Häufigkeit in
Angelica polymorpha Maxim.	Shiranesenkū	absteigender
Saxifraga contusaefolia S. et Z.	Daimonjiso	Skala geord-
Carpesium cernuum L.	Sajigankubisō	net.

<i>Cardiandra alternifolia</i> S. et Z.	Kusaajisai
<i>Ophiopogon japonicus</i> Ker.	Janohige
<i>Cryptogramme japonica</i> Prandl.	Kanshinobu
<i>Lilium cordifolium</i> Thunb.	Ubayuri
<i>Woodwardia radicans</i> Sw. var. <i>orientalis</i> Lüers.	Komochishida
<i>Asarum Blumei</i> Duch.	Kanaoi
<i>Arisaema japonicum</i> Bl.	Tennanshō
<i>Hosta Sieboldiana</i> (H. K.) Engl.	Tōgibōshi
<i>Polygonatum lasianthum</i> Sat. Maxim.	Miyamanarukoguri
<i>Adiantum monocephalum</i> Sat.	Hakoneshida
<i>Carex Morrowi</i> Boott.	Kansuge
<i>Calanthe discolor</i> Lindl.	Ebine
<i>Cirsium spicatum</i> Maxim.	Yamaazami
<i>Cymbidium virens</i> Lindl.	Shunran
<i>Astilbe Thunbergii</i> Miq.	Toriashishōma
<i>Iris japonica</i> Thunb.	Shaga
<i>Senecio Krameri</i> Fr. et Sav.	Yaburegasa
<i>Aster japonicus</i> Miq.	Yamashirogiku
<i>Disporum sessile</i> Don.	Hōchakusō
<i>Cimicifuga foetida</i> L. var. <i>simplex</i> Huth.	Sarashinashōma
<i>Anemone hepatica</i> L.	Suhamasō
<i>Solidago Virga-aurea</i> L.	Awadachisō
<i>Heteropappus hispidus</i> Less. var. <i>isochaetus</i> Fr. et Sav.	Yamajinogiku
<i>Patrinia scabiosaeifolia</i> Link.	Ominaesli
<i>Chimaphila japonica</i> Miq.	Umegasasō
<i>Rhizogonium Dozyanum</i> Lacost.	Itachinoshippo
<i>Usnea longissima</i> Ach.	Saruogase

Im Wald "Sengen" gibt es also nur geringe Mengen von Grass aus natürlichen Gründen und jene Lichtgräser, welche auf jährlich gebrannter *Hara* vorherrschen, können hier nicht gefunden werden.

Beim Eintritt in einen geschlossenen Wald wird man wahrnehmen, dass fast kein Grass auf dem Waldboden sich findet, während in geringer geschlossenen Beständen das Grass sich mehrt. Der Grund davon ist, darin

zu suchen, dass die Sonnenstrahlen in geschlossenem Walde, das ganze Jahr hindurch den Boden wenig erreichen können. Die Gräser, welche in der jüngeren Zeit der Bestände noch zahlreich sind, werden durch den sich verdichtenden Schluss der Bestände von Jahr zu Jahr weniger, da ihnen die zur Existenz nöthige Lichtmenge allmählig zu fehlen beginnt; Aus gleichen Gründen kommen auf der *Hara*, welche über 10 Jahre nicht gebrannt ist und einen grösseren Antheil an Bäumen Sträuchern anweist, verhältnissmässig wenige Gräser vor.

1. *Verminderung der Gewächsarten.*

Die Tabellen zeigen ferner dass die Menge der Gewächsspecies auf oftmals gebrannter *Hara* abnimmt, während sie in der selten gebrannten *Hara* zunimmt, bis auf einer *Hara* die Baumflora vorherrschend wird. Gewächsarten, welche durch häufiges Brennen auf einer *Hara* an Zahl abnehmen, sind :

Disporum sessile Don,	Hōchakasō
Salmia japonica var. buergeri Maxim.	Akinotamura-sō
Pieris hieracioides var. japonica Rge.	Kōzorina
Carex brunnea Thunb.	Nakirisuge
Tupatrium kirilowii Furcz.	Sawahiyodori
Eupotrium japonicum Thunb.	Hiyodoribana
Asarum blumei Duch.	Kanaoi
Polygonatum giganteum var. thunbergii Max.	Narukoyuri
Clematis heracleifolia var. Stans.	Kusabotan
Chrysanthemum sinense var. japonica Max.	Ryūngiku
Ixeris thunbergii A. Gr.	Nigana
Artemisia japonica Thunb.	Otokoyomegi
Kraunhia floribunda Taub.	[Fuji]
Dianthus superbus L.	Kawaranadeshiko
Tricyrtis hirta Hook.	Hototogisusō
Phagopteris totta Mett.	Mizoshida
Aster japonicus Miq.	Yamashirogiku

Carex conica Boott.	Himekansuge
Potentilla fragarioides L.	Kijimushiro
Carpesium cernuum L.	Gankubisō
Cardiandra alternifolia S. et Z.	Kusajisai
Artemisia vulgaris var. indica Max.	Yomogi
etc.	etc.

2. *Unter dem Brennen nicht leidende Gewächsorten.*

Sie sind folgende :

Miscanthus sinensis Anders.	<i>Susuki</i>
Potentilla fragarioides var. ternata Max.	Mitsubatsuchiguri
Serratula atriplicifolia B. et H.	Kumatoribokuchi
Sanguisorba officinalis L.	Waremokō
Thalictrum minus var. elatum Lecoy.	Akikaramatsu
Lysimachia clethroides Duby.	Okatoranoo
Plectranthus inconspicuus Miq.	Yamahakka
Pteris aquilina L.	Warabi
Senecio krameri Fr. et Sav.	Yaburegasa
Polygonum cuspidatum S. et Z.	Itadori
Euphorbia sieboldiana Mor. et Dic.	Natsutōdai
Imperata arundinaceae var. Koenigii.	Chigaya
Astilbe thunbergii Miq.	Toriashishōma
Gerbera anandria Sch. Bip.	Sebonyari
Athyrium nipponicum S. et Z.	Inuwarabi
Atractylodes lyrata S. et Z.	Okera
Dioscorea tokoro Makino.	Onidokoro
Arundinella anomala Steud.	Todashiba
etc.	etc.

diese Grassarten sind eben jene Sichtgräser, welche nur auf alljährlich gebrannter *Hara* sowie auch auf anderem beschädigten z. B. verhagelten Boden noch gedeihen.

(B) *Grasarten der verschiedenen Standorte.*

1. *Grasarten auf dem Rücken der Berge.*

Gerbera anandria Sch. Bip.	Senbonyari
Sanguisorba officinalis L.	Waremokō
Serratula atriplicifolia B. et H.	Kumatorilokeuli
Potentilla fragarioides var. ternata Max.	Mitsubatsuchiguri
Pteris aquilina L.	Warabi
Lysimachia clethroides Duby.	Okatoranoo
Imperata arundinaceæ var. Koenigii.	Chigaya
Astilbe thunbergii Miq.	Toriashishōma
Brachypodium silvaticum R. et S.	Yamakamojigusa
Euphorbia sieboldiana Mor. et Dic.	Natsutolai
Euphorbia Onoei Fr. et Sav.	Takatedai
Arundinella anomala Steud.	Todashiba
Agrimonia viscidula var. japonica Miq.	Kinmizuhiki
Phytolacca acinosa var. esculenta Max.	Yamagobō
Taraxacum officinale Wigg. var. glaucescens.	Tanpopo
etc.	etc.

Diese Arten benötigen zu ihrem Gedeihen eine geringere Feuchtigkeitmenge. Auch sind sie gegen extreme atmosphärische Einwirkungen weniger empfindlich.

2. *Grasarten am Mittelhange.*

Senecio krameri Fr. et Sav.	Yaburegara
Polygonatum cuspidatum S. et Z.	Itadori
Thalictrum minus L. var. elatum Lecoy.	Akikaramatsu
Aster indicus L.	Yomena
Osmunda regalis L. var. japonica Milde.	Zemmai
Plectranthus graucocalyx Max. var. japonica Max.	Hikiokoshi
Aster scaber Thunb.	Shirayamagiku

Patrinia scabiosaeifolia Link.	Ominaeshi
Patrinia villosa Juss.	Otokoeshi
Seseli livanostis var. daucifolia DC.	Ibukibōfu
Artemisia japonica Thunb.	Otokoyomogi
Cirsium spicatum (Maxim.)	Yamaazami
Miscanthus sinensis Anders.	Susuki
Solidago virga aurea L.	Awadachisō
Serratula coronata L.	Tamabōki
Angelica decursiva Miq.	Nodake
Plectranthus inconspicuus Miq.	Yamahakka
etc.	etc.

3. *Grasarten im Thale.*

Aralia cordata Thunb.	Udo
Petasites japonicus Miq.	Fuki
Oenanthe stolonifera DC.	Seri
Acorus gramineus Ait.	Sekishō
Astragalus sinicum L.	Rengesō
Iris japonica Thunb.	Shaga
Equisetum arvense L.	Sugina
Clematis herachipolia var. Stans S. et Z.	Kusabotan
Vicia hirsuta Koch.	Suzumenoendō
Achillea sibirica Ledeb.	Nokogirisō
Ranunculus acer var. japonicus Max.	Umanshigata
Osmorhiza japonica S. et Z.	Yabuninjin
Rumex japonicus Meiser.	Gishigishi
Angelica miqueliana Max.	Serimodoki
Geranium nepalense Sweet.	Furosō
Carex confertiflora Bott.	Shirasuge
Carex gibba Wahl.	Maskkusa
etc.	etc.

Vorgenannte Gräser sind hauptsächlich auf alljährlich gebrannter *Hara* wahrzunehmen.

Am Bergkamme gedeihen nur diejenigen Grassarten, welche grosse Menge von Sonnenlicht zu ihrem Wachstum benöthigen; während im Thale jene Arten fortkommen, welche zu ihrem Gedeihen weniger Sonnenlicht dagegen mehr Feuchtigkeit nöthig haben. Beim Vergleich mit *wenig* gebrannter *Hara* zeigt sich nun die auffallende Thatsache, das auch *jene* Grasarten, welche *sonst* nur geringere Sonnenlichtmenge beanspruchen hier ebefalls am Kamme gefunden werden. Die Eigenschaften resp. Existenzbedingungen der Gräser am Mittelhängen haben entsprechend ihrer Lage weniger ausgesprochene Besonderheiten.

III. KAPITEL: *Wuchszustände der Gräser auf gebrannter Hara.*

Auf selten gebrannter *Hara* ist die Bodendecke dichter, sie schützt den Boden vor direkter Einwirkung der Atmosphärien und gibt in den Verwesungsprodukten ihrer Pflanzen gewissermassen den Düngstoff für die spätere Generation wieder. Solche Bodendecken enthalten eine grosse Menge von Feuchtigkeit, u. verhindern weiterhin eine starke Austrocknung des Bodens durch die Sonnenstrahlen, unter solchen günstigen Umständen leben dann auch Gewächse, welche zu ihrem Gedeihen eine gewisse wenn auch mässige Menge von Nährstoffen und Feuchtigkeit unumgänglich nöthig haben. Auf nackter *Hara* können nur bestimmte Pflanzen leben, denen neben starkem Sonnenlicht, hohe Wärme und ein sehr geringer Procentsatz an Nährstoffen und Feuchtigkeit nöthig ist. Wenn man z. B. die Entwicklungsphasen von *Miscanthus sinensis*, *Pteris aquilina*, *Potentilla fragarioides*, *Euphorbia sieboldiana*, und *Sanguisorba officinalis* autmerksam beobachtet, so mögen sie oft zwar zahlreich vorhanden, aber arm an Arten sein, sowie von sehr kleinem oder zwerghaftem Wuchse. Ich untersuchte das Längenwachstum des Artwuchses auf verschiedenen *Hara*'s. Solche Beobachtungen müssen natürlich in der Zeit gemacht werden, wo die Grass-Pflanzen ihre Lebensthätigkeit eben vollendet haben. Die Entwicklungszustände der betr. Gewächse sind im Einzelnen je nach den Standortsverhältnissen und Jahreszeiten ganz verschieden. So z. B. die Länge-Entwicklung von *Miscanthus sinensis*. Dieses hat im Spätherbst

des vorigen Jahres seine Lebensthätigkeit vollendet und steht jetzt (am Ende April) absterbend auf der *Hara*. Ich dehnte meine Messungen sowohl auf die *jährlich* gebrannte *Hara* "Kiridöschi," als auch in auf "Ömiyama" (woselbst seit 3 Jahre nicht gebrannt wurde) und auf "Nanamagari" woselbst seit 10 Jahren kein Brennen stattfand, aus

Kiridöschi (Nordseite)

Länge (das Mittel von 100)

Kamm	1.6033 m.
Mittelhang	1.9433 m.
Thal	2.0900 m.
Mittel	1.8789 m.

Ömiyama (Nordseite)

Länge (das Mittel von 100)

Kamm	1.9013 m.
Mittelhang	2.3200 m.
Thal	3.0833 m.
Mittel	2.4349 m.

Nanamagari (Nordseite)

Länge (das Mittel von 100)

Kamm	2.2135 m.
Mittlhang	2.5013 m.
Thal	3.4022 m.
Mittel	2.7057 m.

Für andere Grasarten konnte ich kein passendes Untersuchungsmaterial finden; an der kleinen Untersuchung über *Miscanthus sinensis* jedoch lässt sich der ungünstige Einfluss *des jährlichen* Brennens schon *deutlich* erkennen. Richtiges, dickstengeliges Kayagrass kann man nur auf selten gebrannter *Hara* antreffen.

IV. KAPITEL: *Veränderung der Bodenbeschaffenheit durch das Brennen der Hara.*(A) *Veränderung der physikalischen Eigenschaften des Bodens.*

Die Pflanzen bilden sowohl als lebende Individuen, wie auch durch ihre abgestorbenen Körpertheile unter den gewöhnlichen Verhältnissen eine Decke, für jede Bodenart. In dieser Decke finden dann andere Pflanzen wiederum ihr Gedeihen. Es üben zweifellos verschiedene Decken auf den Pflanzenwuchs und den Boden einen günstigen oder ungünstigen Einfluss je nach dem aus. Auf viel gebrannter *Hara*-fläche z. B. in "Kiyosumi," wo man durch das Feuer die Pflanzen-Decke des Bodens oft vernichtete, änderten sich alle Verhältnisse besonders die physikalischen Eigenschaften des Bodens in ungünstiger Weise weil er nunmehr schutzlos den atmosphärischen Einwirkungen direkt wiederholt ausgesetzt wurde. Die flach- und mittelseit gründigen Bodenarten finden sich in der Regel, entweder auf den Rückenplateaus oder an den oberen Gehängen der Gebirge, namentlich wenn die letzteren waldlos sind; der in den unteren Regionen und am Fusse der Gebirge lagernde Boden dagegen ist mittelgründig oder sogar tiefgründig; insbesondere bei steilen Gebirgsgehängen wird dies deutlicher erkennbar. Das kann auch auf der *Hara* von Kiyosumi nachgewiesen werden, besonders auf der häufiger gebrannten *Hara*.

Ich habe schon angedeutet, dass die Gebirge in Kiyosumi sehr steil sind, meist über 35° Neigung; wird nun die Pflanzen-Decke auf der *Hara* durch häufiges Brennen wiederholt fast gänzlich vernichtet, so gelangt der feine gute verwiterte humose Boden immer mehr durch die Regengüsse in grosser Menge thalwärts, so dass die einen Rücken bekleidende *Hara* (d. h. deren Boden) sich als ausserordentlich flachgründig u. nahrungsarm nachweisen lässt; nicht selten tritt sogar bald das Grundgestein zu Tage. Im Thale dagegen ist durchgängig der Boden tief- ja oft sogar sehr tiefgründig zu finden.

Hara-boden muss also umso seichtgründiger werden, je öfter die *Hara* gebrannt wird, Auf selten gebranntem *Hara*-boden wird durch die lebenden

und abgestorbenen Pflanzentheile der Boden gehalten, was in grösserer Tiefgründigkeit zum Ausdruck kommt.

(B) *Bindigkeit.*

Wie erwähnt sind im Allgemeinen die Verwitterungsböden der Tertiärperiodengesteine zu den fruchtbaren zu rechnen. Sobald aber, wie auf der Kiyosumi *Hara*, der Bodenüberzug öfters abgebrannt wird, tritt auffallend rasch Verschlechterung ein; alljährlich gebrannte *Hara*, zeigt sehr lockeren oder oberflächlich grob gekrümelten Boden, welcher gegen Pflanzen- Wuchs sich ungünstig verhält infolge seiner grossen Austrocknung.

Weiterhin hängt die Empfänglichkeit eines Bodens für die Erwärmung durch die Sonnenstrahlen zum grössten Theile von der Beschaffenheit seiner Oberfläche ab; in dieser Beziehung gilt, dass, je grobkörniger das Gemenge eines Bodens ist, desto schneller und stärker er sich erhitzt durch die Insolation, aber dass er auch um so schneller die hierdurch erhaltene Wärmesumme wieder an die Atmosphäre abgibt, sobald die Sonne seine Oberfläche nicht mehr bescheint; je *feinkrümeliger* oder *dichter* dagegen ein Boden-Gemenge sich zusammensetzt, umso langsamer erwärmt es sich, aber um so länger hält auch der Boden die einmal aufgenommene Wärmemenge fest. Der erste Fall ist zweifellos dem Pflanzengedeihen *sehr* schädlich, während der *letztere* als günstig bezeichnet werden muss. Da die selten gebrannte *Hara* den feingekrümelten und milden bindigen Boden enthält, so gedeihen daher auch die Pflanzen auf dieser *Hara* besser, während die jährlich gebrannte *Hara* mit ihrer schlechten Bodenstruktur, nur schlechte Gras-Arten, mit extremen Anpassungsvermögen ein Fortkommen gestattet.

Wasser ist ein wichtiges Lebensmittel für alle Pflanzen. Ohne Wasser, keine Pflanzen! Es ist also der Boden, worin die Pflanze wurzelt, nicht bloss ihr Schützer gegen Winde, ihr Erwärmer in der Kälte, sondern auch ihr *Wasserspender*, wenn die Atmosphäre ihr kein Wasser darreicht; dieses wichtige Amt vollbringt der Boden einerseits durch sein Wasseransaugungsvermögen und anderseits durch seine Wasserhaltungskraft; das erste ist

eine Thätigkeit der Capillarität, welche sich umso günstiger bemerklich macht, je feinkrümiger der Boden ist, im zweiten Sinne wirkt am besten ein an Humus- und Thonsubstanzen reicher Boden.

Den besten Standort für die meisten Gewächse bieten diejenigen Bodenarten, welche unter den gewöhnlichen Verhältnissen bei starker Wasser- Aufsaugung das in ihnen angesammelte Wasser mässig festhalten und mässig verdunsten. Auf der Kiyosumi *Hara* aber, dort wo das Gras jährlich gebrannt wird, kann der Boden diese wichtige Aufgabe nicht leisten, weil die Decke ja beinahe gänzlich vernichtet wird und der Boden direkt den atmosphärischen Einwirkungen preisgegeben wird. In der Oberflächenstruktur des Bodens sind grobe Krümmel keineswegs dem Pflanzengedeihen zusagend. Je häufiger die *Hara* gebrannt wird, desto mehr trocknet der Boden aus und desto gröber wird seine Struktur durch Auswaschung werden; es herrschen Extreme von Trockniss und Feuchtigkeit in solchem Boden. Auf anderer nur selten zum Brande gelangter *Hara* ist der Boden dem Pflanzenwuchse zusagender, weil feinkörniger und mit grossem Wasser- Aufnahme u. Erhaltungs- Vermögen ausgestattet.

(B) *Verminderung der organischen Substanzen im Boden.*

Die organischen Substanzen im Boden bilden für die Pflanzen nützliche ja unentbehrliche Stoffe; man nennt daher einen Boden, welcher an organischen Substanzen reich ist, einen produktiven Boden. Die organischen Substanzen im Boden entstehen durch langsame Oxydation abgestorbener Pflanzentheile der Bodendecke unter dem Einflusse der Bodenfeuchtigkeit und werden "*Humus*" genannt.

Auf solcher *Hara*, wo das Gras alljährlich gebrannt wird, vernichtet das Feuer fast die ganze Bodendecke mit den Humusstoffen, welche die Pflanze so nöthig zum Wachsthum hat. Bei meinen Untersuchungen in Kiyosumi stiess ich demgemäss stets auf nachtheilige Wirkungen des wiederholten *Hara*-Brennens.

I. Bestimmung der Feuchtigkeitsmengen in einzelnen Hara-Böden.

Namen.	Jahre des Hara-brennens.	Böden	Sand (über 0.5 m. m.) in 100 gr. des Bodens.	Feinerde in 100 gr. des Bodens.	Gewicht der genommenen Feinerde.	Gewicht der Feinerde (3 gr.) nach Austrocknung.	Feuchtigkeitsmenge in 3 gr. Feinerde. (A)
Kiridoschi (Nordseite)	jährlich gebrannt.	100 gr.	2.64 gr.	97.36 gr.	3 gr.	2.6226 gr.	0.3774 gr.
" (Südseite)	" "	100 "	3.02 "	96.98 "	3 "	2.6725 "	0.3275 "
Kajisaka (Nordseite)	" "	100 "	2.35 "	97.65 "	3 "	2.6112 "	0.3888 "
" (Südseite)	" "	100 "	2.51 "	97.49 "	3 "	2.5732 "	0.4268 "
Ōmiyama (Nordseite)	{ für 3 Jahre { nicht gebrannt.	100 "	3.76 "	96.24 "	3 "	2.6680 "	0.3320 "
" (Südseite)	" "	100 "	2.24 "	97.76 "	3 "	2.6344 "	0.3656 "
Musado "	{ für 6 Jahre { nicht gebrannt.	100 "	2.19 "	97.81 "	3 "	2.6178 "	0.3822 "
Ōmiyama "	" "	100 "	1.95 "	98.05 "	3 "	2.5752 "	0.4248 "
Suzurischichi "	{ über 8 Jahre { nicht gebrannt. { bis 24 Meiji jährlich u. im { Jahre 33 Meiji gebrannt.	100 "	2.77 "	97.23 "	3 "	2.6411 "	0.3589 "
Sannodai (Nordseite)	gleich.	100 "	3.56 "	96.44 "	3 "	2.6863 "	0.3137 "
" (Südseite)	" "	100 "	2.21 "	97.79 "	2 "	2.6522 "	0.3478 "
Suzurischichi (Nordseite)	{ für 10 Jahre { nicht gebrannt. { über 10 Jahr { nicht gebrannt.	100 "	1.04 "	98.96 "	3 "	2.5785 "	0.4215 "
Nanamagari "	Urwald.	100 "	0.99 "	99.01 "	3 "	2.5641 "	0.4359 "
Musado "	" "	100 "	3.11 "	96.89 "	3 "	2.6012 "	0.3988 "
Sengen "	" "	100 "	1.12 "	98.88 "	3 "	2.6153 "	0.3847 "
" (Südseite)	" "	100 "	1.53 "	98.47 "	3 "	2.5597 "	0.4403 "

Aus Obigem ist die Verminderung der Wasserhaltungskraft des Hara-böden, klar nachweisbar, wenn eine Hara oftmal gebrannt wurde. Die Untersuchung der Mengen organischer Substanzen in den diversen Böden ergab folgende Zahlen.

II. Das Glühen des genommenen Bodens.

Namen.	Feinerde.	Gewicht der Feinerde (3 gr.) nach Glühen.	Verminderung des Gewichte, (B)
Kiridōshi (Nordseite)	3 gr.	2.2436 gr.	0.7564 gr.
„ (Südseite)	3 „	2.2933 „	0.7067 „
Kajisaka (Nordseite)	3 „	2.2118 „	0.7882 „
„ (Südseite)	3 „	2.1998 „	0.8002 „
Ōmiyama (Nordseite)	3 „	2.1704 „	0.8296 „
„ (Südseite)	3 „	2.2388 „	0.7612 „
Musado „	3 „	2.2212 „	0.7788 „
Ōmiyama „	3 „	2.1859 „	0.8141 „
Suzuriishi „	3 „	2.2631 „	0.7369 „
Sannodai (Nordseite)	3 „	2.2664 „	0.7336 „
„ (Südseite)	3 „	2.2913 „	0.7087 „
Suzuriishi (Nordseite)	3 „	2.1780 „	0.8220 „
Nanamagari „	3 „	2.1461 „	0.8539 „
Musado „	3 „	2.1559 „	0.8441 „
Sengen „	3 „	2.1043 „	0.8957 „
„ (Südseite)	3 „	2.0528 „	0.9472 „

III. Bestimmung der organischen Substanzen im Boden.

Aus den Tabellen I. und II. wird die folgende :

Namen.	Die Menge der org. Substanzen sammt dem Ver- bindungswasser in 3 gr. Feinerde. (B—A)	Procent der org. Substanzen sammt dem Ver- bindungswasser in ausgetrockneter Feinerde.	Procent der org. Substanzen sammt dem Ver- bindungswasser in Feinerde.	Procent der org. Substanzen sammt dem Ver- bindungswasser im Originalboden.
Kiridōshi (Nordseite)	0.3790 gr.	14.4513 %	12.6333 %	12.8331 %
„ (Südseite)	0.3792 „	14.1880 „	12.6400 „	12.2583 „
Kajisaka (Nordseite)	0.3994 „	15.2056 „	13.3133 „	12.3338 „
„ (Südseite)	0.3734 „	14.5111 „	12.4467 „	12.1343 „
Ōmiyama (Nordseite)	0.3976 „	18.6567 „	16.5000 „	15.0630 „
„ (Südseite)	0.3956 „	14.8276 „	13.1866 „	12.8913 „

Musado	(Südseite)	0.3966 „	15.1501 „	13.2200 „	12.9305 „
Ōmiyama	„	0.3893 „	15.1173 „	12.9766 „	12.7236 „
Suzuriſchi	„	0.3780 „	14.3122 „	12.6000 „	12.2509 „
Sannodai	(Nordseite)	0.4199 „	15.6311 „	13.8966 „	13.4984 „
„	(Südseite)	0.3609 „	13.6075 „	12.0300 „	11.7641 „
Suzuriſchi	(Nordseite)	0.4011 „	15.5550 „	13.3700 „	13.2304 „
Nanamagari	„	0.4180 „	16.3020 „	13.9333 „	13.7954 „
Musado	„	0.4453 „	17.1190 „	14.8433 „	14.3817 „
Sengen	„	0.5110 „	19.5411 „	19.0333 „	16.8426 „
„	(Südseite)	0.5069 „	19.8631 „	16.8966 „	16.6040 „

Aus der Tabelle kann entnommen werden, dass in jährlich gebranntem *Hara*-boden eine starke Verminderung der organischen Substanzen eintritt, während in selten gebranntem *Hara*-boden diese zunimmt.

VIII. Abschnitt.

Die Einwirkung des Hara-brennens in ökonomischer Beziehung.

In den meisten Gegenden herrscht der Glaube, dass durch das Feuer die Wachstumskraft der *Hara* gesteigert werde. Diese Annahme scheint nur auf ebenem Felde als richtig, während sie an steilem Abhänge nicht zutreffen kann, da im letzteren Falle die Pflanzen-Asche nicht länger am Hänge bleibt, sondern durch die Regengüsse thalwärts geführt wird. Man hat eben nur nach bestimmten *Arten* gesehen, welche sich unter Umständen auf alljährlich gebrannter *Hara* in der Mehrzahl finden, aber nicht nach der *allgemeinen* Beschaffenheit der Bodendeckenvegetation und ihren Wuchszuständen. *Miscanthus sinensis*, welches den Leuten als Dach-Deckmittel unentbehrlich scheint, kann auf gebrannter *Hara* zwar in Masse vorkommen, doch wenn man genauer zusieht, so findet man, dass der einzelne Stamm kürzer und dünner ist, als auf anderem Platze, der selten gebrannt wird. Diese Thatsache beweist, dass das Brennen seinen Zweck nur mangelhaft erfüllt, es kann solch zwerghafte

Miscanthus dem Bedürfnisse der Leute auf die Dauer nicht gerecht werden; die richtigen und langen Halme gewinnt man nur auf *ungebrannter Hara*. Auch die Gräser von alljährlich gebrannter *Hara* sind vorzugsweise "saure" Gräser und für das Vieh *kaum geniessbar*.

Betrachtet man also die Sache unbefangen von allen Seiten, so ist das Brennen der *Hara* nichtsweiter als eine schlechte, durch Nichts begründete Gewohnheit und "*unwirthschaftlich*" zu nennen, weil der Boden, dem sein natürlicher Humus genommen wird, successive zur Inproduktivität herabsinkt. In Kiyosumi hat man sich in Erkenntniss dieses Umstandes zur Aufforstung in planmässiger Weise entschlossen, da dadurch ein weit grösseren Gewinn erzielt werden wird, als bei der bisherigen *Hara*-wirthschaft, welche für das Land nicht die mindeste wirthschaftliche Bedeutung, hat sondern lediglich als eine "*schlechte Gewohnheit*" bezeichnet werden muss.

XI. Abschnitt.

Die Einwirkung des Brennens der Hara im landwirthschaftlichen Sinne.

Das *Hara*-Brennen in Japan entstand hauptsächlich aus den Bedürfnissen der Landwirthschaft, man wollte dadurch eine grosse Menge Gründünger bekommen, aber das Brennen der *Hara* hatte wie überall so auch auf der Kiyosumi *Hara* nur das Gedeihen "*schlechter*" Grasarten, zur Folge welche für den Boden als Düngmittel kaum wirksam zu nennen sind. Auf jener *Hara* dagegen, wo selbst das Gras sehr selten gebrannt wird, finden sich grössere Mengen von guten Species unter anderm z. B. Leguminosen arten in grosser Zahl. Diese Letzteren enthalten grosse Mengen von Stickstoff, welche sie aus der Luft absorbieren, wodurch sie als Düngmittel sehr wirksam sind.

In der Asche, welche durch das Brennen der Bodenüberzuges erzielt wird, fänden sich zwar für die Pflanzen einige gute Nahrstoffe, z. B. das Kali; aber an steilen Abhängen, wie auf der Kiyosumi *Hara* wird es

zusammen mit den feinen produktiven Bodentheilen durch die Regengüsse thalwärts gewaschen und dadurch erst recht ein nahrungsarmer Boden geschaffen. Als Futtermittel sind jene *schlechten* sauren Grasarten, welche sich auf (durch das Feuer) oft verwüsteter *Hara* befinden, für das Vieh kaum verwendbar, während die auf guter, bzw. selten gebrannter *Hara* wachsenden Arten als ein sehr gutes Futtermittel bezeichnet werden müssen. Durch das Brennen der *Hara* werden also gute nahrungsreiche Grasarten vernichtet und der Boden landwirthschaftlich werthlos gemacht, die *Hara* erfüllt also gerade ihren eigentlichen Zweck umso weniger, je öfter sie gebrannt wird.

X. Abschnitt.

Die Einwirkung des Brennens der Hara in forstlicher Hinsicht.

I. KAPITEL.: *Gefährlichkeit des Brennens der Hara.*

Die Forstwirthschaft bedarf im Allgemeinen grosser Vorrath-Kapitalien und längere Zeiträume zu ihrer Produktion, um gute Erträge nachhaltig in ihren Waldungen abzuwerfen. Unter verschiedenen Gefahren wie Insekten, Sturm etc, welche den Wald bedrohen, ist auch das Feuer zu erwähnen. In Japan sind Waldfeuer sehr häufig, was hauptsächlich auf die achtlose Behandlung des Feuers zurück zu führen ist, namentlich ist es das Brennen der *Hara*, welchem grosser Schaden im angrenzenden Wald alljährlich zur Last zu schreiben ist. Die *Hara* wird hauptsächlich im Frühjahr (von Februar bis Ende März) gebrannt; die an solche *Hara* angrenzenden Waldtheile stehen in grosser Gefahr. Es gibt zwar gesetzliche Vorschriften über das Brennen der *Hara*, wonach die *Hara* ohne zuvor erholte Erlaubniss überhaupt nicht gebrannt werden soll, auch muss dem benachbarten Grundstück ein gewisser Schutz gegeben werden durch Ziehen eines sogenannten Schutzgrabens. Da diese Vorschriften nie beachtet werden, tritt das Feuer über die Schutzzone in den benachbarten Wald nur zu oft über.

Die *Hara* in Kiyosumi grenzt an ihrer nördlichen Seite an das Schulwald-Areal an, welches in letztes Zeit aufgeforstet wurde; es bedarf deshalb einer besonderen Aufsicht, wenn man das Brennen gestatten will. Die Vernichtung von Wald ist eine constante Begleiterscheinung des Brennens der *Hara* und auch aus diesem Grunde möchte diese Bodennutzungsmethode zu verwerfen sein.

II. KAPITEL: *Vernichtung der Bodendecke.*

Wie ich schon bemerkt habe, bildet die Pflanzen- Vegetation durch die Humusbildung eine Decke, welche der Erhaltung jeder Verwitterungsschicht günstig ist. An abhängigen Lagen bildet sie einen das Fortschlängen der Erdtheile verhindernden Widerstand und an sonnigen Stellen schützt sie den Boden gegen die Sonnengluth und bewahrt so den von ihr bedekten Boden vor Ausdörrung, im Sommer, wie vor den Einwirkungen des Frostes im Winters. Sie lässt starke Regenmengen nicht direkt zum Boden dringen, sondern gewährleistet deren allmähliche und mehr gleichmässige Aufsaugung in den Boden. Das zu rasche Abfliessen des Wassers mit seinen schädlichen Folgen wird hauptsächlich verhindert. Durch das alljährliche Brennen von *Hara*-flächen wird nun die Humus- und Pflanzendecke gänzlich vernichtet, sie kann also auch ihr wichtiges Amt nicht mehr vollbringen.

III. KAPITEL: *Verminderung der Nahrungsstoffe im Boden.*

Man unterscheidet zwei Arten von Nahrungsstoffen im Boden nämlich die organischen und anorganischen oder mineralischen Stoffe. Die organischen Substanzen im Boden entstehen durch den Verwesungsprocess der abgestorbenen Organismen (d. h. Pflanzen und Thiere). Auf *Hara* und Waldboden sind sie meist von abgestorbenen Pflanzentheilen gebildet.

Der Boden enthält eine um so grössere Menge von Humussubstanzen, je grösser die Zahl der dort lebenden und absterbenden Pflanzen ist; auf *Hara* aber, welche alljährlich gebrannt wird, gibt es nur eine geringe Menge abgestorbener Pflanzen; auf der schutzlosen *Hara* bringen

die Regengüsse etc. unaufhörlich Humussubstanzen, feinen Sand und erdige Theile von den Berggebängen herab in die Thäler zur Ablagerung oder in die Bäche zum weiteren Transport; wenn man also an die Aufforstung oft gebrannter *Hara* geht, so fehlen dem Boden jene Nahrungsstoffe, welche zum Gedeihen der jungen Bestände so notwendig sind; man kann daher auf solchen schlechten Böden auch nur jene Arten von Bäumen pflanzen, welche die geringsten Ansprüche an die Nährkraft des Bodens stellen, auf die besseren Baumarten muss man also von vorn weg verzichten, was einem erheblichen wirtschaftlichen Nachtheil gleichkommt. Auf die traurigen Folgen der Geschiebeführung der Bäche und Flüsse infolge der *Hara*-bedeckung der Gebirge sie hier nur kurz hingewiesen.

IV. KAPITEL: *Trockenheit des Bodens.*

Wo dem Boden die nöthige Menge von Wasser fehlt, da bleiben die Nahrungssubstanzen in ungelöster Form, was wiederum Nahrungsnoth für die Pflanzen bedeutet; auch können die Pflanzen ihre zu ihrem Gedeihen nöthige Transpirationsgrösse bei Wassermangel nicht aufrecht ertalten u. sterben ab. Ist nun der Boden mit Pflanzen und Humusdecke geschützt, so kann er eine bedeutende Wassermenge aufnehmen u. festhalten, welche langsam aber sicher u. wirksam den Pflanzen zu Gute kommt, während auf nackten *Hara's* die meteorischen Niederschläge oberflächlich rasch abfliessen und der Boden der Trockenheit ausliefern. Trockenheit des Bodens aber ist für die Forstwirtschaft oft ein sehr grosses Hinderniss; gute Bestände können nur auf jenen Böden gedeihen, wo ihnen eine gewisse Menge Feuchtigkeit nicht fehlt.

Nachdem die *Hara* in Kiyosumi, alljährlich gebrannt wird, hat sie auch nur eine schwache Pflanzen- & Humusdecke. Aus diesem Grunde kommt dann der fast nackte Boden durch Einwirkung der Atmosphäre, namentlich durch die starke Besonnung mehr u. mehr herab. Wollte man solchen Boden sofort aufforsten, so würden die Pflanzen die nöthige Wasser- und Nahrungsmenge im Boden kaum finden und zu Grunde gehen. Dagegen hat man stets leichteres Spiel mit jener *Hara*, welche seltener gebrannt ist, sie setzt der Aufforstung viel weniger Schwierigkeiten entgegen.

Schluss.

Aus den bisher mitgetheilten Beobachtungen mag erschen werden, dass die Kiyosumi *Hara*, da sie in der subtropischen Zone mit deren grosser Menge an Luftfeuchtigkeit bei seltenem Frost und Schnee liegt, in Bezug auf das Pflanzengedeihen zu den günstigen Plätzen gerechnet werden muss. Trotzdem wird zur Zeit von den grossen Flächen der Kiyosumi *Hara* in ihrer "*Hara*"—Form, nur geringer Nutzen gezogen, sie sind de facto Nichts weiter als unproduktive Flächen. Oftmaliges Brennen der *Hara* verursacht folgende Nachtheile:

1. Die Pflanzen, Bodendecke ist durch das häufige Brennen der *Hara* fast ganz zum Verschwinden gebracht.
2. der Boden wird direkt den Atmosphärien preisgegeben.
3. die Regenwasser fliessen oberflächlich ab und verursachen keine Zunahme der Bodenfeuchtigkeit.
4. der Boden geht in grobe Krümmelstruktur über.
5. die produktive Bodenkrumme wird durch die Regengüsse thalwärts abgeführt.
6. Die Bodenverwitterungsschicht wird dadurch seichter und seichter.
7. Es tritt im Laufe der Zeit Humus-Verarmung im Boden ein.
8. die Lichtgräser kommen auf viel gebrannter *Hara* in Überzahl vor.
9. die Grasspecies werden durch das Brennen in ihrer Zahl vermindert.
10. die Gewächse auf oftmal gebrannter *Hara* neigen zur Degenerirung.
11. Baumarten verschwinden sobald eine *Hara* öfter gebrannt wird.
12. wenn eine *Hara* längere Zeit nicht gebrannt wird, so geht sie wieder in Wald über, zuerst erscheint "*Quercus glandulifera*" in hiesiger Zone wieder.
13. das Hauptprodukt der *Hara*, (d. h. Kaya) Gras, wird durch oftmaliges Brennen weder in richtiger Bonität noch Menge erzeugt.
14. die Asche, welche durch das Brennen der Gräser entsteht, kann auf dem geneigten *Hara*-boden nicht liegen bleiben, sie hat also als Dünger auch keinen Werth.

15. das *Hara*-brennen ist in der Nähe von Waldungen am gefährlichsten.
16. die Wachstumsenergie der Gräser sinkt durch oftcs Brennen herab.

Die Hoffnung, durch Brennen Boden in seiner Produktivität zu heben, ist im Gebirge wenigstens (wie in Kiyosumi *Hara*) eine irrige. Die japanische *Hara*, welche die Haupterscheinungsform der Bodenbenutzung im Berglande ist, verändert den Boden in nachtheiliger Weise, führt Verarmung des ungeschützten Bodens durch Auswaschung und Verhagerung überall herbei, ja nicht selten tritt das nackte Grundgestein auf japanischer *Hara* zu Tage.

Ich schliesse meine Beobachtungen damit, dass ich behaupte, dass das *Hara*-brennen vom Standpunkt der Landwirthschaft wie, der Forstwirthschaft betrachtet, einen entschieden nachtheiligen Einfluss auf die *Hara* selbst ausübt, ja dass es geradezu den *Hara*-boden der Verödung preisgibt.

Es wären nach meiner Ansicht die meisten *Hara* in Japan, weil sie als *Hara* nicht nur *ohne* wirthschaftliche Bedeutung sind, sondern geradezu landverwüstend wirken, *alsbald* aufzuforsten. Wo dies nicht möglich ist, können gute Gräser nur nach Authören jedes Brennens erwartet werden.

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Schluss.

—♦♦♦—

Die zukünftige Bewirtschaftungsform des japanischen Waldes!

VON

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Kgl. bayr. Forstmeister u. Professor in Tokio.

Die Umwälzung des Jahres 1868 ist auch von grossem Einflusse auf die japanischen Waldungen gewesen. Während früher wohl die meisten der Feudalherren die ihnen unterstellten Kronforste und Staatswaldungen wenigstens in einigermassen gutem Zustande erhielten, wenn auch eine besondere Waldwirtschaft sich nicht entfaltete, so ist doch in den letzten Jahrzehnten manches Stück trefflichen Staatswaldes der Begehrlichkeit insbesondere bäuerlicher Kreise und den schwankenden, wechselnden Ausschauungen über die Lösung der volkswirtschaftlichen Fragen nach der Restauration in Japan zum Opfer gefallen. Ausserdem besass die neue Centralgewalt vielfach weder die Macht noch die Organe, ihre Autorität genügend geltend zu machen, um den durch Säkularisirung von Kloster-gütern, Einverleibung von Gemeindsforsten etc. vermehrten Staatswaldbesitz vor Forstfrevel zu schützen.

Auch der Privatwaldbesitz unterlag manigfachen Veränderungen. Armuth und Verschuldung vieler Grund-Besitzer, welche bei der Umgestaltung der allgemeinen Verhältnisse, sich der Neuzeit nicht anzupassen vermochten, führte häufig zum Verkauf, zur Zerstückelung und Vernichtung von Wäldern. Namentlich haben aber Theilungen von Waldgrund unter die ehemals nur zu Nutzungen Berechtigten am vorher gemeinsamen Walde (Gemeindewalde) zur voraussehenden sicheren Vernichtung, Devastirung oder wenigstens Verschlechterung der Theilstücke geführt. Auch will mich bedünken, dass die hier auf dem Papier so geordnet und genau festgestellte Oberaufsicht des Staates über die Gemeindewaldungen in praxi nicht genügend strenge zur Anwendung kommt. Der factische Zustand der meisten bäuerlichen Gemeindewaldungen spricht hierfür.

Habsucht und mangelndes Verständniss veranlassen ferner gar manche kleine Privatwaldbesitzer zu einer Nutzungsweise, welche in vielen Fällen direct der Zerstörung gleichkommt. Die Eigenthümer *grösserer* Privatwaldungen sind bis zu einem gewissen Grade indessen bereits zum tieferen Verständnisse des Werthes und der Bedeutung des Waldes gelangt und wirtschaften im wohlverstandenen eigenen Interesse etwas mehr conservativ. Am besten wurde der Wald wohl da conservirt, wo die fehlende Aufschliessung der Gegend und die geringe Bevölkerungsziffer ihn von selbst bis heute geschützt haben und es gilt dies nicht blos für den Privat-, sondern auch für den Staatswald. Es möchte fraglich erscheinen ob nicht finanzielle Schwierigkeiten des Staates den dort vorhandenen reichen Vorräthen ein schnelles Todesurtheil gesprochen hätten, wäre ihre Lage zum Verkehr nur ein bischen günstiger gewesen.

Darin beruht auch heute noch die Gefahr, dass das Bestreben hohe Erträge *um jeden Preis* aus einem Walde herauszuholen, die heiligste Pflicht gegen das nationale Wohl vergessen lässt, nemlich die Sorge für die ungeschmälerte Überlieferung eines werthvollen Waldbestandes an die Nachwelt durch entsprechende Form der Nutzung & Verjüngung. Liegt ja doch der Werth der Wälder nicht blos in ihrem Rentenertrag; die Überschwemmungen und Wasserkatastrophen, welche der Vernichtung des Bergwaldes z. B. allenthalben zu folgen pflegen, reden eine zu deutliche Sprache, als dass sie misszuverstehen wäre. Wohl sind Gesetze zum Schutze solcher Forste erlassen, aber ein Blick in die reale Wirklichkeit zeigt zur Genüge, dass das Wollen dem Können noch nicht die Wagschale hält.

Der Staatswald leidet abgesehen von einigen grösseren Complexen auch nicht wenig durch die Zersplitterung seines Areales; es wird eine stete Sorge der Regierung sein müssen, eine Verbindung der zerstreuten Theile auf dem Wege des Ankaufes oder Austauschens herzustellen. Wenn man sich mit Recht zur Abstossung einiger sehr abseits liegender Kleinflächen entschliesst, so sollte doch Verschleuderung unter allen Umständen vermieden werden.

Eine intensivere Pflege muss zweifellos durch den nöthigen gesetzmässigen Druck der *Gemeindewald* im getheilten und ungetheilten Besitze

erfahren. Auf welche abschüssiger Ebene man sich hierin momentan befindet, zeigt jede Excursion durch das Land.

Das Beispiel, welches der Staat den Privaten durch seine eigene Wirtschaft zu geben berufen ist, setzt aber voraus, dass er sich selbst über seine einzuschlagende Richtung klar sei und mit allen Mitteln auch Ernst machen will, sowohl durch die nöthige durchgreifende *Reformirung* seiner Verwaltung als auch durch consequente Befolgung gewisser *Wirtschaftsgrundsätze*. Die wichtigste Frage ist hiebei zweifellos jene nach der zweckmässigsten Art der Nutzung und damit zusammenhängend die Untersuchung der den Verhältnissen *am besten gerecht werdenden Verjüngungs- und Bestandsform*. In Übung sind in Japan in der Hauptsache zur Zeit zwei Hauptnutzungsarten, einmal *Kahlschlag* auf grösserer Fläche, und dann eine Art von auszugswaiser Nutzung, welche *fälschlich* den Namen eines *Plenterbetriebs* erhalten hat.

Dem Kahlschlag auf grosser zusammenhängender Fläche musste früher da, wo durch einen Wasserlauf die Waldungen günstige Abfuhrbedingungen für ihre Schlagergebnisse hatten, eine gewisse Berechtigung zugestanden werden, immerhin sind die aus ihm hervorgehenden Nachtheile vom waldbaulichen Standpunkte so bedeutend, dass man von ihm überall, wo es nur irgend möglich ist, abzukommen trachten wird. Nicht blos sind alle Gefahren wie Frost, Dürre, Unkraut (Bambus) im erhöhten Masse bei dieser Verjüngungsform gegeben, sondern die nachgezogenen Bestände nahezu gleichen Alters auf Flächen von grösser Ausdehnung bilden auch einmal später für den Wirtschaftler sehr schwierig zu realisirende Hiebsobjekte; umso schwieriger je weniger etwa die Aufschliessung der jetzigen grossen Verjüngungsschläge solcher Plätze bis zur seinerzeitigen Haubarkeit derselben fortgeschritten sein sollte.

Man darf nicht vergessen, dass es nicht Aufgabe einer Wirtschaft sein kann, nur *Wald schlechthin* nachzuziehen, sondern die Gegenwart hat die heiligste Verpflichtung, dem Stande des Wissens entsprechend, auch *technisch vollkommene* Bestandsbilder der Nachwelt zu überliefern.

Die Gewissensberuhigung, welche vielfach darin gesucht wird, dass man die grossen Schläge mit Nadelholz in irgend einem Verbande durch Pflanzung in Bestockung bringt, ist in vielen Fällen ein grosses Armuths-

zeugniss fehlender Überlegung und Klarheit über die Grundzüge einer geordneten Wirtschaft.

Ziellose Aufforstung gibt wohl Wald, ob aber den nach Betrachtung aller Verhältnisse *richtigen* Wald, darnach fragt man, wie mir auf Grund meiner eigenen Anschauung durch Reisen zweifellos ist, viel zu wenig. Dem *künstlichen* Kulturwald *gleichen* Alters aber haftet ein grosser Nachtheil an; man hat wenigstens in Europa diese Erfahrung aus bitteren Verlusten gesammelt, dass nemlich die Insektencalamitäten in grösserem Umfange und verderblicher aufzutreten pflegen, als sonst.

Japan ist zwar in der glücklichen Lage von solchen Catastrophen bisher verschont zu sein, ob aber die unter dem Drucke der wirtschaftlichen Notwendigkeit eintretende Umgestaltung seiner Waldungen nach innerer Verfassung und nach Holzarten nicht dieselben mit sich bringen werde, dafür fehlt vorderhand jede Erfahrung. Wie erwähnt würde man gut daran thun, die Möglichkeit zu berücksichtigen. Auch die Verlegung des Schwergewichtes nur nach *einer* Holzart hin, z. B. der Cryptomerie, hat, wenn zu uniform zur Geltung gebracht, manche recht bedenkliche Seite. Man sollte stets darnach streben Misch-bestände zu erziehen, um bei einer ev. Änderung der Conjunktur des Absatzes nicht auf das Trockene gesetzt zu sein und eine bessere, intensivere Bodenausnutzung zu erzielen. Das stellenweise bemerkbare *gänzliche* Verdrängen der Laubwaldungen durch Nadelhölzer ist ein später schwer wieder gut zu machender Fehler.

Zweifellos ist ja der Nadelwald in Japan überwiegend werthvoller und man wird deshalb Nichts dagegen einzuwenden haben, wenn der Laubwald praktisch in die Inferiorität gedrängt wird, aber ihn ganz vom Zukunftswalde auszuschliessen, wie man das an verschiedenen Plätzen bereits bemerken kann, ist ebenfalls verfehlt. Ich glaube man gibt ihm am besten die Rolle eines Zwischen-und Unterstandsgliedes, dadurch wird die Bodenthätigkeit besser gewahrt als durch die reinen Nadelholzbestände. Der *reine* Laubwald, wo er allein angängig erscheint, wird zweifellos im hochwaldartigen Mittelwalde seine beste Wirtschaftsform haben.

Auf geeignetem Boden und bei entsprechenden sonstigen Verhältnissen ist auch sicher der Laubwald noch rentabel, sobald nur auf Erziehung eines sehr werthvollen Materiales hingearbeitet wird. Schlechte Böden sind für

Laubwald als Hauptnutzholzwald aufzugeben, da hier die erwähnten Voraussetzungen hochwertiger Produktion nicht vorhanden sind.

Die grosse Ausdehnung der Fläche nach, nimmt in Zukunft der Laubwald in Japan sicher nicht mehr ein, und mit Recht, denn eine bessere Wirtschaft sucht *Rentabilität*, aber auch waldbauliche Gesichtspunkte insbesondere Vielseitigkeit und Nachhaltigkeit mehr in Einklang zu bringen, das führt dann unmittelbar zu *gemischten* Waldungen. Für gemischten Wald ist aber die Grosskahlfächenverjüngung die *ungeeignetste* und muss ihr auch aus diesem Grunde der Werth, den sie in der Vergangenheit hatte, völlig abgesprochen werden.

Ebenso unpassend ist aber der grosse Kahlschlag, der im Wesentlichen seine Heimath in der Ebene hatte, für *gebirgige* Länder, wo eben die Gefährdung des Terranis durch die meteorischen Niederschläge in ganz anderer Art sich äussert.

So viele verödete Plätze des Berglandes, welche heute in ihrem zerrissenen, den Abschwemmungswassern preisgegebenen Böden, die Zufuhrquelle der Geschiebe zu den verderblichen Wildbächen und Flüssen darstellen, sind sicher grossentheils nur auf Kahlschlagwirtschaft im grossen Stile zurückzuführen. Wir hätten gewiss noch viel traurigere Bilder der Verwüstung des Bergterrains in Japan vor uns und eine noch viel drohendere Gefahr der Wildwasser, wenn nicht die beispiellose Reproduktionskraft des Bodens mit ihrer unendlich manigfaltigen Pflanzendecke hier zu Hilfe käme. Dass man aber damit auf die Dauer nicht auskommt, das beweist zur Genüge eine Augenscheinnahme im Gebirge und die Hochwasserschadenstatistik. Die Extreme berühren sich und nach diesem Grundsatz scheint man auch in Japan vorgehen zu wollen, denn nachdem zweifellos die Fehler der alten bis heute im Schwung befindlichen *Grosskahlschlag-damit künstlichen Verjüngungs- sowie Auszugshauungs-* Politik sich nicht mehr läugnen lassen, will man sich zum direkten Gegentheil bekehren, nämlich zur *natürlichen horst- und gruppenweisen* Verjüngung.

Die Nutzung der Altholzbestände in langen Verjüngungszeiträumen und die Nachzucht der neuen Generation auf natürlichem Wege in Gruppen und Horsten, unter Zuhilfenahme stellenweisen künstlichen Anbaues, entspricht ja auch der Bewegungsrichtung welche die *moderne* waldbauliche

Entwicklung in Europa eingeschlagen hat, somit ein Grund mehr, so meint man hier, sofort in radikalster Weise vorzugehen und zwei Treffer auf einmal zu erzielen, nämlich besser zu wirtschaften und dann sich im Glanze des Bewusstseins zu sonnen, dass man auf der Höhe der Zeit ist. Dieser Ansicht wird man heute in Japan nicht selten begegnen und die Verfechter derselben führen als Argument an, dass diese Form der Bestandsnutzung und Verjüngung nicht einmal *neu* sei, sondern lange *hier* zu Lande geübt. Als Beweis wird dann eine vorhandene sogenannte Plenterwirtschaft theoretisch und bei Gelegenheit auch in praxi vorgezeigt.

Wenn man eine *regellose* Ausraubung des werthvollsten Materiales aus alten Beständen und eine *völlige* Gleichgültigkeit über die *innere* Beschaffenheit und den *wirtschaftlichen* Werth der Nachzucht, eine Plenterwirtschaft heisst, dann allerdings haben die Anhänger dieser Richtung Recht. Der Hinweis auf die Natur, welche ja auch in ähnlicher Weise, wenn sie durch die Hand des Menschen nicht gestört wird, den Wald regenerirt hat, ist umso weniger gerechtfertigt, als sie eben den Wald *nur als Selbstzweck* weiter produzirt und verjüngt, *ohne* sich um Zeit und Werth zu kümmern.

Die *Frage nach Zeit u. Werth* ist aber das Grundelement einer heutigen Waldwirtschaft, in der Berücksichtigung dieser Faktoren liegt das Characteristicum des Begriffes "Wirtschaft." Eines der Hauptziele einer *wirklich* ökonomischen Betriebes muss die Erreichung des *besten* Effektes unter Aufwand *geringster* Mittel sein.

Das *technisch Vollkommenste* nach den möglichen Verhältnissen zu leisten, *nicht* aber bloß *überhaupt* Production zu treiben, ist Aufgabe der modernen Forstmannes. *Natürliche horst- und gruppenweise Verjüngung, (Fehmelschagform) und bei Ausdehnung des Verjüngungszeitranmes auf den ganzen Umtrieb (Femel oder Plenterform) kann das Vorhandensein eines guten und entwicklungsfähigen Verwuchses oder die Schaffung schöner guter Verjüngungsgruppen nicht entbehren.* Wo sie kritiklos *jeden* natürlichen Vorwuchs benützt, auch wenn er mit 50 oder 70 Jahren höchstens 1 meter hoch ist, da verdient ein solches Verfahren den Namen einer Wirtschaft überhaupt *nicht* mehr, es wird blanker Raubbau, dessen blenden-sollende geringe Kultur-Kosten nichts weiter als blanke Täuschung Unerfahrener bezwecken.

Glaubt man sich aber damit beruhigen zu können, dass aus solchen schlechten, oben erwähnten Vorwüchsen ein gesunder geschlossener Wirtschaftswald sich entwickeln werde, so würde das die Wahrheit des Satzes voraussetzen, dass eine Jahrzehntelang *unterdrückte* Baumpflanze bei Gewährung von entsprechendem Lichtgenuss und Kronenfreiheit sich ebenso zum Hauptstamm entwickeln könne, wie eine *gesunde*, durch Druck *nicht* degenerirte. Wie grundfalsch eine solche Annahme ist, zeigt schon das Fiasco des auf ähnlichen Voraussetzungen basierenden Borggreve'schen Durchforstungsverfahrens. Vollends in den *höheren* Regionen der Berge ist ein solcher Fehmelschlag oder Plenterbetrieb zwecklos. *Viel* Material kann nicht gewonnen werden und wo man nur mehr einzelne Stämme nutzen kann, da ist man meines Erachtens in die Region des *Schutzwaldes* eingetreten oder ihr bedenklich *nahe* gekommen und da muss, so lange die Waldungen der tieferen Lagen noch keiner richtigen Wirtschaft unterstellt sind, einfach vorerst die Hand davon gelassen werden.

Man übersieht aber auch an *den* Plätzen, wo aus natürlichen Gründen *horst* und *gruppenweise* Natur-Verjüngung möglich wäre, ein paar *Hauptbedingungen* einer solchen Wirtschaft *vollkommen*. Sie setzt nämlich, wenn sie richtig betrieben werden will, einmal eine *weitgehende* Aufschliessung der Waldungen durch Wege etc. voraus und kann ferner auch nur von einem erfahrenen und *geschulten* Personal executiert werden.

Ob diese *conditiones sine qua non* grundlegender Natur für Japan zutreffen oder nicht, darüber glaube ich besser kein Wort verlieren zu sollen. Dinge, welche weitschauenden Blick, Umsicht und praktische Schulung der technisch herangebildeten Beamten sowie eine vorzügliche Dressur der Vollzugsorgane erfordern, können meines Erachtens nicht wie eine Maschine einfach gekauft und in Gang gesetzt werden und im Decretieren ist man zweifellos weniger beengt, als in der Durchführung in praxi. Die *ungeheure* Verantwortung aber, die darin liegt, dass man ein Nationalgut, wie den Wald, der, bei richtiger Behandlung, eine der besten Einnahmequellen des Staates sein kann und in andern Ländern auch ist, zu *gewagten* Experimenten mit zweifellos *schlechtem* Ausgang benützt, gibt doch wohl auch zu denken

Die ganze Frage der Rentabilität des Waldes in Japan ist eine Frage der Entwicklung der Communicationslinien!

Welche Nutzung und Verjüngungsform wäre demnach empfehlenswerth, bis dieser Entwicklung des Verkehrs durch Wege etc. mehr Rechnung getragen ist?

Zweifellos die *Kahlschlagsform in relativ schmalen Schlägen*, im Bergterrain der Hangrichtung folgend, also *Saumschlag* mit *nachfolgender künstlicher Verjüngung durch Saat oder Pflanzung*.

Damit vermeiden wir einerseits die Gefahren des Grosskahlschlages, und bekommen *mehr* Material auf jedem gegebenen Hiebsplatze, als wie dies bei richtiger Fehmelschlag oder Fehmelwirtschaft möglich ist; *darauf* muss aber gesehen werden, da eben die geringe Ausdehnung des Wegenetzes für manche substituierend eintretende Bringungsmethoden einen *hinreichenden* Materialanfall als Bedingung für die Rentabilität der Bringungsanlage erfordert. Die Saumkahlschlagform ist ferner technisch einfach und also mit dem vorhandenen Personale eher durchführbar, sie ermöglicht die Bringung über die Schlagfläche *selbst* in ungenirter Weise, und ist z. B. die günstigste Abholzungsform in Bergländern, denen gute Hangaufschliessung durch Wege mangelt. Unter Zuhilfenahme entsprechenden Hiebswechsels kann auch die sichere Nachzucht von Beständen jeder Holzart oder Mischung div. Holzarten sehr gut ausgeführt werden.

Das m. E. nach den dermaligen Verhältnissen auf lange Zeit hinaus in Japan *Erreichbare* ist damit gekennzeichnet. Wird einmal ein wohlüberlegtes richtiges Fundament, ein solider Unterbau, für die weitere Entwicklung durch *Reorganisation* der Waldwirtschaft Grundsätze mit zielbewustem Ersatz der *überstürzten, weder genügend gekannten, noch in ihrer Wirkung genügend geschätzten Massnahmen* gegeben, dann wird auch die Zeit sich nähern, wo der primären *Fundamentalaufgabe, der Aufschliessung der Waldungen* durch Wege etc., *ohne* Schwierigkeit eine *natürliche* Verjüngungsform europ. Stils wird folgen können.

Techniker, welche selbst praktische Wirtschaftler sind, müssen aber erst das Personal für diese hohe Anforderung mittlerweile *schulen*, was bei localer *langsamer* Überleitung (der Aufschliessung entsprechend) vom *Saumschlag* zur *femelschlagweisen Verjüngung* und *zuerst an kleinen*

Objecten probirt, bei consequenter *richtiger* Methode auch erreicht werden kann.

Schwierigkeiten können nicht schrecken, wenn es das Wohl des Staates gilt, aber *Hindernisse* in waldwirtschaftlichen Dingen lassen sich nicht *überspringen*, sondern nur *langsam und schrittweise besiegen!*





Wald und Wasserwirtschaft.

VON

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*(Vortrag gehalten in der deutsch. Ost-Asiat. Gesellschaft für Natur u. Völkerkunde
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Jeder Kulturfortschritt im Leben der Völker beruht nicht in letzter Linie auf einer intensiveren Ausnützung der natürlichen Güter, wie sie durch klimatische, oro- und hydrographische Verhältnisse des von einem Volke bewohnten Landes dem Menschen von der göttlichen Vorsehung beschieden wurden.

Die notwendige Voraussetzung hierfür ist zweifellos die Erkenntniss des Werthes derselben und die menschliche Einwirkung ist mitunter in ausgedehntem, ja staunenswerthem Masse möglich, häufig findet sie aber auch ein schnelles Ende, je nachdem sie in Übereinstimmung oder in Dissonanz mit den "ewigen Gesetzen" der Natur ihre Wege einschlug. Das Grundfundament eines *wahren* Fortschrittes muss also die Erforschung der Ursachen einer Erscheinung bilden und so sehen wir, dass mit der zunehmenden Durchleuchtung bisher dunkler Gebiete durch die alles durchdringenden Strahlen einer logisch vorgehenden exacten Wissenschaft, natürlich auch der Weg zum Ziele gerader und freier von irreführenden Seitenpfaden wird.

Gerade die Entwicklung der Naturwissenschaften in ihrem rapiden Gange hat der unanhaltsam vorwärts hastenden Zeit bei der Kulturmission die unschätzbaren Dienste geleistet, wie ein Blick ins tägliche Leben ohne weiters lehrt.

Dass manch alte liebgewohnte Vorurtheile oder Annahmen dabei zu Fall kommen und manche trotzig eingelegte Burg einer Scheinwahrheit ihre

Jahrhunderte alten Grundfesten untergraben findet, ist nur natürlich, aber ungern trennt sich der Mensch von einer durch Alter geheiligten Überlieferung.

Die Besprechung des vorliegenden Themas wird zeigen, dass es in seinem Gegenstande zu einem der heissumstrittenen gehört, da eben eine allseitig einwandlose Lösung der damit vorknüpften Fragen noch weiterer exacter Forschungen bedarf, wenn auch das Endziel in groben Umrissen schon jetzt *klar* vor Augen steht.

Wie aber die Ungleichartigkeit von Kräften allein eine heilsame Störung des starren Gleichgewichtes veranlasst und damit als Grundelement der Bewegung im Sinne der Bildung neuer Phasen betrachtet werden muss, so kann auch nur durch den Widerstreit des Meinungen, basirt auf gründlichen Untersuchungen, die Vorbedingung geschaffen werden für das Aufsteigen des Phönix "Wahrheit und Fortschritt" aus der Asche der im Kampfe zerstörten Vorurtheile.

Der Grund warum bei der eigentlich beabsichtigten Ausprache über die "*Wasserwirtschaft*" und deren Werth für ein Land, der "*Wald*" als gleichberechtigt, ja voranstehend genannt wird, liegt in der Abhängigkeit der Letzteren zum grossen Theile von den Verhältnissen des Ersteren. Im *Wasser* ist den Menschen eine Naturgabe von der Schöpfung verliehen, welche nicht nur in fundamentalster Weise das organische Leben auf dem Erdball beeinflusst, sondern in weiterer Ausgestaltung seiner Benutzung dem schwachen Geschlechte der Erdenbewohner jene Riesenkräfte leiht, welche zur Durchführung der immensen Pläne seiner nimmer rastenden geistigen Capacität eines der grössten Hilfsmittel darstellt.

Gewissermassen schon der blosser Anblick der Weltkugel scheint uns die Bedeutung des Wassers auf unserem Planeten ziemlich eindringlich zum Bewusstsein bringen zu wollen, denn $\frac{3}{4}$ der Oberfläche ist allein von den *Meeren* eingenommen. Die Bedeutung dieser internationalen Handelsstrasse der Völker wächst mit jedem Tage und die Ausgestaltung der Verkehrsmittel durch die Technik macht sozusagen die Welt nach und nach kleiner und die Mission der Kulturstaaten grösser und grösser, bringt das *Ziel der geistigen und sittlichen Hebung* der Völker der Erde näher und näher.

Um aber in diesem Austausch der realen und geistigen Produkte einen

durch historische Vergangenheit namentlich aber den verlichenen Talenten entsprechenden Platz im Rathe der Völker für den Verkehr über die Meer zu behaupten, ist die *Entwicklung* der Produktion im Innern der Länder eine bindende Voraussetzung. Und hier kommen die Binnengewässer *die Seen, die Flüsse und Bäche zur Geltung*. Sie bilden den Kernpunkt der Gütererzeugung sei es direct durch Fructificirung der im natürlichen Boden vorhandenen Nährstoffe für das Wachstum von Feldfrüchten, wie im Agrarstaat oder indirect, als Lieferanten motorischer Kraft für die Maschinen des Industriestaates. Stets aber ist die Erzielung einer segensreichen Wirkung geknüpft an ein gewisses Ebenmaas, eine gewisse continuirliche Beständigkeit, nirgends sind Extreme nach dem Zuviel wie Zuwenig von solch einschneidenden Folgen begleitet, wie hier.

Die Ausstattung eines Landes mit zahlreichen guten Flüssen und Wasserläufen gibt einen guten Masstab der Beurtheilung seiner Entwicklungsfähigkeit gegenüber einem anderen Lande bei sonst gleichen Bedingungen. Je grösser die Ströme, je tiefer dieselben sind, je leichter und je weiter sie vom Meere landeinwärts befahren werden können mit grossen Schiffen, desto *werthvollere Verkehrsmittel* stellen sie dar und, wenn es auch eine Zeitlang schien, als ob ihre Bedeutung durch die Eisenbahnen in den Hintergrund gedrängt würde, so hat eben die neueste Zeit mit ihren Riesenprojekten, z. B. dem Mittellandkanal in Deutschland bewiesen, wie gerade bei stark entwickeltem Verkehr und wirtschaftlichem Aufschwung die Wasserstrasse der grossen Flüsse u. Kanäle wegen ihrer hohen Leistungsfähigkeit und Billigkeit, speciell bei im Eigenwerth niedrigen Gütern, nicht entbehrt werden kann.

Wo *Mangel* an natürlichen Wasserwegen herrscht, wird sich das Eisenbahnnetz bei günstiger Aussicht der Produktion verdichten aber immer wird es auch mit dem Nachtheile grösserer Kosten behaftet sein. Die gute Benutzbarkeit der Wasserstrassen hängt jedoch nicht zum geringsten Masse von regelmässigen *Wasserständen* und möglichst *unveränderlichen* Sohlenverhältnissen ab. Da es bei den grossen Strömen vor allem der Oberlauf und die Seitenflüsse sind, welche die günstigen oder *ungünstigen* Veränderungen veranlassen, und eine Einflussnahme von

Seiten des Menschen im Grossen Ganzen *hier* einsetzen muss, so ergibt sich von selbst *deren* grosse Wichtigkeit im Systeme der Wasserwirtschaft. Die im Nachfolgenden betrachteten *wirtschaftlichen* Vortheile der kleineren Flüsse und Wasserläufe werden von den *grossen* Verkehrsströmen, bis zu einem ausgiebigen Grade getheilt, so dass ihre gemeinschaftliche Anführung zweckmässig erscheint.

Während bei den kleineren Flüssen und Wasserläufen der Werth als *grosses Verkehrsmittel des Handels* wegfällt, leisten *alle* Wasserläufe für *Landwirtschaft* und die *Industrie* nach mehrfachen Richtungen Unersetzliches.

Die Landwirtschaft kann sie nicht entbehren im Sinne:

- 1) der Entwässerung. 2) Zum Zwecke der Bewässerung
& Fructification des Bodens,

während die vom Gewerbe u. der Industrie so sehr gesuchte *billige* Betriebskraft und die Möglichkeit eines billigen Lokaltransportes für Rohprodukte, bei *grösserer* Entwicklung des *landwirtschaftlichen* Betriebes selbstverständlich von demselben durch die Zuhilfenahme der Maschinen etc. im Sinne der gleichen Vortheile, wie sie die industrielle Thatigkeit sucht, ebenfalls getheilt wird. Die *Entwässerung des Landes durch die Flüsse* ist gewissermassen ihre natürlichste Aufgabe, denn das meteorisch niederströmende Wasser muss diesen Ausweg haben, wenn nicht ein Hinderniss der Kultur und damit der Bewohnbarkeit eines Landes durch Versumpfung geschaffen werden soll. Die *Abflussgrössen* der Flüsse sind von verschiedenen Umständen bedingt. In erster Linie von der *Menge der fallenden Niederschläge* in ihrem Einzugsgebiete. Wo diese in einem Lande nur *gering* sind, wird man vergeblich nach Wasserläufen von einiger Bedeutung suchen.

In Aden z. B. fällt durchschnittlich alle drei Jahre einmal ausgiebiger Regen und die Vorkehrungen durch künstlichen Ausban natürlicher Felsbecken im Gebirge als Wasserreservoirs, um nur das nöthige Trinkwasser für die spärliche Bevölkerung zu erhalten, sind weltbekannt. Der Mississippi der gewaltigste Strom Nordamerikas mit dem ungeheuren Einzugsgebiete von 3,150,000 q. Km. führt dagegen von den pro Jahr ca 90,000. Millionen engl.-Cubicfuss betragenden Niederschlägen auf diesem

Territorium, ca 19,500 Millionen Cubicfuss dem Meere zu. (25% der Niederschläge.)

Die Riesenströme Südamerikas (Amazonen & Laplata-Strom) und des Ob, des grössten asiatischen Stromes in Russland, des Jantzekiang in China lassen in ihren gewaltigen Wassermassen einen Rückschluss auf die Menge der Niederschläge in ihrem Einzugsgebiete sowie auf dessen Grösse zu. Freilich ist die Vertheilung der Niederschlagsmengen in den angeführten Strom-einzugsgebieten selbst eine umso ungleichmässiger, je klimatisch und orographisch verschiedenere Regionen umschlossen bzw. drainirt werden.

Das *Verhältniss* zwischen *Niederschlagsmenge* und *Abfuhrmassen* ist keineswegs ein *konstantes* sondern, wie leicht erklärlich, von den variablen Verhältnissen des Bodens nach natürlicher Form, physikalischer Beschaffenheit etc. abhängig und am *meisten* bedingt durch das *Fehlen* oder *Vorhandensein* einer Pflanzendecke und deren *Zusammensetzung*. Bei der Besprechung des Einflusses des Waldes werde ich hierauf ausführlicher zurückkommen.

Was die drainierende Thätigkeit der Flüsse stört, wird naturnotwendiger Weise auch die günstige *Wirkung* der Drainage beeinträchtigen. So sehen wir denn überall, namentlich in den mittleren und unteren Flussläufen mit den geringen Gefällen, woselbst in der Regel die Ebene das Characteristicum der anstossenden Landschaft bilden wird, durch *Verwilderung der Flüsse*, also bei *mangelndem* Eingreifen des Menschen Überschwemmungen, Eisstopfungen beim Eisgang, Neigung zur Bettverlegung, Versandung etc, in allen Fällen aber eine starke Verminderung der Abführung des Wassers eintreten. Es muss also die Sorge einer Landesverwaltung auf eine *Correction* und *Überwachung* ihrer Wasserläufe gerichtet sein, natürliche Abflusshindernisse müssen beseitigt und künstliche Einbauten auf ihre Wirkung in etwa nachtheiligem Sinne zuvor wohl geprüft werden.

Die zweite wichtige Aufgabe des Flussläufe nämlich der "*Bekanntmachung*" des Kulturlands hat eine weitaus *grössere* Beachtung von Seite des Menschen gefunden und wird dies Verhältniss wohl auch in Zukunft bestehen; die Möglichkeit der Fructificirung ungeheurer Flächen ist in vielen Fällen nichts weiter als eine Frage der Einleitung von genügend Wasser

um mineralisch tauglichen Boden durch die Zuführung der Pflanze nöthigen, bisher fehlenden, Feuchtigkeit, in ertragsreiche Kultur umzuwandeln.

Das grösste Project der Neuzeit dieser Art, dürften die Nilsperrdämme bei *Assuan* bezw. *Sint* im oberen resp. mittleren Aegypten sein, welche in Verbindung mit den bereits bestehenden bei Kairo eine systematische Ausnützung der Nilfluth im Frühjahr in grossartiger Weise bezwecken. Durch Sperrdämme sollen Stauseen geschaffen werden, deren Wasser nach Bedarf zur Abgabe kommt und die Bodenbenutzung in ausgedehntem Masstabe, namentlich für höher gelegene Ländereien, welche mit den bisherigen Staumitteln Wasser nicht erhalten konnten, garantiert. Den riesigen Kosten von 2 Mill. Pfund stehen dauernde Erhöhung des Nationalvermögens, der Steuerkraft des Landes und Einnahmen aus dem zunehmenden Verkehre in überlegener Grösse gegenüber.

Bereits ertragsfähiger Boden wird in seinem Ertrage durch *dauernde Bewässerung* gesteigert, das wird namentlich dort zur Geltung kommen, wo *sonstige günstige klimatische Faktoren noch unterstützend eingreifen*. In Europa hat Frankreich den 31. Theil, Italien den zwangigsten Theil seiner Landfläche zur Bewässerung eingerichtet, während in unserem Heimathlande Deutschland diese Ziffer nicht erreicht ist.

Je mehr bei wachsender Bevölkerung und nicht beliebig *vermehr-bezw. cultivirbarem* Boden *geringere* Böden der Kultur unterworfen werden, desto mehr wird auch das billige Düngemittel des Wassers in Anspruch genommen werden.

Im Allgemeinen sind es auch hier wiederum die kleineren u. mittleren Wasserläufe welche für solche Zwecke in Betracht kommen, denn die grossen Flüsse bedürfen zu ihrer Nutzbarmachung kostspieliger Anlagen, welchen nicht immer die entsprechenden Gegenwerthe gegenüberstehen. Was endlich den *Werth* von Wasserläufen für *Gewerbe und Industrien* betrifft, so streben Gewerbe, wie industrieller Betrieb im engeren Sinne nach Ersatz der menschlichen Arbeitskraft durch den *Motor*, dessen Antrieb von einer möglichst billigen, leicht erhältlichen und nachhaltigen Kraftquelle i. e. dem Wasser erfolgen soll. Auch diesen Anforderungen entsprechen die geringeren Wasserläufe besser im Durchschnitt als grosse Ströme, da letztere wohl immense Kräfte liefern aber auch enorme Anlagen zur Realisirung derselben

erfordern. (Nutzbarmachung des Niagarafalls in Amerika.) Die Bedeutung der Kraft des Wassers als Antriebsmittel nimmt zu, je mehr der Grossbetrieb decentralisirt wird, was hinsichtlich einer Anzahl Industrien z. B. Holzindustrien schon der Fall ist.

Die Möglichkeit der electricischen Kraftübertragung ist zweifellos als die Hinwegräumung eines Hindernisses für die Nutzbarmachung mancher Wasserkraft zu betrachten.

Die Wasserleitungen der grossen Städte greifen endlich zurück bis in die Quellengebiete, also den Anfang aller Wasserläufe, und die Wirkungen derselben in sanitärer Hinsicht bedürfen keiner näheren Begründung.

Den Seen Kommt jene Bedeutung wie den Flüssen & Bächen weniger zu, sie sind mehr lokalisirt, es fehlt ihnen die Modulation der Längenenwicklung, obwohl sie unter Umständen im gleichen Sinne wie laufende Wasser nutzbar gemacht werden können.

Diesen *segensreichen* Wirkungen des Wassers der Binnenflüsse etc. von denen eine einzige genügt, um ihnen die Eigenschaft der Unentbehrlichkeit zu verleihen, stehen Verheerungen und Katastrophen gegenüber, welche in manchen Ländern durch ihre häufige Wiederkehr die Bewohnbarkeit und Cultur weiter Landesstrecken annullierten und ganze Gegenden der Verödung preisgaben.

Je mehr die Völker der Erde dieselbe occupieren, je dichter sie auf gegebenem Raume sich concentriren, desto mehr wird man sich der Bekämpfung des den Boden bedrohenden Ungeheuers widmen.

Wohl wird es niemals möglich sein, eigentliche Katastrophen grössten Stils, welche in Störungen der Atmosphäre etc. ihre Ursache haben oder einer Veränderung von bisherigen Gleichgewichtsfaktoren im Weltsysteme zur Last fallen mögen (man denke nur an die Theorie der allmählichen Abkühlung der Erde bis zur Vereisung, an den Einfluss der Sonnenflecken etc.) abzuwenden, aber bis zu einem gewissen Grade besitzt der denkende Mensch Mittel, um sich zu schützen und zu wehren gegen den Vernichtungskampf, den ungezügelte Naturkräfte gegen ihn führen, u. wenn auch nach dem Dichterworte "denn die Elemente hassen das Gebild von Menschenhand," alles irdische Streben eitel zu sein *scheint*, so dürfen wir doch die Hände nicht in den Schooss legen und müssen das *Menschen-*

mögliche zu erreichen streben.

Man hat sich in neuerer Zeit nicht mehr begnügt mit der Bekämpfung der Folgeerscheinung, *dem Hochwasser* als solchem, sondern ist der Frage nach den *Ursachen* nähergerückt. Hier findet man nun in der Litteratur den vom Volksglauben längst als Ursache bezeichneten Hauptfaktor, nemlich den Zustand des Ursprungsgebietes der Flüsse hinsichtlich seiner *Pflanzendecke*, und in der Hauptsache ist der "Wald" damit gemeint, in Herz und Nieren geprüft auf den Zusammenhang mit den verheerenden Wirkungen der Wasser in den Flüssen.

Wir sind damit unwillkürlich in jenes Gebiet der Wasserläufe eingetreten, das uns Forstleute naturgemäss am meisten interessirt, in den *Oberlauf*, das aber auch, wie ich darzuthun hoffe, die meiste Aussicht *auf erfolgreiche Beeinflussung* der heute so brennenden "Wasserfrage" gibt. Ich beschränke mich daher im grossen Ganzen auf die Besprechung der möglichen Einwirkungen auf die Wasserläufe, im *Entstehungs-Gebiete*, welches wohl meistens im Gebirge zu suchen ist und auf die Bekämpfung der Übel in den *oberen* im Gebirge gelegenen Theile der Flüsse, wo die *grossen Gefälle* und insbesondere die *Geschiebeführung* die meisten Schäden verursachen, während im mittleren und unteren Laufe der Ströme *mehr* die *Menge* der zugeführten Wassermassen verderblich wird.

Der erstere Theil besitzt vielleicht nicht immer jene räumliche Ausdehnung der Schäden, wie sie die Niederungen der Mündungsgebiete und die Mittelläufe der Flüsse aufweisen, aber an *Intensität* der Verwüstung kommt kein anderer Theil ihm gleich. Hier ist das Terrain der Wildwasser, der "*Wildbäche*" und ihrer Vermehrungen und jeder Erfolg der hier errungen wird, kommt den Niederungen der Ebene zu Gute und zwar in potenzirtem Maasse.

Frägt man zunächst, ehe man den Wert der "Bewaldung" untersucht, ob und in *wieweit* eine Pflanzendecke im Ursprungsgebiete überhaupt einen Einfluss ausübt auf das Regime der Wasser, so kommt man auf Grund der Untersuchungen des bayrischen Prof. Ebermayer, des leider zu früh verstorbenen E. Wollny in München u. Anderer zu dem Schlusse, dass *jede* Pflanzendecke (Gras, *Hara*) einen *Vorzug* gegenüber *kahlem* Gebirge bedeutet.

Unschwer ist einzusehen, dass *jeder* Bodenüberzug perennirender Gewächse einen gewissen Ausgleich in der Wasserführung hervorruft, indem die ober- wie unterirdische Wasserableitung verzögert wird, was in der Gesamtwirkung *gleichmässigerer* Wasserstände seinen schliesslichen Ausdruck findet.

Eine Verlangsamung der oberirdischen Abfuhr bedeutet *eben* ein besseres *Einsickern* der meteorischen Wasser in den durch die Pflanzenwurzeln physikalisch günstiger d. h. lockerer gemachten Boden, wodurch *Durchlässigkeit und Aufnahmefähigkeit* sich steigern.

Für die wichtige Frage der *Quellenbildung- u. Erhaltung* an den Hängen der Gebirge kommt in erster Linie das längere Verweilen des oberirdischen Wassers auf der betr. Fläche in Betracht, denn *je mehr* davon in den Boden einsickert, desto *besser* wird die *Speisung der Quellen* erfolgen, die ja nichts weiter sind, als tiefer zu Tage tretendes Sickerwasser böherer Lagen. Sieht man von "*Wald*" vorerst ab, so ist im Übrigen bei der *Höhen-Lage* der *Einzugsgebiete* meist nur eine *Buschvegetation* und in der *Hauptsache* "*Gras*" als Bodendecke *vorhanden*.

Gras erfüllt aber den Zweck der Wasserverlangsamung u. der Verhütung des Terrainangriffes *nur sehr mangelhaft*, denn ein *dichter* Grassüß ist nicht genügend durchlässig und bringt namentlich in steilen Lagen ein *rapides oberflächliches Abfließen* mit sich, ist also zweifellos für Gleichmässigkeit der Wasserführung der Flüsse und für Quellen von *inferiorer* Werthe, wenn auch besser als *nakter* Boden. *Lockere Grasdecken Hara* sind aber keineswegs gegen den Angriff des Wassers gesichert, wie der Augenschein in Japan lehrt.

Als man in Frankreich 1864 hoffte, eine *Beruhigung* der gefährdeten Flächen im Berglande *durch BERASUNG der von Wald entblössen*, durch die meteorischen Wasser in Bewegung gerathenen, und die Wildbäche mit Schutt füllenden Berghänge zu erzielen, da erwiesen sich die daran geknüpften Hoffnungen als trügerisch und es blieb nichts anderes übrig als zur *Aufforstung* zu greifen. Ich denke, das ist ein deutlicher Fingerzeig für die Zukunft der wir z. B. in Japan entgegenstehen, wenn die rücksichtslos- Verschlechterung der *Hara* in steilen Lagen weiter geführt wird, abgesehen davon, dass, wie erwähnt, der *Graswuchs an sich*, auch wenn er den

Boden im concreten Falle hält, dennoch für eine *grössere* Regelmässigkeit der Wasserstände im Sinne der Verlangsamung des Abflusses des Atmosphärischen und somit der *zeitlichen Vertheilung* der Wassermassen starker Regen etc. *nichts Hervorragendes* leistet.

In Japan muss aber der *Frage zwischen dem Zusammenhange der Bedeckung der Gebirge mit entsprechender Vegetation und den Wasser-Verhältnissen* eine erhöhte Aufmerksamkeit geschenkt werden aus *zwei* Gründen.

- 1) Infolge der *schmalen Ausformung* des Landes mit seinen der Längsrichtung folgenden ziemlich hohen Gebirgszügen ist die Längsentwicklung der meisten Flüsse eine sehr *geringe*, es herrscht das bei grosser Länge von Flüssen in anderen Ländern auf den oberen Lauf beschränkte *steile* Gefälle und der "*Wildbach-artige Charakter*" vor, ja verlässt sie zumeist *nicht* bis zu ihrer Einmündung ins Meer, wie man an den Verhältnissen einer Anzahl derselben Fugigawa, Oigawa, Kisogawa etc. etc. beobachten kann.
- 2) Die *Ausformung* der Hänge im Gebirge ist in diesem vulkanischen Lande eine extra *steile*; die erodierende Thätigkeit des auf diese Art *rascher* wie sonstwo zum Abflusse kommenden Niederschlagswassers wird im *ungünstigen* Sinne *noch* unterstützt durch die durchgängig sehr weiche Verwitterungsdecke alter Schiefer etc, welche dem Grundgesteine aufliegt.

Wenn unter der bisherigen Benutzung als *Hara* ein grosser Theile des Berglandes in Japan scheinbar noch *nicht so* sichtbar gelitten hat, als man nach dem Gesagten vermuthen könnte, so ist zu bedenken, dass wir für *die* dem Durchschnittsreisenden zu Augen kommenden niedrigeren Vorberge eine durch das Klima *bedingte* ausserordentliche *Regenerationskraft* der Vegetation haben. Diese Kommt aber für hohe, kühlere Lagen nicht mehr so sehr in Betracht. Deutlich erkennbar sind auch schon für den Laien die umfangreichen Terrainzerstörungen im Bergland des mittleren und südlichen Japan. Von Kobe bis Shimonoseki präsentiren sich die Berge im *räudigen* Zustande d. h. es blinkt der rothgelbe, nakte Grund der Hänge durch die zerstörte *Hara* und den Wald. Wer aber erst einen Blick in das *Innere*

gethan hat, dem ist kein Zweifel über die unheimliche Thätigkeit des Rachegeistes des untergegangenen Waldes.

Es bleibt nun zu untersuchen, in wie weit der "*Wald*" als der Ausdruck der höchsten organischen Gestaltung der Bodendecke der Erde einen *Einfluss* auf die *Wasserhältnisse* eines Landes hat. Diese Frage lässt sich nicht beantworten, ohne dass man die Sache in zwei Abschnitte theilt, nämlich in die sog

- I. "*Waldklimafrage*" d. h. den Zusammenhang des Waldes mit dem Klima eines Landes im Allgemeinen oder auf concreten Örtlichkeiten und,
- II. *Die Wirkung des Waldes*, u. zwar des *Bergwaldes* in erster Linie, auf Regelung des *Wasserabflusses* u. Geschiebeführung.

Der Glaube an eine heilsame Wirkung des Waldes hinsichtlich einer Verbesserung des Klimas durch Milderung der Temperaturextreme, Vermehrung von Niederschlägen, Verminderung der Hagelgefahr etc., kurz als klimatischen Factor ist ein sehr *alter*, nichtsdestoweniger jedoch jetzt als eines der unter dem Ansturm der exacten wissenschaftlichen Forschung gefallenen Vorurtheile zu betrachten.

Man hat auf die Trockenheit resp. Regenarmuth der Mittelmeerländer im Zusammenhange mit ihrem geringen Waldreichthum hingewiesen, das Beispiel von Italien & Griechenland angeführt, wo grosse Länderstrecken durch die mangelnden Niederschläge zu Wüsten geworden seien, als man den schützenden Wald zerstört hatte und kein Geringerer als ein Alexander von Humboldt hat auf die Abnahme der Regenmenge und der Luftfeuchtigkeit durch die Zerstörungen der Waldungen aufmerksam gemacht. Die reiche Zahl der Anhänger dieser Anschauungen wies die hervorragendsten Namen der Wissenschaft auf, aber als man der Sache durch die meteorologischen Stationen und deren Beobachtungsergebnisse nähertrat, namentlich aber durch die Ergebnisse der Untersuchungen über die Existenzbedingungen des Waldes, da gelangte zu dem anfangs Staunen erregenden Resultate, dass nicht der Wald einen Einfluss auf das *Klima* habe, sondern, dass die Baum- und Waldvegetation vollständig von den klimatischen Verhältnissen einer Gegend abhängig sei, so dass die bisher üblichen obenerwähnten Annahmen nur theilweise richtig sind.

Der Wald ist nämlich weder natürlich vorhanden noch jemals künstlich bei sonst günstigsten Verhältnissen dauernd begründbar, wenn z. B. während der Hauptvegetationszeit (in der nördl. gemässigten Zone Mai—August) nicht in minimo 50 millimeter Niederschlag auf die betreffende Gegend fallen. In Aden 3 B. wird niemals eine Baumvegetation ohne künstliche Bewässerung denkbar sein; die Ebene Californiens verdankt ihren Ruf als Obstkammer Amerikas nur den künstlichen Bewässerungsanlagen; die ersten Ansiedler trafen *keine* Bäume an, da eben das *nöthige Minimum an Wasser* dem Boden bei sonst eminent klimatischen Vorzügen nicht geboten wurde.

Baumwuchs ist aber trotz genügender Bodenfeuchtigkeit (:Niederschläge) *ebenso wenig denkbar, wenn* die notwendige *relative Luftfeuchtigkeit von mindestens 50% während der Vegetationszeit* nicht vorhanden ist.

Da wir auf diese zweite Bedingung kaum je Einfluss gewinnen, so ist das Bestreben der Gründung von Wald *absolut* aussichtslos in diesem Falle, mag die Bodenfeuchtigkeit noch so reichlich vorhanden sein.

Beweise für diese Thesen finden sich durch die ganze Welt in prägnantem Ausdrücke.

Die Grasprärien im Centrum Nordamerikas und die Pampas Südamerikas und Australiens, die *Wüsten* Afrikas, *Asiens*, (Gobi in China) sie können nie und nimmer durch des Menschen Hand auch mit Aufwand aller Mittel, Baum oder Waldvegetation tragen, da die Luftfeuchtigkeit in genügender Menge nicht gegeben werden kann.

Das Sinken der relat. Luftfeuchtigkeit unter 50% während der Vegetationszeit kann nur von Gras oder Staudengewächsen ertragen werden, (Prärien Amerikas u. Australiens) während ein Herabgehen der Luftfeuchtigkeit unter 40% rel. Feuchtigkeit und eine Niederschlagsmenge unter 20 *m/m* während der Haupt-Vegetationsmonate die vegetationslosen Wüsten (Asiens u. Afrikas) zur Folge hat.

Nur auf den Grenzgebieten von Prärie und Wald ist anscheinend eine *geringe* Verschiebung in Sinne der Ausdehnung des *Waldes möglich*, aber bei genauerer Untersuchung zeigt sich immer, dass man ehemaligen Waldgrund vor sich hat, der durch irgend eine gewaltsame Einwirkung zu Grunde ging, was beispielsweise in Amerika im ausgiebigsten Masse bemerkt werden kann, indem es durch die Hilfe des Feuers leider

gelang, den Wald auf einer direkt nord-südlich streichenden Linie, parallel dem Mississippilaufe zurückzudrängen u. zwar um volle 10 Längengrade (100° - 90° westl. L.), eine gewiss nicht zu unterschätzende Leistung!

Dass man *hierzulande* in z. B. Hokkaido durch das schonungslose und unvernünftige Anzünden der Bodenüberzüge *des Waldes* in der Nähe der der Ackerkultur gewonnenen Thäler das amerikanische Beispiel, wenn auch im schwächerem Masstabe, aber mit einer für den *Nadelwald* ebenso tödlichen Wirkung wiederholt, habe ich schon in einem früheren Vortrage erwähnt.

Weder die hohen *Temperaturgrade* im *positiven* Sinne, welche in den sonnigen Wüsten Afrikas herrschen, schliessen bei gegebenen Bedingungen *der Feuchtigkeit* den Wald aus (Oasen), noch *Wintertemperaturen* von -30 u. 40° C, wie man sie *in America* beobachtet hat. Ein grosser Theil Sibiriens mit seinen Wintertemperaturen von oft -45° C. ist voll der reichsten Waldschätze. Erforderniss ist einzig und allein eine mittl. Temperatur von $+12$ bis 14° C. während der Hauptvegetationszeit. Wird diese nicht erreicht ($+8$ bis 12° C), so sinkt der Wald zur Stauden u. Buschform herab.

Tritt *jeden* Monat des Jahres Frost ein, so ist *Vegetationslosigkeit* die Folge. *Innerhalb* dieser natürlichen Existenzfaktoren gliedert sich der Wald nach *Form* und *Zusammensetzung* aus *Arten*, den *lokalen klimatischen Verhältnissen entsprechend*, und da die *jeweils nördlicher* bzw. *südlicher* vom Aequator gelegene Zone mit einer *näher gelegenen, aber in grösserer Elevation befindlichen correspondirt*, so ist leicht zu erkennen, wie nach relativ einfachen Gesetzen die Bedeckung der *Continente* durch *Waldflora* zu denken ist.

Wenn dem Walde auch ein *genereller Einfluss auf das Klima* über ganze Flussgebiete und Landstriche oder *Continente abgesprochen* werden muss, so darf doch die Möglichkeit einer *Einwirkung* auf klimatische Verhältnisse im Sinne *localer* Modulation ebenfalls nicht übersehen werden. Ein sogenanntes "*Lokalklima*" kann, ähnlich wie an einem grösseren See besondere Luftströmungen herrschen, vom Walde *beeinflusst* werden und wird sich natürlich dies umso mehr geltend machen, um je grössere Waldflächen es sich handelt.

Die Klarlegung aller *dieser* Verhältnisse muss den eingeleiteten Versuchen überlassen bleiben und wenn der Kampf der Meinungen darüber

auch in den berufenen Kreisen noch heftig tobt, an dem entscheidenden Hauptmomente, dass der Wald klimatische Katastrophen *nicht* verhindern kann und *keinen* Einfluss auf *Vermehrung* u. *Vertheilung der Niederschläge etc. hat*, ist mit allen Consequenzen *kein* Zweifel.

Der Hauptwerth der Waldbestockung für *geregeltere* Wasserstände der fließenden Gewässer und damit für die Verhinderung der zerstörenden und verderblichen Hochwasser oder für Erhöhung der für Kultur und Industrie *gleich* misslichen, *zu niedrigen* Wasserstände, liegt auf einem *anderen* Gebiete.

Bewaldung erhöht nämlich unter Bedingungen einerseits die *Sickerwassermenge*, was von hervorragendem *Einfluss* auf die *Speisung* der *Quellen* ist, und *stellt anderseits* von allen Bodendecken *das bedeutendste mechanische Hinderniss gegen die Abschwemmung der Bodens* u. *Abrutschung der Schecdecke bei Lawinenbildung etc. dar.*

Die erste Behauptung der Erhöhung der Sickerwassermenge, also der Quellenvermehrung, kann in ihrem *vollen* Umfange nach dem heutigen Stande der Wissenschaft *nicht* mehr aufrechterhalten werden und *damit fällt wiederum* eine seit alten Zeiten gehegte Ansicht. Wie weit sie noch Geltung hat, werden wir sehen. Die Untersuchungen von Ebermayer in München und Ototzkij's in Petersburg haben den Nachweis geliefert, dass der Untergrund unter Wald in der *Ebene* unter allem Umständen *ärmer* an Feuchtigkeit ist, als auf freiem Lande, dass also dem Walde *der Ebene* ein Einfluss auf *Vermehrung* der Bodenwasser im Sinne etwa der Erhöhung des Grundwasserspiegels u. der besseren Speisung von Quelle *nicht* zukommt. Das scheint im ersten Augenblicke *stark zu contrastiren* mit Ergebnissen von Versuchen, welche zahlreiche Autoritäten hinsichtlich der Wasser-Absorption und Retention des Waldes und namentlich seiner *Streu* angestellt haben.

Durch die *Überdeckung* des Bodens mit Wald wird in erster Linie dessen oberflächliche Verkrustung, teilweise erzeugt durch *mechanische* Wirkung (Festschlagen durch Regentropfen) verhindert, ferner, als eine Folge der durch die Humuserzeugung der Streudecke bewirkten besseren Krümmelung und Lockerung der Struktur des Bodens bei sonst gleicher mineralischer Beschaffenheit die *Aufnahmefähigkeit* für meteorische *Nieder-*

schläge erhöht. Die herrschenden *niederen* Temperaturen im Walde während der hauptsächlich wichtigen wärmeren Jahreszeit,—im Winter ist der Boden an sich durch Frost undurchlässig oder die Bedeckung mit Schnee als eine momentan latente Wasserquelle zu betrachten,—und die dazu kommende aus der Transpiration der Pflanzen resultierende grössere Feuchtigkeit der Luft (3–10% im Mittel mehr gegenüber dem Freilande) wirken im Sinne einer Vermehrung der Wassermenge des Bodens indirekt, indem dadurch die Verdunstung des atmosphärisch zu Boden gelangten Wassers verhindert wird.

Eine gute, in richtigem Zersetzungsgrade befindliche *Streudecke*, welche im wohlgepflegten Walde niemals fehlt, vermindert die Verdunstung des der Bodenfeuchtigkeit nochmals ganz bedeutend, so dass die Verdunstung im Walde etwa nur 20% von jener einer Freilandsfläche beträgt.

Diesen günstigen Faktoren gegenüber, welche die *Sickerwassermenge* experimentell nachgewiesenermassen um nicht weniger als 24% für den *streubedeckten Waldboden* gegenüber dem *waldlosen erhöhen*, steht nun der aufstockende Holzbestand bis zu einem gewissen Grade *feindlich* gegenüber. In erster Linie werden von dem atmosphärischen Niederschlagswasser ca. 25% desselben durch die Baumkronen absorbiert, welche überhaupt *nicht* an den *Boden gelangen* können und ist diese Ziffer natürlich nach Waldzustand Holzart, Alter etc. einer gewissen Veränderung unterliegend.

In dichten Fichtenbeständen werden die Absorptionsprocente der Kronen bis zu 40 und 45% ansteigen und in lichten Lanbwaldbeständen umgekehrt niedrige Beträge aufweisen. Das bisher nicht genügend in Rechnung gezogene Abflusswasser an den Zweigen und Schäften, welches bei länger dauerndem Regen nachträglich diese Procente um einige Einheiten verbessert, ist wegen der Schwierigkeit der Untersuchung für verschiedene Verhältnisse noch *nicht* hinlänglich einwandfrei bestimmt.

Die Vervielfältigung der Oberfläche durch die Blätter namentlich aber die unzähligen Nadeln der Bäume ist der raschen Verdunstung des aufgefundenen Wassers und damit dem definitiven Verluste desselben für den Boden ungemein günstig. Von *dem*—nach Abzug von etwa 25–30% des Niederschlagswassers, welches an den Kronen der Waldbäume hängen blieb, und weiteren 8–10%, welche durch Verdunstung aus dem Boden in die Luft verloren gehen,—übrigen Quantum von etwa rund 60% werden ca

25% durch den *Boden* entgeltig *aufgesogen*, u. der Rest zur oberirdischen Abfuhr gebracht, Jeder pflanzenbedeckte Boden nun, ganz besonders aber der durch die Vegetation des *Waldes* in Anspruch genommene, erfährt eine kolossale *Verringerung* seiner Feuchtigkeit, denn die Bäume sind die *grössten Wasserconsumenten* und darauf ist eine Thatsache zurückzuführen, welche geeignet ist, durch fälschliche Auslegung eine *gewisse Unruhe in waldkonservativ handelnden Kreisen* hervorzurufen, ja vielleicht in der Hand gewissenloser Volksagitatoren und habgieriger Waldschlächter zu einem allerdings nicht einwandfreien Sturmmittel gegen die *Erhaltung der Wälder* benutzt zu werden.

Prof. Ototzkij in Petersburg fand, wie bemerkt, die übrigens aus kleineren Versuchen längst vermuthete Thatsache, dass der *Grundwasserspiegel in der Ebene* unter Waldungen eine *bedeutende Senkung erfahre* und somit praktisch das *Gegentheil* von der behaupteten Bodenfeuchtigkeitsbewahrung durch den Wald *darthue*. Sicherlich kann nicht erwartet werden, dass diese oft sehr bedeutende Differenz des Wasserstandes in tieferen Schichten zwischen Freiland und Wald zu *Gunsten* einer *reichlichen* Speisung der Sickerwasser und damit der Quellen durch den Wald spricht. Bei gleicher geo-physikalischer Beschaffenheit leistet also die baumlose *Ebene* mehr für eine continuirliche Unterstützung des Grundwasserstandes und der Quellen als der *Wald*. Man ist für den ersten Lugenblick erstaunt über die *drainirende* Wirkung der *Waldvegetation*, aber *einige* Zahlen mögen beweisen, wie kolossal der *Wasserverbrauch* durch die *vegetativen Prozesse der Bäume* sich stellt.

An sich ist die Produktion der organischen Substanz pro Jahr bei den Bäumen schon *grösser* als bei allen *übrigen* Kulturgewächsen und daraus erklärt sich auch der entsprechend höhere Wasserverbrauch durch Transpiration zur Erfüllung dieser gesteigerten Arbeitsleitung. Die im Baumkörper u. Blättern *selbst aufgespeicherte* Wassermenge ist ebenfalls sehr bedeutend, sie beträgt nach Ebermayer bei einer kräftig entwickelten 85 jg. Fichte (im Holzkörper und in den Nadeln) ca 1000 Liter; eine gleichalterige Tanne hatte 1200 Liter Wasser.

Zur Produktion der organischen Substanz verbrauchte eine grosse

Birke in 6 Monaten nicht weniger als 7080 kg. oder pro. Tag. 38 Liter im Verdunstungswege und eine 115 jg. Rothbuche beanspruchte ca 50 Liter in gleicher Zeit, während bei jüngerem Alter (50-60 Jahre) eine Rothbuche pro. Tag. 10 Liter benöthigte. Ein *Buchenhochwald* producirt auf *gutem* Standorte jährlich durchschnittlich 7057 kg. Trockensubstanz, was einer jährlichen Wasserconsumption von etwa 2,187,670 kg. oder = 218 *m. m* Wasserhöhe gleichkommt.

Diese Zahlen eröffnen Einblick in den Haushalt der Natur von geradazu verblüffender relativer Einfachheit und lassen die entsprechenden Schlüsse auf den *inneren* Zusammenhang der äusserlichen Erscheinungen *zu-Nimmermehr* wird es gelingen einen schönen alten Buchenbestand zu erziehen, *wo* ihm das Minimum seiner zum Wachsthum nöthigen Wassermasse nicht zu gute kommen kann. Es *basirt* somit die ganze *Abstufung der Bonität* bei gleicher physikalisch-chemischer Bodenbeschaffenheit für eine Species *zu einem guten Theile* auf den *Grundwasser-bezw. Niederschlagsverhältnissen*. Was nun für die *Ebene* vom Einfluss der Waldes auf die Wasserverhältnisse gilt, ist *keineswegs* zutreffend mit Zunahme der Erhebung des Bodens, also im *Berglande* und *Gebirge!*

Mag in den Ebenen immerhin der Wald *verringert* werden auf Grund *dieser* Beobachtungen, besondere Nachtheile *grossen* Stils werden daraus nicht erwachsen, höchstens dass einige Quellen niederen Ursprunges versiegen. Grössere Quellen verdanken ihre Entstehung und ihre Speisung meist dem *Druckwasser der höheren Lagen* und werden dadurch, dass *tiefer* Plätze *entwaldet* werden, kaum in ihrer Stärke und in dem hervorragenden Werthe, der ihnen für Wasserversorgung der *Flüsse*, Wiesenbewässerung etc zukommt, beeinträchtigt werden.

Mit *jedem meter* höherer Bodenerhebung aber gestalten sich die Verhältnissheinsichtlich der Bodenfeuchtigkeit: im Walde *günstiger*, *wächst* die *Bedeutung*, der *Werth des Waldes*. Nicht nur *die Niederschläge* *mehren* sich, sondern auch durch die *Abnahme der Temperatur* und damit der Verdunstung, sowie durch die *grössere Lockerheit* des Waldbestandes die *Summe* der der dem *Boden verbleibenden Feuchtigkeit* im *Wachsen be-griffen* ist. Die Vegetationsdauer in den Hochlagen ist *kürzer* und damit die *Ansprüche* der Baumevegetation an das *Bodenwasser* *geringer*, die *sinkende*

Durchschnittstemperatur und Zunahme der relativen *Feuchtigkeit* bewirken auch eine *geringere* Transpirationsgrösse.

In je höhere Lagen man daher in den *Bergen ansteigt*, desto *weniger* wird die in der Ebene an sich *bedeutende Differenz* im Boden-Wassergehalt einer *bewaldeten* und *unbewaldeten* Fläche, *desto mehr entkräften sich die Vorwürfe gegen der Wald als einen Feuchtigkeitsverzehrer* ohne Gleichen, *desto mehr bekommt der Wald das Recht der Existenz und überall, wo man gegen dieses Recht sündigte, waren die Folgen schreckliche*. Die *konstante* Speisung der Bäche & Flüsse ohne *excessive schädliche* Extreme wich stets *nach* der Entwaldung den abnormsten Gegensätzen und die Geschichte der Schweiz, Tirols, des südlichen Frankreichs (Provence) Italiens, Griechenlands (und teilweise auch Japans) lehrt jedem Unbefangenen die traurigen Folgen, welche die Vernichtung des dem Menschen von der Natur gegebenen schützenden Waldes nach sich zieht.

Im Bergland und Gebirge besitzt der *Wald* eben nicht *blos* das *Recht der Existenz*, sondern er wird zur *zwingenden Notwendigkeit*, da sich der *Faktor des mechanischen Hindernisses gegen die oberflächlich zum Abfluss kommenden Wasser hinzugesellt*. Die *Menge des in ihrem Oberflächenablaufe zerstörend wirkenden Niederschlagswassers ist experimentell auf ca 35% der Totalniederschlagsgrösse ermittelt* und einer *Vergrösserung* oder *Verminderung* in *zweifacher* Weise *unterworfen*. Je *absorptionsfähiger* der Boden ist, auf dem der Oberflächenabfluss *erfolgt* desto, mehr wird sie *verringert* und die *Aufsaugungsmenge erhöht*, von der *hinwiederum das für die Quellenspeisung so wichtige Sickerwasser abhängt*, welches im Durchschnitt 17-20% des Niederschlages beziffert.

In positivem Sinne wirkt nun auf das oberflächlich zum Ablauf kommende Wasserquantum und zwar in *ganz* bedeutendem Masse die *Neigung* des in Frage kommenden Terrains. Je *weniger* lang infolge der Schwerkraft das Wasser auf dem *concreten* Boden verweilt, desto *weniger* versickert, desto mehr nimmt die *Oberflächenablaufwassermenge* zu.

Wo Wald fehlt, ist im geneigten Terrain, also im *Bergland*, ein Ansteigen der *oberflächlich* abfliessenden Wasserquanta auf 45-55% der Niederschläge, je nach dem Terrainwinkel, zu erwarten und in *vegetationslosen* Gebieten der Gebirge gehören 60% und mehr keineswegs zu den Seltenheiten. Die

zerstörende Kraft bewegten Wassers wächst natürlich *mit seiner Menge* und alles was dieser Thätigkeit einen Hemmschuh auferlegt, muss in *conservirenden* Sinne wirken.

Hier greift nun der *Wald* wie keine andere Vegetationsdecke wirksam ein, theilt durch sein *Wurzelsystem* und seine *Streudecke* u. durch die hiebei implicite geschaffenen tausendfachen *mechanischen Hindernisse* die abfließenden *Wasserfäden* und zwingt sie durch Erschöpfung *ihrer Kraft* zur *Unschädlichkeit*. *Runsenbildung, Anrisse, Abschweemmung der Feinerde, Auswaschung der Verwitterungsschicht*, kurz *jede* Art von Terrainangriff mit all den nachtheiligen Folgen werden durch *sein Vorhandensein* *vermieden* und die *Umwandlung* von normalen *Bächen* in *Wildbäche* mit ihrer verderblichen Geschiefbeführung und den *gefährlichen* Begleit- und Folgeerscheinungen *verhindert*.

Unumstösslich ist durch die direkte Beobachtung und lange Erfahrung bewiesen, dass der Zerstörung des *Bergwaldes* die *Vertrocknung, Verödung* der Hänge und im Weiteren unter dem Einfluss der meteorischen Niederschläge die *rapid zunehmende Abfuhr* der *Bodenkrumme* bis zum *nakten Felsen* folgt. Damit entfallen in erster Linie die *Sickerwassermengen* für die *Quellenspeisung* und gleichen Schrittes mit der *Deterioration des Terrains* ist die *Versiegung* dieser für den *Haushalt* und die *Bewässerung* von *Ackergründen etc* so nöthigen und wichtigen *Feuchtigkeitsspender* zu *konstatieren*. Das fällt umso schwerer ins Gewicht als die oberflächlich liegenden und durch kein so *niederschlagsreiches Einsickerterrain* unterstützten *Quellen der tieferen Lagen* erfahrungsgemäss während der trockenen Jahreszeit ihre Thätigkeit erheblich verringern oder *gar einstellen*, wenn in den tieferen Lage etwa der *Wald* fehlen sollte.

Die unschädlichen *Bergbäche* der *bewaldeten* Epoche erleiden eine *successive Veränderung* in *trockene* Rinnsale welche nur zu Zeiten heftiger Regen Wasser führen, dann aber in *gewaltiger Masse*, da die zeitlich und räumlich verzögerte Zufuhr der Feuchtigkeit zu ihnen von den Hängen *nach dem Untergange des Waldes, proportional zum Verwilderungs und Verkahlungsprocess ihres Einzugs Terrains, impetuos* und *kurz dauernd* wurde.

Diese *Umwandlung zu echten Wildbächen* mit immenser *Geschiefbeführung* tritt naturgemäss zuerst im Oberlaufe aller Flüsse auf, bleibt so

lange dortselbst bis zu einem gewissen Grade localisirt, bis der Hauptabflusskanal im Gebirge mit all seinen Seitenbächen durch seine erodirende Thätigkeit, als Folge der grossen irregulären Wasser und Geschiebeführung immer weiter und weiter die seinen Lauf begleitenden Einhänge und Ufer in ähnlichem Masse beeinflusst, wie die feinsten Runsen es erstmals auf dem ehemaligen Waldterrain thaten.

Uferabbrüche, Hangeinstürze, kurz weitgehende Verwüstungen durch Unterwaschung und Corrosion führen immer mehr Geschiebe und Fels-trümmer dem Mittel- und Unterlaufe zu, bis endlich die Schutt und Trümmerwelle die Ebene erreicht, und der an sich viel weniger schädlichen Überschwemmung des Landes die Überlagerung mit Schuttmassen hinzugesellt.

Die Zeitdauer bis dieses Stadium erreicht ist, bei dem die Aufmerksamkeit der Bewohner der Ebene auf die unaufhaltsam progressive katastrophale Gestaltung der Wasserführung gelenkt wird, hängt natürlich ab von der Grösse des Einzugsgebietes und namentlich von dessen geologischer Formation.

Weiche Schiefer und Mergelgebirge oder sandige Verwitterungsschichten untermischt mit gröberen Gesteinstrümmen, geben nicht selten Veranlassung zum Niedergang einer "Muhre." Hier mengt sich das rasch abfliessende Niederschlagswasser so stark mit dem leicht abschwemmbaren Detritus bei kurzen aber heftigen Gewitterregen (Wolkenbrüchen), dass ein lava-artiger Brei statt eines Wasserlaufes zu Thale eilt. Diese spezifisch schwerere Masse besitzt ein potenziertes Angriffs- und Transportvermögen für loses Gestein jeder Grösse vom hausgrossen Felsstück bis zum kleinen Kiesel und die Verwüstungen des ihren Lauf einsäumenden Terrains sind entsprechend gesteigerte, abgesehen davon, dass kein Wasser im Stande wäre, solche Steinmengen nach Zahl und bes. Grösse in gleicher Zeit* zu Thale zu fördern.

Treffen solche elementare Gewalten mit ihrem Endeffekt in bewohnte Gegenden so ist die Verwüstung von Eigenthumwerthen und die Gefährdung von Menschenleben im ausgiebigsten Maasse zu fürchten.

* Demontzey berichtet uns, von einer solchen Muhre, welche in einem Gange mit 65000 cbm Wasser nicht weniger denn 160000 cbm, feste Masse zu Thal brachte.

Die Schweiz Tirol und Frankreich weisen genug verwüstete Plätze an *den Mündungen solcher Wildwasser* auf, der Ortschaften sind nicht wenige, welche aus dem gleichen *Grunde verlassen* werden mussten!

In gemässigt kühleren Klimaten oder in den wärmeren, wo die Elevation der Gebirge eine genügend grosse ist, füllt die *Verlangsamung der Schneeschmelze* durch Waldbestockung ebenfalls bedeutend ins Gewicht; die *ungeheuren Hochwasser aus naktem Terrain bei rascher Schneeschmelze* und die *Gefahren der Lawinen* unterstützen den aus dem vorigen wohl schon genügend gerechtfertigten *Anspruch auf Bewaldung des Berglandes*.

Ich denke, der Hinweis auf die *Folgen der Entwaldung* reicht hin, um das Mittel zu zeigen, wie all diesen Übelständen begegnet werden kann, wenn auch die Zerstörung leichter war als die Wiederherstellung.

Man *muss* im jedem *bergigen Lande*, das eine *gesunde und produktive Wasserwirtschaft* im Interesse von Ackerbau Industrie und Landwirtschaft aufrecht erhalten will, eine entsprechende *Behandlung und Bewirtschaftung des Waldes* als *fundamentale Forderung für die Erreichung dieses Zieles verlangen*.

Wo der Wald im Berg u. Hügelland auf seinem natürlichen Standorte durch Eingriff des Menschen verschwand und verschlechtert wurde, muss die *schleunigste Wiederaufforstung* in weitmöglichstem Umfange befürwortet werden, *wenn sich nachweisen lässt, dass Verschwinden von Wald und locale Störungen der Wasserführung* von Bächen und Flüssen im ursächlichen Zusammenhange stehen.

Dabei sollte wohl gedacht werden, dass eine Bedeckung mit *Wald schlechthin nicht* genügt, um die entsprechende Schutzwirkung auf das Terrain auszuüben; *schlechte Waldbestockung* hat eben auch nur *teilweise* die günstigen Wirkungen zu verzeichnen, welche dem gut geschlossenen und gepflegten Walde zukommen.

Alle Manipulationen und Neben-Nutzungen, welche den Wald in seinem Gedeihen beeinträchtigen, sind auch *als eine Beeinträchtigung seiner Wirkung aufzufassen*. *Übermässige und unvernünftige Weide* hat in Frankreich wie in Italien, Tirol und der Schweiz den Bergwald herabgebracht und damit auch seine wohlthätige Kraft nicht zum kleinsten Theile *illusorisch* gemacht. Die *Schutzwaldgesetzgebung*, welche eine Nutzung des Waldes

beschränkt in solchen Lagen oder direkt verbietet und auf welche z. B. auch in Japan mit solchen Stolze hingewiesen wird, hat nur einen Werth, wenn sie sich auch auf einen wirklichen Wald und nicht auf das Zerrbild eines solchen bezieht und notabene auch der Wille und die Organe vorhanden sind, um seine richtige Behandlung zu überwachen.

Es ist nicht *die Nutzung als solche* in der Regel, welche Nachteile zeitigt, sondern *die Art der Nutzung*. Von diesem Standpunkt ist auch die Bewirtschaftung solcher Waldungen zu regeln.

Kahlschläge sind namentlich in hohen und steilen Lagen unter allen Umständen zu vermeiden; ein *regelloser Planterbetrieb* mit seiner schlecht verdeckten Ausschlachtung und Verwahrlosung verdient eben so wenig den Namen einer *Wirtschaft* wenn er gar noch wie hierzulande, oft *unter dem Deckmantel einer natürlichen Verjüngung, besser gesagt Verwüstung*, segelt.

Der *Saumlieb* mag auch hier wohl die besten Dienste leisten, wenn die Aufforstung *ihm auf dem Fusse folgt*.

Wo die *Wiederaufforstung* nicht umgangen werden kann, wird man zu unterscheiden haben *zwischen normalem Terrain*, dessen Wiederbestockung ohne weiteres eine mechanische Manipulation der künstlichen Holzzucht ist, und *zwischen einem durch die Atmosphärlilien zerstörtem Terrain*, dessen *Abnormität in Frankreich dem klassischen Lande* solcher Verwüstungen, eine eigenartige höchst wirksame Technik zeitigte.

Nach dem Vorbilde Frankreichs bezüglich der *Wildbachverbauung und Wiederaufforstung* der Gebirge haben Schweiz, Tirol, Bayern und *Österreich* seit langen Jahren denselben oder ähnlichen Wegen folgend, Erfahrungen gesammelt, welche man gegebenen Falles sich unter allen Umständen zu Nutzen machen sollte.

Auch in dieser Hinsicht möchte *für Japan*, das ja erst am Anfange einer *derartigen Thätigkeit* steht, gar Manches zu lernen sein.

Die Notwendigkeit zu *energischem Vorgehen* wird kaum mehr bezweifelt werden, *angesichts der Verwüstungen seiner Flüsse*, welchen alle Jahre eine stattliche Anzahl von Millionen Yen (10) an Werth, zum Opfer fallen, und abgesehen von dem dauernden Verluste an kulturfähigem Boden in den *Mündungsthälern* der Flüsse.

Mehr wie sonstwo hängt *hier* die Bebauung des Landes (Reisbau) von der geregelten Versorgung mit Wasser ab und *ebenso mehr wie* sonstwo hindert der *Wildbachcharakter* die Aufschliessung der Innenlandes innerhalb gewisser Grenzen durch den billigen und einfachen Wasser-verkehrsweg.

Die *Geschiebeführung* und das *beständige Schwanken* zwischen *Wasserlosigkeit oder doch sehr niedrigen Wasserständen und reissenden Hochwassern*, in der Hauptsache zurück zu führen auf die *fehlende richtige Behandlung oder gar Verwüstung* des *Waldes* und des *Terrains* im Innern der Berglandschaft, sie haben einen erheblichen Antheil an der *geringen Rente* sehr wohl nutzbarer Wälder, da das Holz auf den Wasserläufen nicht in *richtiger* Weise gebracht werden kann.

Was hat man von der Zukunft zu erwarten, wenn man bei Fahrten, der Ost-Küste der Hauptinsel Hondo entlang, *sieht*, wie die Eisenbahnen auf endlosen eisernen Brücken die enormen und in keinem Verhältniss zu ihrer Wasserführung erweiterten Schuttbette diverser Wildwasserflüsse wie (Oigawa, Tenriugawa, Fujigawa etc überquert, oder gar, wie zwischen Osaka und Kobe mehreremale *unter* den Flussbetten hindurch führt, da die enormen Schuttkegel die oberirdische Führung der Linie verbieten.

Die Eindämmung der Flüsse auf ihren Schuttkegeln, wie z. B. des *Minatogawa*, *der durch* Hiogo in einer Höhe von mehreren Metern *über* dem Nivean der Strassen bei Regenzeiten seine schuttbeschwerten Wogen wälzt, ist ein allerdings nicht mehr zu änderndes aber gefährliches, verzweifelttes letztes Hilfsmittel, denn ein einziger Dammbbruch ist im Stande, unsägliches Unglück zu vollbringen.

Wird *hier* nicht die *Zufuhr* der Geschiebe durch Aufforstung und durch Verbauung der Zuflüsse im Bergland unterbunden, so kann der Fluss sich niemals in seinen Schuttkegel einschneiden und so selbst seine Gefährlichkeit vermindern, sondern die fortdauernde Auffüllung des Flussbettes bedingt die correspondirende Erhöhung der *Dämme bei diesem, wie bei jedem anderen Wildwasserfluss*, und mit der Erhöhung der Schutzwehren wächst in Potenzen die Katastrophe beim Bruche derselben. *In anerkennenswerthester Weise ist bereits all dem Erwähnten in Japan die Aufmerksamkeit von zuständiger Seite zugewendet worden* und es sind

Wildbachverbauungen und Wiederaufforstungen völlig zerstörter Hänge im Eingangsgebiete diverser Wildwasser im Gange.

Was man dabei vermisst, ist der auch in anderen Sparten so peinlich sich geltend machende *Mangel grossen Zuges*.

Ich würdige hiebei sehr wohl den Umstand, dass die übrigens in abschbarer Zeit dennoch nolens volens sich von selbst mehr u. mehr aufdrängende Inangriffnahme solcher *Wiederaufforstungen und Verbauungen im grossen Stil*, namhafte Millionen an Geldmitteln erfordern wird, die zur Zeit nicht beschafft werden können, aber die *gesetzlichen Grundlagen, die Frage nach der Beitragsleistung der* beteiligten und Interessenten nach der Beschränkung des Eigenthumsrechtes, Expropriationen etc etc kurzum *die breitere Basis, die Anbahnung der Wege für das einheitliche Vorgehen* und für die *definitive unaufhaltsame consequente Ausführung* dieser *nur vom Staat durchführbaren Arbeiten* müssten meines Erachtens eine *baldmöglichste* Würdigung erfahren, wenn nicht zusammenhanglose Stückarbeit das Resultat sein soll.

Die Ausscheidung jener Flächen in Japan, *welche wieder bewaldet werden müssen*, dünkt mich *eine der nöthigsten Aufgaben* der nächsten Zeit für planmässiges Vorgehen, ebenso wie *die Dispositionen für das in erster Linie dringend Notwendige* und *das etwa Aufschiebbare* und die *Sorge für Beistellung von Mitteln*.

Die Frage wird zweifellos hier noch verwickelter durch den Umstand, dass ein *Theil der Hara* unbedingt in *diese Sphäre einbezogen* werden muss, nämlich da wo die *enorme Steilheit* der Hänge die Abfuhr der meteorischen Niederschläge in *einer für das Terrain gefahrdrohenden* Weise beschleunigt.

Hara und *Reisbau* *scheinen* aber vorerst in unauflösbarer Verbindung zu sein und *doch kann bei näherem Zusehen* eine Landwirtschaft nicht produktiv genannt werden, die *direkt* sich die Düngstoffe vom Walde und der *Hara* ohne eigentliche Gegenleistung holt und *indirekt* später durch die daraus resultierende *Zerstörung des Waldes* und *schliesslich des Terrains* dem Lande Millionen kostet.

Es ist eine konstatierte Thatsache, dass die *Beanspruchung der Hara* resp. ihres Grases für die Reiskultur das *wirkliche Bedürfniss namhaft*

überschreitet; *vielleicht*, und ich zweifle nicht daran, würde schon eine *Reduktion* auf das *Nöthige die Ausscheidung der gefährlichsten Plätze zur Wiederaufforstung* ermöglichen. Die Untersuchung, in wie weit *eine andere Düngungsart* in der Landwirtschaft Platz greifen kann und *welche Theile von Hara etwa* für die Umformung in spätere *Weideplätze* passend sind, wenn einmal die doch kommende *Viehzucht* einen grösseren Umfang angenommen hat, das sind Fragen, *deren* Beantwortung so recht eigentlich in das *Gebiet einer landwirtschaftlichen Akademie* fallen würden.

Ich halte das Scheitern der vielen seitherigen Versuche zur Hebung der Viehzucht, welche dem Ackerban eine rationellere Düngerquelle liefern würde, nicht zum *geringsten Theile* dadurch veranlasst, dass man der *Basis für deren natürliche Fortentwicklung* d. h. der Frage nach Weidetermin, dessen Beschaffenheit, mögliche Ausdehnung, Umwandlung von *Hara* in solches etc. etc nicht die *nöthige fundamentale Aufmerksamkeit* geschenkt hat.

Der Fleischkonsum ist steigend und samit die Berechtigung zur *Einführung u. Entwicklung von Viehzucht* gegeben.

Kehren wir zurück zu unserm eigentlichen Thema und kommen wir zum Schlusse!

Wald und geregelte Wasserverhältnisse, wie sie die *Landwirtschaft* für eine gute *Bewässerung des Landes* und wie sie *Handel Gewerbe* und *Industrie als Transport und Kraftmittel* verlangen, stehen *in engster* Beziehung, wie ich hoffe dargethan zu haben.

Vermag der Wald auch auf abnorme Niederschlagsverhältnisse und deren *Folgen, die Hochwasser*, einen *universalen und nie versagenden* Einfluss *nicht* auszuüben, so gibt doch gerade Bewaldung ein Mittel zur *völligen* Durchführung des Einflusses, den der Mensch *überhaupt auf* diese Naturgabe auszuüben vermag.

Gerade für Japan, auf dessen Industriezunahme ein gut Theil seiner Zukunftsentwicklung beruht, ist die Herbeiführung einer *möglichst regelmässigen* Wasserführung seiner Flüsse umso mehr von einschneidendem Interesse, da *deren natürliche* Beschaffenheit durch die Besonderheiten des Terrains keineswegs *eine an sich günstige* genannt werden kann.

Die *Ausnützung* der *Wasserkraft* in der Industrie ist zur Zeit eine *minimale*, ungefähr 8000 Pferdekräfte in allem.

Bewaldung des Einzugsterrains im Gebirge *allein* vermag eine bessere Wasserregelung namentlich, wo die Flussläufe durch lange Vernachlässigung eine weitgehende Verwilderung erfahren haben, nicht ohne *weiteres* zu garantiren, es müssen in den *Bächen und Flüssen selbst* auch die entsprechenden technischen Wasser-Bauten *correct und technisch* richtig ausgeführt werden durch Errichtung von *Thalsperren, Sammelweihern, Uferversicherungen* etc, *um mit dem Walde die so verderbliche Geschiefeführung*, zu der speziell die japanischen Flüsse infolge tektonischer Verhältnisse neigen, *hintanzuhalten*.

Die wassertechnischen Bauten sind also als *unumgänglich nöthige Stützen der Waldwirkung* zu erachten!

Während in den Oberläufen mit den starken Gefällen der Hauptwerth auf *Vertheilung und Verzögerung des Wassers* zu legen ist, bezwecken die *Flusskorrekturen, Deichbauten* etc im *Uterlaufe die raschere Abführung der zufließenden Wassermengen*.

Das einträchtige Zusammenwirken *des Technikers* und *des Forstmanns* wird den *gewünschten Effect gleichmässigerer, unschädlicher Wasserführung* zeitigen.

M. H. Die Natur hat ewige an Weisheit nicht übertroffene Gesetze zu ihrer Grundlage und Nichts geschieht von ungefähr! Sie hat die Quellen und Wasserläufe zu *Füssen des Bergwaldes* und nicht eines *kahlen Terrains* gesetzt, ihr Fingerzeig spricht dem Denkenden *deutlich* genug.

Unterstützen sie den Fachmann, wenn er sie überzeugen konnte, durch das Gewicht ihrer Anschauung in dem Bestreben, Klarheit über den Werth des Bergwaldes für ein geordnetes Wasserregime zu verbreiten.

Dann wird nicht ausbleiben, dass sich der alte Spruch mit einer allerdings etwas freien Anwendung auch hier wiederholt!

„*Ἀσιστον μὴν ὕδατος*“

Benützte Literatur:—Weber, Aufgaben der Forstwirtschaft.

Ney: Der Wald und die Quellen.

Frauenholz: Bessere Benützung des Wassers und der Wasserläufe.

Coaz: Lawinen.

Koch: Das schnelle Anschwellen der Gebirgswasser.

Ebermayer: Einfluss der Wälder auf Bodenfeuchtigkeit etc etc.

Wese: Ueber die Wasserabnahme in den Quellen etc.

Wang: Grundriss der Wildbachverbauung.

„ Bewegungsgesetze des Wassers.

Demontzey: Wiederbewaldung der Gebirge.

e'tc, etc.



Ueber Entstehung und Vertheilung des Kamphers im Kampherbaume.

VON

Homi Shirasawa, *Ringakuhakushi*.

(mit Tafeln XXI—XXIII.)

I. Einleitung.

Der Kampher verdankt seinen Ursprung dem Sekret von *Cinnamomum Camphora* Nees. Diese Pflanze wächst in tropischen und subtropischen Ländern. In Japan kommt sie ungefähr bis 36° nördl. Breite vor, besonders aber an der Meeresküste; Shikoku, Kiushiu, Izu, Suruga und Kii bieten ihr den besten Boden. In diesen gegen Kälte gut geschützten Gegenden, kommt sie neben anderen immergrünen Laubhölzern in ansehnlichen Mengen vor. Leider gehen mit der immer zunehmenden Vermehrung des Kampherbedarfes diese Bestände allmählig zu Grunde; die grossen prächtigen Exemplare trifft man nur mehr in Tempelhainen. Manchesmal erreicht der Kampherbaum eine Höhe von 20 m. und einen Durchmesser vor über 2 m. In Formosa kommt er in grossen Beständen in den Urwäldern vor; die Kamphergewinnung ist dort eine bedeutende Einnahmequelle der Regierung.

Der Kampherbaum wächst auch im östlichen China in Tsching und Kiangsi auf der Insel Chusan, südlich von Shanghai, Itschung in der Provinz Hupe und auch der Insel Hainan. In diesen Gegenden kommt er in mehr oder weniger grossen Massen vor, während Formosa als das produktivste Land in Kampher betrachtet wird.

Ueber die Ausfuhr von Kampher und Kampheroel von Japan vom Jahre 1888 bis 1901 gibt uns das Kaiserl. Jap. statistische Bureau folgende Angabe :

	aus Japan ;		aus Formosa ;	
	Kampher, Kampheroel Kin	Kin	Kampher, Kampheroel	
1888	4,555,469	1,149,299		
1889	4,971,849	867,414		
1890	4,463,881	778,902		
1891	4,429,050	821,537		
1892	3,064,005	699,836		
1893	2,487,485	444,184		
1894	2,071,378	427,251		
1895	2,238,386	516,792		
1896	1,617,660	558,858	4,395,921	5,761.
1897	2,608,242	1,094,910	3,174,206	65,753.
1898	2,434,028	684,037	2,292,098	15,954.
1899	2,758,625	1,100,226	3,198,740	6,440.
1900	3,280,715	450,973	1,571,200	
1901	4,165,757	1,561,970		

(1 Kin ist ungefähr 1 englisches Pfund.)

Da von fast allen Ländern der Welt, Japan die Hauptkampherproduktion hat und der Kampherbaum hier so weit verbreitet ist, dürfte es für Forsttechniker und Botaniker speziell in wissenschaftlicher und praktischer Hinsicht von grösstem Interesse und von Wichtigkeit sein, ueber Herkunft, Entwicklungsweise und Vertheilung des Sekretes, resp. des Kamphers etwas zu erfahren. Bei der Wichtigkeit des Kamphers für die Technik und die Weiterentwicklung seiner Produktion insbesondere, wünsche ich mit diesem Gegenstande mich zu beschäftigen.

Was nun die Behälter des Sekretes in den Pflanzenorganen anbelangt, so schrieb zuerst de Bary¹ in seiner „Vergleichenden Anatomie“: „Nach dem Bau sind die Sekretbehälter zu unterscheiden: in schläuche d. h. aus

¹ De Bary, Vergleichende Anatomie S. 143, 152 und 209.

Zellen, welche ihre Wände beibehalten, hervorgegangene, daher gewöhnlich als Zellen bezeichnete und intercellulare, ihrer Gestalt nach entweder, Gänge oder Lücken, Höhlen zu nennende.“ Erstere unterscheidet er nach den Formen in „kurze und lange“ und letztere in „schizogene“ und „lysigene“ „oder rhexigene“. A. Tschirch¹ welcher sich in den letzten zehn Jahren speciell mit den Sekreten der Pflanzen beschäftigt hat, nennt die oelführenden Zellen „Oelzellen“²; die schizogenen Lücken „Oel-oder Harzbehälter“ (auch Oelraum); die lysigenen „Oel oder Harzlücken“. Für die schizogen entstandenen und später auflysigene Art erweiterten Behälter hat er den Namen „Schizo-lysigene Räume“ eingeführt. „Oblitoschizogene Gänge“ hat er sie genannt, wenn die Sezernierungszellen der Oelbehälter erst nach Bildung des Belages obliterieren.

Ueber diese Sekretbehälter, speciell die Entstehungsorte des Sekretes, haben in letzter Zeit Tschirch³ und dessen Schüler eingehende Studien gemacht. Becheraz, „Ueber die Sekretbildung in schizogenen Gängen“, Bern 1893; Sieck, „Die schizogenen Sekretbehälter, vornehmlich tropischer Heilpflanzen“, Bern 1894; Lutz, „die oblito-schizogenen Sekretbehälter der Myrtaceen“, Bern 1895; Bierman, „Ueber Bau und Entwicklungsgeschichte der Oelzellen und die Oelbildung in ihnen“, 1898.

Unter diesen Arbeiten hat Bierman im Speciellen über Oelzellen und die Oelbildung in ihnen bei den Pflanzen von den Familien der Lauraceen, (Cinnamomum Camphora ausgenommen) Canellaceen, Valerianaceen, Zingiberaceen, Magnoliaceen, Myristiaceen, Piperaceen, Calycanthaceen, Umbelliferae, Convolvulaceen, Papilionaceen, Untersuchungen angestellt.

In gar manchen chemischen und pharmazeutischen Werken finden wir Abhandlungen über den Kampher; aber da die Quellen zu diesen meistens dieselben sind, beschränken sie sich auf seine chemischen Eigenschaften und seine Anwendung, etc. Was aber die Entstehung und Vertheilung des Kamphers im Kampherbaum anbetrifft, so ist eine eingehende Untersuchung, besonders die mikroskopische Forschung, noch nicht bekannt.

¹ Tschirch, Angewandte Pflanzen Anatomie S. 460-530.

² A. Meyer hat früher diesen Namen eingeführt; Wissenschaftlich Drogenkunde 183, S. 79.

³ Tschirch, Harz und Harzbehälter, 1900.

Tschirch und Oesterle, Anatomischer Atlas der Pharmakognosie und Nahrungsmittelkunde, 1900.

Flückiger¹ schreibt: „der Kampher findet sich auskrystallisiert in Spalten des Stammes, sowie aufgelöst in dem Oel, welches in allen Theilen des Baumes (mit Ausnahme der Blüten) verbreitet ist. Pfeffer² hat die Ansicht, dass das aetherische Oel der lebenden Zellen des Kampherbaumes durch eine postmortale Sauerstoffaufnahme im Kampher verwandelt wird. In Weisner's Buch steht³; „alle Theile des Baumes enthalten in besonderen Sekretzellen ein aetherisches Oel, aus welchem zum Theil schon in der lebenden Pflanze der Kampher im krystallinischen Zustande sich ausscheidet.

In Bezug auf den Gehalt von Kampher im Kampherbaum haben neuerdings Moriya⁴ und Mimura durch Destillation von Holz und Blättern werthvolle Ergebnisse, besonders mit Rücksicht auf Kamphergewinnung gehabt. Sie schreiben: „der Kamphergehalt im Kampherbaum ist sehr verschieden je nach dem Alter und Standorte des Baumes. Bei demselben Exemplar nimmt er vom Wurzelstock nach den Aesten hin ab. Das Kernholz enthält mehr Kampher als das Splintholz. Aus dem aus Formosa stammenden ungefähr 500 jährigen Holz haben sie 7.13 proz. Rohkampher gewonnen, während sie aus dem 70 jährigen Holz, welches in der Provinz Bingo gewachsen ist, nur 4,73 proz. Rohkampher gewonnen haben. Die lufttrockenen Blätter enthalten durchschnittlich 3,75 proz. Rohkampher und 1,25 proz. Kampheroel.

Ueber den Kamphergehalt in den Blättern von *Cinnamomum Camphora* theilt uns M. Kelway Bamber⁵ im Royal Botanical Garden, Ceylon mit, dass er durch Destillation von lufttrockenen Blättern durchschnittlich 2,080 bis 2,425 proz. Kampher und 1,96 bis 1,05 proz. Kampheroel gewonnen hat.

In meiner vorliegenden Arbeit wird es sich hauptsächlich darum handeln, nach *mikroskopischer* Untersuchung folgende Fragen zu beantworten.

¹ Pharmacognosie des Pflanzenreiches, 1891, S. 150.

² Pflanzenphysiologie I, 2. Auf, S. 501.

³ Die Rohstoffe des Pflanzenreiches 1900.

⁴ Journal of the chemical Society Tokyo, Vol. 21 No. 6 und Ringakushikai-Zasshi^{II}.

⁵ The Indian Forester Vol. 28 No. 4, 1902 S. 163.

1. Aus welcher Substanz scheidet sich der Kampher im Kampherbaum aus?
2. Wann macht sich das erste Produkt, aus dem sich der Kampher bildet, bei der wachsenden Pflanze bemerkbar?
3. In welche Form geht das zuerst entstandene Produkt secundär über, und was sind seine mikroskopischen Eigenschaften?
4. Wo wird das erste Produkt angelegt und wodurch charakterisiert es sich?
5. Wo vertheilt es sich in den Pflanzenorganen und in welcher Beziehung zu klimatischen und sonstigen Verhältnissen steht seine Bildung?
6. Kann man vom Standpunkte der Kamphergewinnung aus, den Kamphergehalt im Baume mikroskopisch bestimmen?

Diese Untersuchungen wurden im Jahre 1900-1901 in der forstlichen Versuchsanstalt in München und hauptsächlich im Jahre 1901-1902 im pharmazeutischen Institut der Universität Bern und später im forstlichen Versuchsgarten in Meguro bei Tokio, ausgeführt.

Das lebende Versuchsmaterial stammte aus dem botanischen Garten in München, und Bern sowie auch aus dem forstlichen Versuchsgarten in Meguro bei Tokio; das trockene Material aus der Sammlung des Herrn Prof. Dr. A. Tschirch und aus meiner eigenen. Herr Prof. Dr. H. Mayr überreichte mir auch das trockene Material aus der Sammlung, die er auf seiner Reise in Japan angelegt hatte. Mein College Herr a. o. Prof. Mimura, Tokio hat mir verschiedenes Holzmaterial aus der Provinz Fukuoka besorgt.

Es ist mir eine angenehme Pflicht diesen Herrn, vor Allem Herrn Prof. Dr. A. Tschirch, unter dessen freundlicher Leitung ich hauptsächlich diese Untersuchungen im pharmazeutischen Institut der Universität Bern ausgeführt habe, dem inzwischen verstorbenen Herrn Prof. Dr. R. Hartig, und Herrn Prof. Dr. H. Mayr an der Universität München, welche während meines Aufenthaltes in München für diese Arbeit ihre gütige Hilfe mir gewährten, meinen wärmsten und verbindlichsten Dank auszusprechen.

Tokio, September 1902.

II. Entstehung.

Knospen.

Um festzustellen, wo sich die erste Anlage der Oelzellen vollzieht, und wo man ihre früheste Entwicklungsgeschichte wahrnehmen kann, habe ich zuerst an den Knospen Untersuchungen ausgeführt. Dazu diente mir frisches Material aus dem botanischen Garten Bern. Die dünnen wie auch die dickeren Schnitte in der Quer- und Längsrichtung wurden direct in etwas verdünnte 1 proc. Osmiumsäure oder stark verdünnte Alkannatinktur getaucht. In ersterer Lösung wurden die Schnitte nach einigen Minuten, in letzterer nach einigen Stunden untersucht. Bei meinen mehrmaligen Untersuchungen konnte ich die Oelzelle oder eine der letztern entsprechende Zelle niemals finden. Die flachkegelige Spitze wird vollständig aus weichem meristematischen Gewebe gebildet. Schon direct hinter dem Vegetationspunkte treten in unregelmässiger Vertheilung einige Zellen auf, welche sich durch Grösse und rundliche Form von den übrigen Zellen auszeichnen. Sie reagieren noch nicht mit Osmiumsäure oder Alkanmatinktur, und der Zellinhalt ist etwas durchsichtig. Betrachtet man diese Zellen an den in Alkohol liegenden Präparaten so bemerkt man, dass ihre Zellräume mit einer farblosen homogenen Masse erfüllt sind. Diese Masse quillt durch vorsichtiges Zufließenlassen von Wasser und schwindet in Alkohol. Mit Jodjodkali nimmt sie eine hellgelbe Färbung an. Dieses deutet auf Schleim. -- Wir wissen ja durch die Untersuchungen von Tschirch¹ und Biermann², dass die Oelzellen als Schleimzellen angelegt werden. Unmittelbar hinter dem Vegetationspunkte, besonders an den Randpartien der Knospenachse finden sich die ganz differenzierten Oelzellen, welche durch Osmiumsäure oder Alkannatinktur nachgewiesen werden können, Fig. 1.

Unter denselben machen sich aber noch andere Zellen bemerkbar,

¹ Die Harz und Harzbehälter.

² Ueber Bau und Entwicklungsgeschichte der Oelzellen.

welche in den verschiedenen Stadien der Entwicklung der Oelzellen stehen. In das erste Entwicklungsstadium, also in die Zeit des Uebergehens der Schleimzelle zur Oelzelle, fällt das Auftreten der „resinogenen Schicht“ Tschirch's,—diese Schicht stellt eine feinkörnige vacuolige Masse dar. In der Zelle ist die Wand mit einer Schleimmembran dicht belegt, während die resinogene Schicht inwendig derselben aufliegt, Fig. 2, a.

Die von den verschiedenen Partien des Schleimbeleges herausgehenden, bandförmigen Schläuche treten in der Mitte des Zellraumes zusammen, Fig. 2, b.

Im Zusammenhang mit der successiven Entwicklung der resinogenen Schicht steht das allmähliche Verschmelzen des Schleimbeleges. Es ist jedoch auch möglich, dass dünne Schichten des Schleimbeleges oder Reste desselben zurückbleiben.

Das erste Auftreten von Oeltropfen konnte ich bald nach dem Entstehen der resinogenen Schicht feststellen. In letzterer fanden sich die Oeltropfen häufig in der Mitte, jedoch auch in beliebigen anderen Punkten. Diese Oeltropfen wachsen bald zu ansehnlicher Grösse heran, was bewirkt, dass die betreffenden Stellen der resinogenen Schicht aufgeblasen werden und das Ganze in den Zellraum hineintritt. Die aufgeblasenen Partien zeigen manigfache Gestaltungen Fig. 3—7.

Im weiteren Fortschritte der Oelbildung treten die genannten Partien miteinander in Contact und erfüllen den ganzen Zellraum, lassen aber nicht selten Lumen in der Mitte oder an der Zellwand zurück, Fig. 8—9. Diese Masse,—sammt dem in ihr befindlichen Oel—ist ziemlich fest. Bei Untersuchung der Knospennachse bei trockenem Material konnte ich sie mit dem Messer zertheilen und ohne Formveränderung aus der Zelle herausnehmen.

In einigen Fällen habe ich im Zellraum die resinogene Schicht stark aufgeblasen gesehen, welche dadurch ein beutelförmiges Ansehn erhielt, Fig. 10. Bringt man eine solche Bildung mit Alkohol in Berührung, so schrumpft sie bald zusammen. Ohne Behandlung mit einer Reagenz hat die obenerwähnte Masse ein hellgelbes, homogenes etwas lichtbrechendes oder schaumiges Aussehn.

Osmiumsäure färbt diese Masse dunkelbraun und kontrahiert etwas. Mit Alkannatinktur nimmt sie eine intensive rothe Färbung an. Durch die

Behandlung mit Alkohol dehnt sie sich aus und es bleibt eine feinkörnige Masse zurück. Zusatz von Wasser hat zunächst zur Folge, dass die Masse zu einem ovalen oder rundlichem Gebilde kontrahiert wird, welches ein trübes Aussehn besitzt.

Diese Tatsache, welche den allgemeinen Gesetzen entgegengesetzt zu sein scheint, lässt sich vielleicht auf folgende Art erklären. Bei Hinzufügung von Alkohol tritt momentan Volumenvermehrung der schleimigen Masse durch die Lösung des festes Oels ein. Setzt man Wasser hinzu, so wird infolge Quellung die Oellösung herausgepresst, und tritt eine Zusammenziehung derselben ein.

Durch fortdauernde Einwirkung von Alkohol und Wasser wurde sie nach und nach klarer und zuletzt blieb eine schwammige, oft grobporige oder netzartige etwas lichtbrechende Masse zurück. Diese Masse speichert sehr begierig Anilinfarben auf. So färbte sie sich mit Jodgrün-Eisessig leicht grün, auch nach Abwaschen mit Alkohol oder Glycerin wurde die Färbung ganz energisch zurück gehalten, während das andere Gewebe farblos blieb. Mit Jodgrün und Fuchsin färbte sie sich schön violett. In Chloralhydrat, conc. Schwefelsäure oder Salpetersäure ist die Masse unlöslich, Chlorzinkjod gab ihr eine gelbe Färbung.

Diese Reactionen berechtigen mich, sie als „resinogene Schicht“ zu betrachten, welche Tschirch als das Laboratorium der Oelbildung (Harzbildung) bezeichnet hat.

Was für eine Rolle diese resinogene Schicht in der Oelbildung spielt, ist noch ein Rätsel, wir müssen heutzutage noch der Kraft des lebenden Protoplasmas die Fähigkeit der Oelbildung zusprechen. Zur Zeit kann ich jedoch als sicher erklären, dass der Ort der Sekretbildung bei dem Kampherbaum, wie bei den anderen Lauraceen in Tschirch's resinogener Schicht zu suchen ist.

Bei den Untersuchungen von Knospen habe ich bei der ersten und zweiten Blattanlage, d. h. bei den innersten Schuppen nur Schleimzellen bemerkt. Auf dem Längsschnitte sind sie meistens am äusseren Teile in verticalen Reihen angeordnet. In einigen Fällen aber waren bei der dritten Blattanlage die bereits ausgebildeten Oelzellen oder die Schleimzellen mit

der resinogenen Schicht bemerkbar. Von hier nach den äusseren Blattlagen zu ist die Oelzellenbildung eine intensive.

Was die Entwicklungsgeschichte der Oelbildung anbetrifft, so lässt sich ein wesentlicher Unterschied bei diesen zwei beschriebenen Fällen nicht erkennen.

Blätter.

Bei dem kaum entfalteten Blättchen von etwa $1\frac{1}{2}$ —2cm Gesamtlänge sind bereits die Schleim- und Oelzellen vollständig ausgebildet und zwar in Blattspreite und Blattstiel.

Die Oelzellen sind mittelst Alkannatinktur oder Osmiumsäure nachweisbar. Die Zahl der Oelzellen ist jedoch meistens noch verhältnissmässig gering, während sich vielmehr die Schleimzellen in grösserer Zahl bemerkbar machen.

Bei den einjährigen Blättern, welche sich bereits völlig entwickelt haben, weisen die Oelzellen schon ihre charakteristische Ausgestaltung und völlig entwickelte Grösse auf. Die verschiedenen Stadien der Oelbildung sind jedoch bei diesen nicht nachweisbar. Die Oelbildung in der resinogenen Schicht hat zur Folge, dass dieselbe stellenweise aufgeblasen wird und nach innen in den Zellraum eindringt und zwar von der Seite der Zellwand, der die resinogene Schicht entweder unmittelbar angelagert oder vor der sie durch eine Schleimmembranschicht getrennt ist. Diese blasenartigen Gebilde sehen rundlich, eiförmige oder wurstförmig aus, Fig. 11, 12, 13, 14, 15, 16. Manchmal sind eine grosse Menge derselben beieinander angelegt und sie erfüllen dann den Zellraum, Fig. 17. Ohne Zusatz eines Reagens haben sie ein fast farbloses oder hellgelbes, stark lichtbrechendes Aussehn. Durch Zufließenlassen von Alkohol kontrahieren sie sich bald und bleiben hierbei Fetzen der resinogenen Schicht zurück. Dieser Fetzen des resinogenen Häutchens färbt sich mit einer Lösung von Jodgrüneisessig, aber die Färbung ist keine intensive, mit Jodjodkali nimmt er eine orangegelbe Färbung an.

Unter den Sekretzellen dieser Blätter trifft man noch viel Oelzellen, welche auf ihrer Innenseite mit einer dicken Schleimmembran nebst der

resinogenen Schicht versehen sind. Bisweilen erscheint die resinogene Schicht beutelförmig. Hierbei bleibt die farblose Membran durchsichtig und die resinogene Schicht sieht etwas trüb aus. Mit Jodjodkali färbt sich die erstere hellgelb, während die letztere eine orange gelbe Färbung annimmt, wodurch man das Vorhandensein der Schleimmembran und zugleich die Abgrenzung zwischen den beiden leicht wahrnehmen kann.

Bei den zweijährigen Blättern sieht man an der Blattspreite ueberhaupt, und auch an dem einzelnen Geweben z. B. Epidermis, Zellwand, etc. eine bedeutend dickere und festere Struktur als bei den einjährigen Blättern. Die Oelzellen sind bei zweijährigen Blättern noch in den verschiedenen Stadien ihrer Entwicklung. Sie zeigen noch meistens Schleimmembran oder die Reste desselben, obwohl dünner und weniger zahlreich als in einjährigen Blättern. Tropfenweise vorhandenes Oel habe ich nur in wenigen Fällen gefunden. Es bildet sich wie bei den einjährigen Blättern in der schwammigen resinogenen Schicht, oder in blasenartigen Beutelchen. Ich konnte einen grossen Unterschied in der Entwicklung nicht bemerken; ein Unterschied besteht jedoch darin, dass in den zweijährigen weniger Schleim, hingegen mehr Oel in der resinogenen Schicht vorhanden war als bei den einjährigen. Ich bin daher der Ansicht, dass der Prozess der Oelbildung in den Blättern ein fortdauernder ist und zwar während ihrer ganzen Lebensdauer, denn unter den zu meinen Untersuchungen benützten zweijährigen Blättern zeigten sich stets die gleichen Entwicklungserscheinungen, wiewohl einzelne der Blätter schon die auf baldiges Abfallen hindeutende charakteristische mattgelbe Färbung hatten. Meist war entsprechend dem Entwicklungsstadium der Blätter ein successiver Fortschritt in der Oelbildung zu bemerken.

Bei den Blattstielen steht das Fortschreiten der Oelbildung fast immer im entsprechenden Verhältniss zu den zugehörigen Blättern. Bei diesen habe ich wiederholt den tropfenförmigen Oelinhalt in den Oelzellen bemerkt, Fig. 18, und zwar ist derselbe in zweijährigen Blättern reichlicher als in einjährigen. In mehreren Fällen war aber die resinogene Masse mit Oel durchtränkt vorhanden.

Durch Behandlung mit Alkohol lösten sich die Oeltröpfchen sehr bald, während in den resinogenen Schläuchen sich das Oel erst unter dauernder Einwirkung von Alkohol löste. Schleimmembran oder die Reste derselben bleiben zurück, jedoch fehlten sie auch manchmal, oder waren überhaupt dünner und geringer als in den Blättern. Die Blattstiele geben immer ein sehr zweckmässiges Material für Untersuchung der Oelzellen, da wir bei denselben je nach Belieben einen dünnen oder dicken Schnitt bekommen können und da auch wegen des geringen Vorhandenseins von gefärbtem Zellinhalte im Gewebe immer ein klares Bild wahrzunehmen ist.

Bei der Untersuchung von Blättern und Blattstielen des trockenen Materiales aus Java stammend, habe ich auch Oel in tropfenförmigem Zustande gesehen, Fig. 19. Auf Osmiumsäure reagiert es schwach, aber mit Alkannatinktur färbt es sich schön rot. In Alkohol löst es sich bald, unter Zurücklassung von nur wenigem Rückstand. Reste der Schleimmembran waren bei diesen kaum bemerkbar.

Auffallend ist, dass man oft in Knospennachsen, Blattstielen, Rinde, auch im Holz, netzförmige oder hautartige resinogene Masse findet, welche fast die ganzen Zellräume ausfüllt. Bei Blättern fehlt dieselbe in der Oelzelle fast immer und ist bei frischem Material durch dicke Schleimmembran ersetzt. Für den Nachweis der Verkorkung der Oelzellwand, auf welche Zacharias¹ zuerst hingewiesen hat, habe ich Schwefelsäure, Chlorzinkjod oder Jodjodkali benutzt. Bei den jüngeren Pflanzenorganen wurde durch conc. Schwefelsäure das ganze Gewebe zerstört, wobei die feine Zellwandlamelle der Oelzellen zurückblieb. Bei den älteren Organen färbte sich die Zellwand durch Jodjodkali orange-gelb.

Rinde.

Die von mir vorgenommenen Untersuchungen erstrecken sich auf frisches und trockenes Material, und zwar auf die jüngsten Teile einjähriger Triebe.

¹ Botanische Zeitung 1870, S. 617-645.

Bezüglich der Ölbildung treten fast die gleichen Verhältnisse hervor, wie bei den einjährigen Blättern.

Gelegentlich der Untersuchung der Rinde 5 jähriger Triebe war zu constatiren, dass der Prozess der Oelbildung schon zum Abschluss gekommen war. Hellgelb aussehende oft schaumige Oelmassen erfüllten das Zellinnere.

Durch Behandlung der Oeltropfen mit Alkohol lösen sich dieselben unter Zurücklassung einer farblosen schwammigen oder einer hautartigen Masse

Holz.

Ueber die Entwicklungsgeschichte der Oelzellen beziehungsweise die Oelbildung in demselben erfährt man nur wenig, dagegen kann man bei demselben ausschliesslich die interessanten Umwandlungsformen des entstandenen Oels zum krystallinischen Kampher studiren.

Im Wasserpräparat des jungen 2, 3 oder 5 jährigen Holzes sieht man nur den hellgelben Oelinhalt in der von den Nachbarzellen ausgezeichneten Oelzelle. Er erfüllt den Zellraum oder einen Teil desselben.

Mit Alkannatinktur färbt das Oel sich rot, durch Osmiumsäure wird es gebräunt. Alkohol löst das Oel leicht und bleiben manchmal membranartige oder schwammige Klumpen zurück.

Beim ungefähr 80 Jahre alten und vor 12 Jahren gefällten Holz, welches aus einer bekannten Kampherproduktionsprovinz stammte, habe ich hauptsächlich die Bildung vom Kampher untersucht. Wenn man dünne Schnitte dieses Holzes im Wasser betrachtet, so sieht man vier verschiedene Formen:

- a. Dunkles oder orangegelbes Oel
- b. hellgelbes Oel
- c. farbloses Oel
- d. Krystalle.

Das dunkle Oel ist eine balsamartige Masse; bei der mikroskopischen Untersuchung tritt es oft aus der Zelle aus, wobei es seine

ursprüngliche Form beibehält. Dasselbe hat meistens ein schaumiges, trübes Ansehn, Fig. 20 und erfüllt fast immer den ganzen Zellraum. Alkohol löst es langsam unter Bildung von zahlreichen feinen Hohlräumen. Durch andauerndes Zufließenlassen von Alkohol nehmen diese an Zahl und Grösse zu, und bleibt zuletzt ein schwammiges netzartiges Skelett zurück. Durch Aether oder Chloroform geht die Lösung schneller vor sich. Mit Alkannatinktur färbt es sich intensiv rot, mit Osmiumsäure braun. Das hellgelbe Oel ist dagegen etwas durchsichtiger und schliesst meistens in der Mitte oder in den der Zellwand anliegenden Partien grosse Luftblasen ein, Fig. 21. Alkohol löst es leichter als das vorige, bei der Lösung bleibt ebenfalls ein Rückstand.

Das farblose Oel ist eine ganz durchsichtige homogene sehr flüchtige Flüssigkeit. Es kommt in der Zelle tropfenweise vor, und habe ich nur in zwei Fällen ganz mit demselben erfüllte Zellräume angetroffen.

Es kommt bald in grossen bald in kleinen Tropfen vor, die in der Regel, der Zellwand anliegen, Fig. 27. Der Rand der Tropfen ist stark lichtbrechend. Oft treten in dem Tropfen kleine Luftblasen auf. Seine flüchtigen Eigenschaften kann man gut feststellen. Unter dem Mikroskop betrachtet verflüchtigt es sich schon in einigen Minuten, wobei ein in Alkohol löslicher kleiner Klumpen zurück bleibt.

Nicht selten findet man in der Mitte dieser Oeltropfen oder am Rande derselben kleine Krystalle, Fig. 28. Bringt man diese Krystalle oder die Tropfen selbst mit Wasser direct in Berührung, so drehen sie sich sehr lebhaft und verschwinden schliesslich vollständig. Dieses Oel färbt sich sehr schwach mit Alkannatinktur oder Osmiumsäure. In Alkohol oder Aether ist es sehr leicht löslich.

Die Krystalle treten öfters in den Oelzellen des Holzparenchyms auf, während in demselben Schnitte die Oelzellen der Markstrahlen und des Libriforms mit gelbem oder farblosem Oel erfüllt sind.

Diese Krystalle sind farblos, weich und ist die Krystallform sehr undeutlich. Viele dieser Krystalle sind zu Aggregaten vereinigt, Fig. 29, a. b. Dieselben lösen sich sehr leicht in Alkohol oder Aether ohne Rückstand. Durch Erwärmen verflüchtigen sie sich vollständig.

Die bei der Untersuchung angewendeten Methoden und Reaktionen sind folgende:

1. *Kochen.*

Kleine Holzspäne wurden in einem Becher zwei Stunden lang mit Wasser stark gekocht. Das farblose Oel und die Krystalle waren vollständig vorflüchtig, aber das gelbe Oel fast unverändert. Bei der Untersuchung von im eisernen Kessel mit Wasserdampf destillierten Holzspänen, habe ich dieselbe Erfahrung gemacht.

2. *Erhitzen.*

Die Schnitte wurden auf dem Objectträger einige Minuten lang langsam erhitzt. Hierbei verflüchtigte sich das Oel und die Krystallmasse gänzlich, während der in Alkohol unlösliche mit Jodgrün und Fuchsin färbbare schwammige Klumpen zurückblieb.

3. *Sublimation*

Erhitzt man kleine Schnitte des Holzes im Sublimiergläschen schwach und langsam einige Stunden lang, so sublimiert die krystallinische Substanz (Kampher) und bildet feine Krystalle auf der kälteren Fläche des Gläschens; zugleich bilden sich dort farblose Oeltropfen, während das gelbe Oel fast unverändert in der Zelle zurückbleibt.

Anstatt des Sublimiergläschens lassen sich auch ausgehöhlte Objectträger verwenden, die Höhlung muss mit einem Deckgläschen bedeckt werden, welches sorgfältig anzudichten ist. Der Kampher krystallisiert auf der Innenseite des Deckglases, wo sich auch das flüchtige Oel niederschlägt.

4. *Färbungsreagentien.*

a. *Alkannatinktur.*

Die in der sehr verdünnten fast alkoholfreien Lösung über 10 Stunden

lang liegenden Schnitte zeigen das gelbe Oel intensiv rot gefärbt. Die Krystalle und das farblose Oel sind durch das lange Liegen in der Tinktur nicht mehr sichtbar.

b. Osmiumsäure.

Die 1% Lösung derselben bräunt des gelbe Oel, dagegen wirkt sie kaum auf das farblose Oel ein. Jedenfalls ist die Einwirkung auf das in den jüngeren Organen enthaltene Oel stärker als auf dasjenige des alten Holzes.

5. Lösungsmittel.

Die Löslichkeit des im alten Holz vorkommenden Oels ist sehr verschieden. Im folgenden ist das Verhalten des Oels gegenüber den einzelnen Lösungsmitteln wiedergegeben (Die Löslichkeit des Handelskamphers ist bei jedem Lösungsmittel angegeben).

a. Alkohol löst:

das gelbe Oel, wobei eine farblose schwammige oder netzartige Masse zurück bleibt; das farblose Oel leicht, ohne Rückstand; die Krystalle leicht, ohne Rückstand; den Handelskampher leicht, ohne Rückstand.

b. Aether löst:

das gelbe Oel leicht, mit Rückstand; das farblose Oel sehr leicht, ohne Rückstand; die Krystalle sehr leicht, ohne Rückstand; den Handelskampher sehr leicht, ohne Rückstand.

c. Chloroform löst:

das gelbe Oel leicht, mit Rückstand; das farblose Oel sehr leicht, ohne Rückstand; die Krystalle sehr leicht, ohne Rückstand; den Handelskampher sehr leicht, ohne Rückstand.

d. Aceton löst:

das gelbe Oel, mit Rückstand; das farblose Oel leicht, ohne Rückstand; die Krystalle leicht, ohne Rückstand; den Handelskampher leicht, ohne Rückstand.

e. Benzol löst:

das gelbe Oel nach längerem Einwirken, mit Rückstand; das farblose Oel,

ohne Rückstand; die Krystalle leicht, ohne Rückstand; den Handelskampher leicht, ohne Rückstand.

f. Petrolaether löst :

das gelbe Oel schwer mit Rückstand; das farblose Oel leichter, mit Rückstand; die Krystalle, ohne Rückstand; den Handelskampher, ohne Rückstand.

g. Eisessig löst :

das gelbe Oel erst nach der einigen Minuten, mit Rückstand; das farblose Oel leicht, ohne Rückstand, die Krystalle leicht, ohne Rückstand; den Handelskampher leicht, ohne Rückstand.

h. Chloralhydrat (80%) löst :

das gelbe Oel langsam, mit Rückstand, das farblose Oel ohne Rückstand; die Krystalle leicht, ohne Rückstand, den Handelskampher leicht, ohne Rückstand.

6. Verhalten gegen Säuren und Alkalien.

a. In conc. Schwefelsäure ist :

das gelbe Oel theilweise löslich und bleibt eine schwammige Masse zurück; das farblose Oel löslich, ohne Rückstand, die Krystalle löslich, ohne Rückstand; der Handelskampher löslich, ohne Rückstand.

b. In Salzsäure ist :

das gelbe Oel fast unlöslich, das farblose Oel schwer oder kaum löslich, die Krystalle kaum löslich; den Handelskampher kaum löslich.

c. In Salpetersäure ist :

das gelbe Oel theilweise löslich und quillt die Oelmasse auf, infolgedessen sie oft aus der Zelle hervortritt; das farblose Oel leicht löslich, ohne Rückstand; die Krystalle leicht löslich, ohne Rückstand; der Handels-Kampher leicht löslich ohne Rückstand.

d. in Kalilauge ist :

das gelbe Oel unlöslich, jedoch tritt Bräunung desselben ein; das farblose Oel unlöslich, ohne Färbung; die Krystalle kaum löslich, ohne Färbung;

der Handerskampher kaum löslich, ohne Färbung.

Es fragt sich nun, in welcher Beziehung diese verschiedene Oelinhalte zu einander stehen. Es scheint mir, dass dieselben höchst wahrscheinlich Uebergangsstadien zu einander darstellen.

Tritt Luft zum gelben Oel hinzu, so bilden sich in demselben zahlreiche, feine Blasen, welche ihm ein undurchsichtiges, trübes Ansehn verleihen Fig. 20. Diese Erscheinung ist vielleicht auf eine Oxidation zurückzuführen.

Bei längerer Luftereinwirkung vergrössern sich die Bläschen, fliessen zusammen und bilden grosse Blasen entweder in der Mitte der Zelle oder an der Zellwand, Fig. 21. Von diesem Zeitpunkte an wird das Oel hellgelb und etwas durchsichtiger. Im weiteren Verlaufe wird es immer klarer und geht in eine farblose flüchtige, in Alkohol leicht lösliche Flüssigkeit über, d. h. es wird zu farblosem Oel Fig. 22-27. Ob bei diesem Prozesse die Menge des Oels in der Zelle abnimmt, oder ob erst nach der Umwandlung in flüchtiges, farbloses Oel eine Abnehmen desselben zu verzeichnen ist, kann ich nicht bestimmt sagen.

Die Krystalle bilden sich in diesen Oel, und unterliegt es mir keinen Zweifel, dass diese Krystalle „*Kampher*“ sind, Fig. 28-29.

Die Oelzellen, welche für die frühe Krystallbildung in Betracht kommen, liegen, wie ich konstatieren konnte, im Parenchymgewebe. Das Vorkommen daselbst lässt sich dadurch erklären, dass im Parenchym der Luftzutritt wegen seines lockeren und dünnwandigen Gewebes sehr erleichtert ist.

III. Vertheilung.

Knospen und Blätter.

Wie ich früher gezeigt habe, sind die Oelzellen in den frühesten Stadien der Entwicklung, unmittelbar hinter dem Vegetationspunkte und auch in den innersten Blattanlagen angelegt worden; dergleichen auch bereits die Schleimmembran und die resinogene Schicht.

Diese Theile der Pflanzenorgane bestehen vollständig aus meristematischem Gewebe, welches durch conc. Schwefelsäure völlig zerstört wird.

Darin finden sich aber bedeutend grössere rundliche Zellen. Sie sind in der Nähe des Vegetationspunktes im mittleren, bei der Knospennachse im äusseren Theile zu finden, Fig. 1.

Bei den Knospen nimmt die Zellenzahl mit der Grösse der Blattanlagen zu; also erhalten die äusseren Schuppen eine grössere Anzahl Zellen als die innern. Auf einem Querschnitt durch die Mitte der Knospe habe ich in einigen Fällen in der fünften Blattanlage 5 bis 10 Zellen gefunden, während sich in der zweiten auf demselben Schnitt nur 2 Zellen vorfanden.

Bei dem weiter nach aussen liegenden Blattanlagen, in welchen schon die rudimentären Gefässbündel entstanden waren, machte sich eine sehr bedeutende Zahl von Oelzellen bemerkbar, und letztere waren in den inneren und äusseren oder auch in den mittleren Partien der Blattanlagen vertheilt.

Die mittleren oder die sich denselben nach aussen anschliessenden Theile sind jedoch reicher mit Oelzellen versehen.

Bei den Deckschuppen, welche durch die dickwandigen und auf ihrem Querschnitt vierkantig erscheinenden Sclereiden und auch durch die lange Behaarung auf der Aussenseite—besonders am Rande derselben—charakterisiert werden, sind die Oelzellen meistens im äusseren Theile angelegt. Die Sclereiden der inneren Schuppen weisen, in der Regel, noch eine dünne Wandung auf, und sind in ihrem Auftreten sehr spärlich. In den äusseren Schuppen ist ihre Zellwandung jedoch stark verdickt und hat manchmal gabelförmige Kanäle. Solche Sclereiden, liegen besonders in den inneren Randpartien der Deckschuppen zahlreich beieinander.

Bei jüngeren Blättern, welche sich bis circa 2,5 cm. Länge entfaltet haben, sind die Oelzellen unmittelbar unter der Epidermis zwischen dem Palissadengewebe gelegen, zeichnen sich durch eine eigenartige Form, analog den Palissadenzellen aus, sind aber immer breiter als die letzteren und besitzen eine dickere Zellwand. Die, welche zwischen dem Schwammparenchym auftreten, sind meistens kreisrund oder ellipsoidisch und etwas kantig.

Bei den vollkommen entwickelten Blättern zeigen die Oelzellen eine ganz ausgeprägte Gestalt, und zwar erscheinen sie zwischen dem Palissadengewebe ovalrund, im Schwammparenchym rundlich oder ellipsoidisch mit

grosser Achse parallel der Blattfläche. Auch sind sie nicht mehr kantig, sondern völlig abgerundet. Diese Formänderung verdankt vielleicht ihre Ursprung der Spannung der Zelle, welche durch die Oelbildung in dem Zellraum verursacht wurde. So sehen wir, nicht selten, die Palissadenzellen oder die Schwammparenchymzellen durch die gross entwickelte Oelzelle an den Nachbar gedrückt.

Vom morphologischen Standpunkte der Ausgestaltung der Oelzellen aus kann man sagen, dass ihre Gestaltung immer mit den umgebenden Geweben in Zusammenhang steht, z. B. die Oelzellen sind alle in ihren frühesten Entwicklungsstadien bei den jüngsten Blattanlagen und Knospenschuppen und dem Vegetationspunkte etc. fast ausnahmslos rundlich; bei den bereits entwickelten Pflanzenorganen, je nach der Stelle ihres Vorhandenseins, weisen sie dagegen eine differente Gestalt auf, behalten aber noch die charakteristische Gestaltung ihrer Abstammungszelle in dem entsprechenden Gewebe.

Was die Zahl, Vertheilung und Anlage der Oelzellen in der Blattspreite anbetrifft, so habe ich vom Rande ausgehend, bei völlig entwickelten einjährigen Blättern das auf folgender Tabelle dargestellte gefunden.

Länge das Schnittes m.m.	Die Zahl der Oelzellen zwischen dem Palisadengewebe.	Die Zahl der Oelzellen unmittelbar den Palissadengewebe anschliessend.	Die Zahl der Oelzellen zwischen dem Schwammparenchym.	Die Zahl der Oelzellen unmittelbar hinter der Epidermis der Blattunterseite.	Die Zahl der Oelzellen am Randgewebe anliegend.	Gesammt	Durchschnittliche Zahl der Oelzellen auf 1mm Länge des Schnittes.
5	8	3	8	1	1	20	4
6	10	5	12			27	4.5
	7	7	9			23	3.8
7	6	8	9		2	25	3.6
8	11	7	15			33	4.1
	6	11	15			32	4
9	18	3	10	1	1	42	4.6
	12	10	17			39	4.3

Die vorliegende Tabelle zeigt, dass das Auftreten der Oelzellen zwischen oder direct beim Palissadengewebe häufiger ist als bei den anderen Geweben, und dass auch auf der Blattunterseite, unmittelbar unter der Epidermis oder zwischen den Epidermiszellen sich nur ausnahmsweise Oelzellen finden.

Bei den Blattstielen liegt das Gefässbündel in der Mitte und die Bastzellen umschliessen den Sieb- und Holztheil. Die Sclereiden sind rings um das Gefässbündel unregelmässig verstreut. Sie sind bald zahlreich bald spärlich vorhanden. Ich habe bei dem trockenen Material aus Java ein häufigers Vorhandensein derselben bemerkt, als bei dem frischen Material vom botanischen Garten Bern. In letztem Falle war ihre Zahl unbedeutend.

Die Oelzellen finden sich peripherisch zwischen den Subepidermalzellen oder unmittelbar hinter denselben. Sonst sind sie zwischen dem Rindenparenchym vertheilt. Auf dem Längsschnitt sind sie oft in verticalen Reihen, aber niemals in Gruppen vereinigt. Letzteres ist besonders bei den Randpartien leicht bemerkbar, Fig. 30.

Im Gefässbündel zeigen sie sich zwischen dem Parenchym des Siebtheiles, in Holztheil dagegen nicht. Sie sind ziemlich klein und erscheinen auf den Längsschnitt cylindrisch oder länglich oval.

Diejenigen Oelzellen, welche bei den Subepidermalzellen angelegt worden sind, zeichnen sich in der Regel, durch ihre Grösse gegenüber den Nachbarzellen aus, während die im Rindenparenchym ihnen an Grösse fast gleich kommen. Auf dem Querschnitt erscheinen sie meistens rundlich; auf den Längsschnitt weisen sie dagegen eine ovale oder elliptische Gestalt auf.

Die Oelzellen der Blattstiele zeichnen sich oft durch ihre Umgebung aus; die Parenchymzellen sind nämlich rings um die Oelzellen strahlenförmig angeordnet, so dass die Oelzellen in der Mitte der Zellgruppe sitzen. Dieses kann man aus den Quer- oder Längseschnitten ersehen, Fig. 31 a, b.

In Bezug auf die Zahl der Oelzellen der Blattstiele ergab eine Untersuchung vermittelst dünner Querschnitte bei einjährigen frischen Blättern folgendes:

Im unteren und mittleren Teile des Blattstieles enthalten die peripherischen Reihen 23-30 Oelzellen, die der Blattspreite sich anschliessenden

Theile nur 15-23. Beim trockenen Material aus Java oder aus Japan zählte ich dagegen 29-35, was mit davon überzeugte, dass das in einheimischen Gegenden gewachsene Exemplar mehr Oelzellen zu erzeugen vermag, als dasjenige im Treibhaus des botanischen Gartens. Diese Tatsache beweist uns, dass die klimatische, und Standortsverhältnisse zur Oelzellbildung in Beziehung stehen, was Tschirch¹ schon bemerkt hat.

Die Parenchymzellen des Blattstieles weisen Tüpfel auf. Bei den Oelzellen fehlen aber diese Tüpfel vollständig, wengleich man nicht selten die Tüpfel der Parenchymzellen bemerkt, welche bis an die Mitte der gemeinsamen Wandung heranreichen.

Die derbe Wandung, das Fehlen der Tüpfel, und die Korkbildung in der Zellwand bilden die charakteristischen Eigenschaften der Oelzellen. Vielleicht wird damit der Wechselverkehr mit den Nachbarzellen erschwert und die Ablagerung und Zurückhaltung des Oels am Orte der Erzeugung möglich gemacht.

Rinde.

Die Oberhaut der Rinde des einjährigen Triebes ist stark cuticularisiert. Sie färbt sich mit Alkannatinktur intensiv. Beim Abwaschen mit Alkohol und Wasser hält die Färbung ziemlich lange Zeit an. Die Rindenparenchymzellen sind meistens tangential gestreckt und ziemlich dickwandig. Zwischen den Parenchymzellen finden sich sehr zahlreiche Schleimzellen. Die Oelzellen sind noch spärlich entwickelt. Sie sind meistens peripherisch an die Epidermalzellen angeordnet, und auf dem Querschnitt rundlich, auf dem Längsschnitt länglich oval oder oft rundlich und bedeutend grösser als die benachbarten Zellen, Fig. 32, 33.

Im Siebtheil des Gefässbündels kommen sie selten vor, im Holztheil dagegen noch gar nicht. Anschliessend an die äussere Randpartie der Bastzellgruppe treten oft bedeutend grosse Oelzellen auf.

Bei der Rinde des 5 jährigen Triebes ist des Periderm noch nicht

¹ Tschirch, Anatomischer Atlas S. 132 und Harz und Harzbehälter, S. 386.

aufgetreten. Die Epidermis ist sehr stark verdickt, besonders beim Material aus Java, und ragt auf der Innenseite buckelig hervor.

Die Schleimzellen sind hier sehr reichlich entwickelt. Die Oelzellen kommen sowohl in der primären als auch in der secundären Rinde vor. In der letzteren treten sie, in der Regel, häufiger als in der ersteren auf. Diejenigen, welche in der primären Rinde vorkommen, sind etwas abgeplattet, und in tangentialer Richtung lang gestreckt, während sie in der secundären Rinde eine rundliche oder länglich ovale Form zeigen.

Auf der alten Rinde bildet sich eine starke Borke. In der sekundären Rinde kommen stark verdickte, in radialer Richtung gestreckte Sclereiden vor. Sie vereinigen sich meistens reihenweise in tangentialer Richtung.

Die Oelzellen mit einem gelben Inhalt, sowohl, als auch Schleimzellen treten häufig in sekundärer Rinde, sehr selten in primärer auf.

Holz.

Bei der Untersuchung des frischen Materiales habe ich niemals das Vorkommen von Oel in den Zellen des Holztheiles der einjährigen Triebe beobachtet, wengleich im Mark, besonders in den äusseren Theilen desselben ziemlich viele Oelzellen vorhanden waren, Fig. 34.

Im Mark treten oft sehr verdickte Sclereiden auf. Sie kommen vereinzelt oder zu Nestern vereinigt vor.

Beim zweijährigen Triebe aus Java treten die Oelzellen im ersten Jahresringe auf, während im zweiten Jahresringe ihr Vorkommen noch kaum bemerkbar ist.

Im Triebe des dritten Jahres habe ich dieselbe Erfahrung gemacht; in ersten und zweiten Jahresringe kommen schon zahlreiche Oelzellen vor, dagegen im dritten d. h. im jüngsten Jahresringe sind noch keine Oelzellen vorhanden.

Im Mark tritt das Entgegengesetzte ein, indem in dem einjährigen Trieben die Oelzellen verhältnissmässig zahlreicher als in den zweijährigen Trieben und in letzteren zahlreicher als in dreijährigen auftreten, sodass mit dem Alter der Triebe die Anzahl der Oelzellen abnimmt.

Was das Vorkommen und die Vertheilung der Oelzellen in Holz anbetrifft, so habe ich hauptsächlich das alte Holz zur Untersuchung benutzt; da in diesem das Gewebe den Endpunkt seiner Ausbildung erreicht hat, und daher für die Untersuchung ein sehr bequemes und klares Object abgibt.

Die Oelzellen treten im Holz zwischen den primären und sekundären Markstrahlen, im Holzparenchym und im Libriform auf.

Sie finden sich ausschliesslich am Rande der Markstrahlen, niemals in der Mitte derselben. Sie sind meistens kegelförmig mit der in das aussen liegende Gewebe eindringenden Spitze, und bedeutend grösser als die übrigen Zellen der Markstrahlen Fig. 35, 36, 37, 40.

Diejenigen, welche zwischen das Libriform eingebettet sind, sind länglich oval und etwas zugespitzt. Ihre Zellwand ist ziemlich verdickt, wie die der Libriformfasern Fig. 38, 39. Die in dem Parenchym liegenden Oelzellen haben die grösste Ausdehnung. Sie sind elliptisch und finden sich manchmal mehrere neben einander liegend Fig. 41, 42.

Bei diesem dreifachen Vorkommen der Oelzellen treten die im Parenchym liegenden in den Vordergrund, da dieselben sowohl an Zahl als auch an Grösse die andern übertreffen.

In einem Jahresringe enthält das Herbstholz mehr Oelzellen als das Frühjahrsholz. Bei meiner Untersuchung der Jahresringe habe ich auch bemerkt, dass von einer bestimmten Grenze an im Frühjahrsholz zum Herbstholz hin die Oelzellen zahlreicher auftreten. Diese Tatsache erklärt sich dadurch, dass für die Oelbildung gewisse Faktoren vorhanden sein müssen, wobei wahrscheinlich die Erhöhung der Temperatur keine geringe Rolle spielt.

Wurzel.

Bei der Wurzelspitze, welche ausschliesslich aus weichem meristematischen Gewebe besteht, fand ich einige Zellen, welche durch ihre Form von den uebrigen Zellen ausgezeichnet sind. Der Inhalt derselben reagierte weder mit Alkannatinktur noch mit Osmiumsäure.

Bei der etwas entwickelten Wurzel waren die Oelzellen schon bemerkbar. Sie sind im Rindenparenchym nebeneinander angelegt worden. Der Oelgehalt in ihnen ist noch gering. In der einjährigen Wurzel ist das Vorkommen der Oelzellen bedeutend, wenngleich sie hauptsächlich im Rindenparenchym aber nicht im Holztheile des Gefässbündels erscheinen. Der Oelinhalt ist gelb und bedeutender als in der jüngeren Wurzel.

Bei der alten Wurzel ist das Verhalten der Oelzellen ähnlich wie bei dem alten Holz.

IV. Zusammenfassung der Resultate.

Fasst man die vorstehenden Untersuchungen zusammen, so lassen sich folgende Schlüsse ziehen:

A. Ueber die Entstehung.

1. Bei *Cinnamomum Camphora* entstehen die Oelzellen schon früh unmittelbar hinter dem Vegetationspunkte.

2. Bei jüngeren Pflanzenorganen ist der Inhalt der Oelzelle „Aetherisches Oel“.

3. Dieses Oel bildet sich in der von Tschirch benannten „resinogenen Schicht“, wie bei den anderen Laurineen-Pflanzen und diese resinogene Masse bleibt sehr lange Zeit in der Zelle erhalten.

4. In den jüngeren Pflanzenorganen durchtränkt das Oel die resinogene Masse. Im tropfenförmigen Zustande kommt es sehr selten vor.

5. Bei der in tropischen Gegenden (Java) gewachsenen Pflanzen hat das Oel resp. die resinogene Masse eine dichtere Consistenz, und die Menge desselben ist grösser als bei den im Treibhaus (im botanischen Garten Bern und München) gezüchteten Exemplaren.

6. In Blättern kommt das Sekret oft in beutelförmigen Häutchen vor. (bei der Untersuchung von frischem Material).

7. Bei den älteren Blättern tritt das Oel reichlicher als in jüngeren Blättern auf.

8. Im alten Holz nimmt das Oel eine orangegelbe Färbung an; dieses Oel geht später (durch Sauerstoffaufnahme?) in das farblose Oel über. Aus diesem bildet sich der krystallinische Kampher.

9. Dieser Umwandlungsprozess geht erst nach einigen Jahren vor sich; jedenfalls erst lange nach dem Abschluss der Oelbildung in der Oelzelle. So ist im alten Holz die Relativmenge von farblosem Oel und der Krystalle bedeutend grösser als die von gelbem Oel; dagegen im jungen Holz übertrifft die Menge des letzteren die erstere.

10. Die Oelzellen, welche zwischen dem Parenchym liegen, enthalten mehr farbloses Oel und Krystalle als die in anderen Geweben.

11. Wenn sich bei alten Stämmen Kampher Massen in Höhlungen und Spalten des Holzes finden, so können sie dorthin nur aus den Oelzellen durch Sublimation gelangt sein. Sie befinden sich also an „Secundärer Lagerstätte“.

12. Durch die jetzt übliche Methode der Kamphergewinnung ist es kaum möglich, das gelbe Oel aus dem Holz zu erhalten, wiewohl das farblose Oel und die Krystalle leicht destilliert werden können.

B. Ueber die Vertheilung.

1. Die Oelzellen kommen vor:
 - a. bei den jüngsten Blattanlagen in der Mitte oder den derselben anliegenden Theilen;
 - b. bei den Deckschuppen der Knospen vorwiegend in den äusseren Partien.
2. Bei völlig entwickelten Blättern findet man sie im Palissaden- und Schwammparenchym. Am Schlusse ihrer Entwicklung besitzen sie eine abgerundete Form.
3. Bei den in tropischen Gegenden gewachsenen Exemplaren sind die

Oelzellen zahlreicher als bei den im Treibhaus gezogenen. Dieser Unterschied fällt hauptsächlich bei den Blattstielen ins Auge, während bei der Blattspreite derselbe weniger wahrnehmbar ist.

4. In den Blattstielen sind die Oelzellen verhältnissmässig zahlreicher als in den anderen Organen.

5. Im Holztheile des jungen Gefässbündels und zwischen den Epidermiszellen habe ich niemals Oelzellen bemerken können.

6. In der secundären Rinde befinden sich mehr Oelzellen als in der primären Rinde.

7. Im Blattstiel und in der Rinde des jüngsten Triebes sind zahlreiche Schleimzellen vorhanden.

8. Im jungen Mark sind die Oelzellen sehr zahlreich, mit dem Alter desselben nehmen sie ab.

9. Im Holz des einjährigen Triebes sind die Oelzellen kaum vorhanden, wengleich im zweijährigen Holz ihr Auftreten sehr bedeutend ist.

Sie befinden sich zwischen den Markstrahlzellen, dem Holzparenchym und dem Libriform.

In den Markstrahlen treten sie ausschliesslich an dem Rande auf. Das Holzparenchym enthält in der Regel mehr Oelzellen als die anderen Gewebe.

10. Im Herbstholz des Jahresringes sind mehr Oelzellen als im Frühjahrsholz.

11. In der Wurzel kommen die Oelzellen auch vor, und ihre Verteilung und Entwicklungsgeschichte ist dieselbe wie bei den oberirdischen Theilen der Pflanze.

Figuren-Erklärung.

Abkürzungen:	C = Cambium.
Ep. = Epidermis.	Epz. = Epidermiszelle
foe. = farbloses Oel.	goc. = gelbes Oel.
gf. = Gefäss.	hp. = Holzparenchym.
kr. = Krystall.	L. = Lumen.

- | | |
|----------------------------|----------------------|
| lb. = Luftblasen. | lib. = Libriform. |
| ms. = Markstrahlen. | Oe. = Oel. |
| Oez. = Oelzelle. | p. = Plasma. |
| reg. = resinogene Schicht. | |
| Schl. = Schleim, | Scler. = Sclereiden. |

Fig. 1. Längsschnitt einer Knospe, um die Vertheilung der Schleim- resp. Oelzellen und Sclereiden in der Knospenanlage und Knospenachse zu zeigen.

Fig. 2. Erste Stadien der Entwicklung von Oelzellen:

- a. Die resinogene Schicht tritt auf.
- b. Bandförmige Schleimfäden treten in der Mitte des Zellraumes zusammen. (Beide sind an Alkoholpräparaten beobachtet).

Fig. 3. Erstes Auftreten von Sekret in der resinogenen Schicht. Die resinogene Schicht ist durch die Oelbildung aufgeblasen.

Fig. 4. Die resinogene Schicht contrahiert sich und bildet Lumen zwischen derselben und dem Schleimbelege. (Die Präparate sind aus der Knospenachse).

Fig. 5, 6. Dieselben Stadien der Oelbildung in den Oelzellen aus einem Blattstiele des einjährigen Blattes.

Fig. 7. Dieselbe aus einem einjährigen Blatt.

Fig. 8, 9. Fast fertige Oelzellen aus der Knospenachse. Die Reste der Schleimmembran sind bemerkbar. Die resinogene Schicht ist mit dem Sekret durchtränkt.

Fig. 10. Oelzelle aus derselben. Oel ist in einem grossen beutel-förmigen Schlauche vorhanden.

Fig. 11-14. Oelzellen aus einjährigen Blättern:

Fig. 11, 12. Die resinogene Schicht mit Oel ist contrahiert.

Fig. 13, 14. Die durch Oelbildung aufgeblasene resinogene Schicht. Die Reste der Schleimmembran sind noch vorhanden und die resinogenen Schläuche in das mittlere Lumen hervortretend.

Fig. 15, 16. Oelzellen aus zweijährigen Blättern.

Fig. 17. Dieselben mit zahlreichen eiförmig aufgeblasenen resinogenen Schläuchen.

Fig. 18. Oelzellen mit tropfenförmigen Oelinhalt; aus einem zwei-jährigen Blattstiele.

Fig. 19. Oelzelle aus einem Blattstiele des trockenen Materiales aus Java. Das Oel ist im schaumigen Zustande vorhanden.

Fig. 20-29. Oelzellen aus altem Holze, bei welchen die successiven Umwandlungsstadien von gelben Oel zu Krystallen zu sehen sind:

Fig. 20. Das durch die zahlreichen feinen Luftbläschen trüb aussehende gelbe Oel erfüllt den ganzen Zellraum.

Fig. 21. Die kleinen Luftbläschen haben sich zu einer grossen Luftblase in der Mitte des Oelinhaltes vereinigt. Das Oel hat ein klares Aussehen.

Fig. 22. Erste Stadien der Umwandlung von gelben Oel zu farblosen Oel.

Fig. 23, 24, 25. Die weiteren Stadien desselben. Das farblose Oel ist anfangs schaumig (Fig. 24) und dann klarer (Fig. 25).

Fig. 26. Im farblosen Oel sind noch die Reste von gelbem Oel bemerkbar.

Fig. 27. Ganz klares farbloses Oel.

Fig. 28. In demselben bilden sich Krystalle.

Fig. 29. a. b. Krystalle aus dem farblosen Oel.

Fig. 30. Längsschnitt durch einen Randtheil vom Blattstiele. Die Oelzellen sind in Verticalreihen angeordnet.

Fig. 31, a. Oelzelle, welche von den Parenchymzellen strahlenförmig umgeben ist. (Aus dem Querschnitte des Blattstieles).

b. Derselbe aus dem Längsschnitte desselben.

Fig. 32. Längsschnitt der Rinde des jüngsten Triebes. Die Oelzellen sind an die Epidermiszellen in Verticalreihen anliegend.

Fig. 33. Querschnitt desselben.

Fig. 34. Querschnitt eines jüngsten Triebes, bei dem die Vertheilung der Oelzellen bemerkbar ist. (Mit Alkannatinktur).

Fig. 35. Querschnitt des alten Holzes. Die Oelzelle kommt zwischen den Markstrahlzellen vor.

Fig. 36. Radialschnitt desselben. Die Oelzellen liegen zwischen Randzellen der Markstrahlen.

Fig. 37. Tangentialschnitt desselben.

Fig. 38. Querschnitt desselben, wobei die Oelzellen, welche zwischen dem Libriform liegen, sichtbar sind.

Fig. 39. Radialschnitt desselben.

Fig. 40. Tangentialschnitt desselben. Die Randzellen der Markstrahlen sind durch die Sekretbildung stark vergrößert.

Fig. 41. Querschnitt desselben. Die Oelzellen sind zwischen dem Parenchym entstanden.

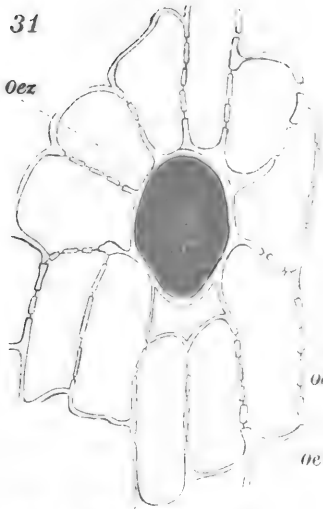
Fig. 42. Radialschnitt desselben.



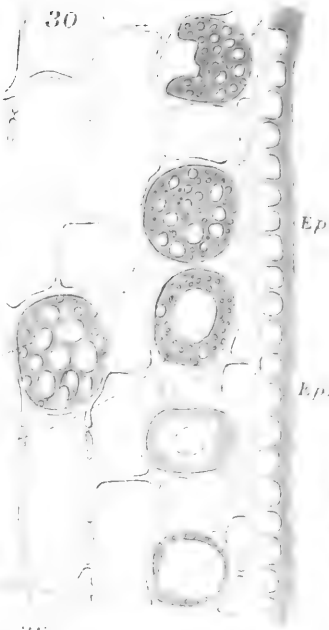




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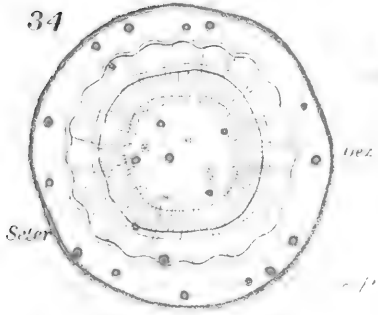
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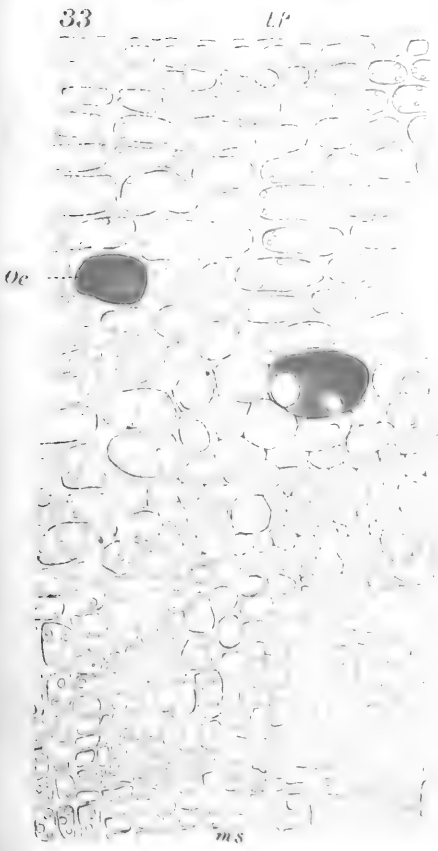
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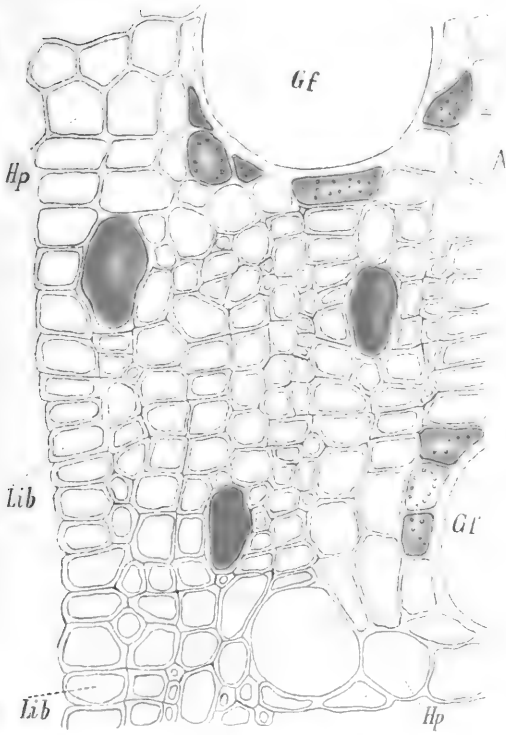
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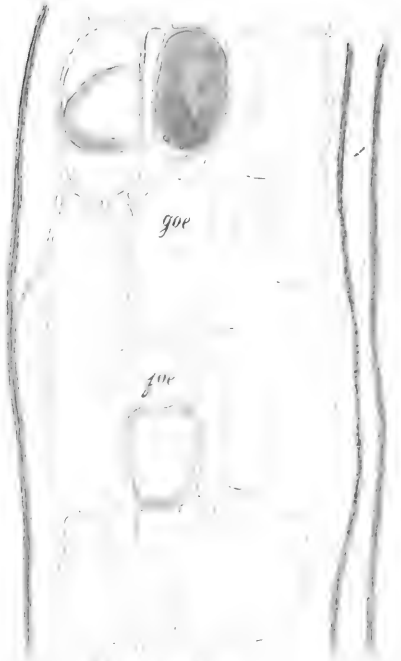




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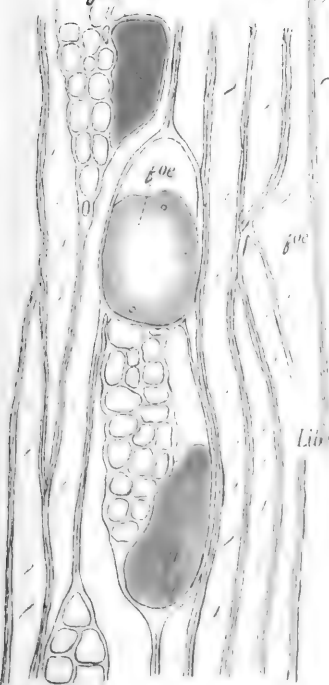


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Investigations on Flacherie.

BY

S. Sawamura.

Chapter I. Introduction.

Flacherie is a frequent disease of the silk-worm, causing great damage to sericulturists. The first observation was made by *Pasteur* who regarded this malady as being caused by three species of bacilli and a micrococcus. These microorganisms penetrated into the tissues of the larva and even into the eggs. *Cuboni*, however, was of the opinion that the pathogenic bacterium of this disease was a micrococcus which produced black spots on mulberry-leaves. Recently *Macchiati*¹ made investigations on this disease and concluded that the malady was caused by a streptococcus quite different from that found on mulberry-leaves which was a diplococcus. He recognized this streptococcus as the *Streptococcus bombycis*. He found besides this a bacillus in the digestive canal of the diseased larva, but he thought this bacillus could not cause the malady, its action consisting merely in the acceleration of the malady.

According to *Macchiati* the streptococcus and the bacillus have the following properties.

Streptococcus bombycis *Macchiati*.

The cell is round or oval, the diameter being 1.25—1.5 μ . It appears never isolated, but two, five or more unite in chains. It is aërobic; the propagation on potato is very quick and the colony thereon is greenish-yellow and has a metallic lustre. Colony on gelatine is light yellow, and gelatine is completely liquefied.

¹ Contribuzione alla Biologia dei Batteri nei Bachi affetti da flaccidezza. Le stazioni sperimentali Agrarie Italiane, Vol. XX, Part. II.

Bacillus bombycis Macchiati.

This bacillus exists not only in the larva, but also in the cocoon, crysalis and imago of silk-worm. The length is $1-3\mu$ or often more; the ends of the rod are round, and two or more unite forming a long thread. The cell-membrane consists of cellulose, as it is coloured blue by iodine and sulphuric acid. It is motile and produces spores usually in the middle part of the rod. Colony on potato is yellowish-brown and elevated, which turns afterwards light brown.

Colony on gelatine is light yellow, and has a milky appearance, and gelatine is quickly liquefied. In gelatine stab-culture gelatine is completely liquefied, and flake-like precipitates are produced.

After the investigations of *Macchiati*, *Krassiltschik*¹ in Pasteur's Institute made some investigations on this malady, and found that it is caused by a micrococcus quite different from that of *Macchiati*. This micrococcus has the following properties.

Streptococcus Pastorianus Krassiltschik.

The cell is round, and has a diameter of $1-1.1\mu$. It occurs in the form of diplococcus. Colony on gelatine is round and gray, and gelatine is not liquefied. On gelatine stab-culture colony grows in nail-like form without liquefying it.

*Macchiati*² assumed that the micrococcus found by *Krassiltschik* was the same as his streptococcus, disregarding the great difference of the properties between his and *Krassiltschik's* microbe.

*Macchiati*³ proposed to examine with a microscope the moth to be used for laying the eggs and to wash the eggs with sublimate solution, since the streptococcus exists usually in the moth and eggs. He said that very good results were obtained in various places by practising his proposal.

¹ Comptes rendus 1896. II, P. 427.

² Societa botanica italiana 1896.

³ " " " "

The microbes of flacherie were sometimes used for killing insects. In 1890 *Hoffmann*¹ used various species of microorganisms to kill *Laparis monacha*, injurious insect of forests, and found that *Botrytis* and the microbe of flacherie could become parasitic on this insect and kill it.

Tangl,² however, denied *Hoffmann's* report, since he did not believe *Bacillus bombycis* was the true pathogenic microbe of flacherie, and he said that bacteria could not be used for killing *Laparis*, as there was not known any microbes that were parasitic on that insect.

*Tubenf*³ described a certain bacterium which became parasitic on insects of pine-trees and also caused flacherie. The infected insect lost appetite and died finally. The contents of the intestines were brown, and numerous bacilli were found in it especially in the fore-intestines. He gave this bacillus the name of *Bacillus monachae*. Its width is 0.5μ and length 1μ . It was present also in the blood of the dead larva. This disease broke out more frequently, when the climate was cold and moist. *Tubenf*, henceforth, explained the reason, that the disease was produced, because when it was cold and moist, the food was not digested well and remained long in the intestines, thus offering an opportunity for the growth of the bacillus.

In 1901 the Austrian Agricultural Experimental Station⁴ reported that flacherie could not be infected to healthy silk-worms, neither by giving together with mulberry-leaves the intestinal juice of the diseased larva, nor the pure-culture of the microbe.

*Omori*⁵ in Japan made investigations on this disease, and found that it was caused by four kinds of special micrococci and two kinds of special bacilli, and as the symptoms of the disease caused respectively by these microbes were different, he distinguished three kinds of flacherie.

¹ Central-Blatt für Bakteriologie XI. P. 341.

² " " " XVI. P. 660.

³ " " " XII. P. 269.

⁴ Oesterreich. Versuchsstationen IV. Part 3.

⁵ Nihon Sanbyoron.

Chapter II,

Description of the Bacteria found in the Diseased Silk-worm.

The diseased larva lose appetite, vomit a viscous fluid and suffer from diarrhea or excrete a viscous fluid in most cases. When they are dead, the third and fourth segments become somewhat elongated and the whole body softens. But there rarely occurs a case in which the dead body shrinks and becomes rather hard. The dead worm turns black usually very soon.¹ Sometimes while the diseased larvae are still living, the third and fourth segments are coloured black. In the digestive canal there are found usually a viscous fluid containing very little fragments of mulberry-leaves, but sometimes the digestive canal is filled with the fragments of mulberry leaves compressed to a hard mass. The color of mulberry-leaves therein is usually brown, while that in healthy animals is green. But rarely the mulberry-leaves in the intestines retain the original green color. While the reaction of the intestinal juice of the healthy larva is strongly alkaline, that of the diseased is in most cases neutral or only very faintly alkaline.² The fluid excreted shows often an acid reaction.

In the intestinal juice there are found a great number of bacteria. That abundantly found in many cases is a micrococcus. In some cases only micrococci are found, but in many cases there exist along with them large and short bacilli. There is sometimes a case in which short motile bacilli are seen, but it is very rarely observed, except in silk-worms reared in summer, that the large bacilli alone occur. Although these organisms exist abundantly in the intestinal juice of the diseased larvae, they can not easily

¹ According to *Dewitz* (*Arch. für Anatomie und Physiologie* 1902, P. 328) the turning black of the insect larva is due to oxydases. The fact that the diseased larvae turn usually very quickly black after death, or show black spots while still living, is probably due to the increased production of oxydizing enzymes accelerated by the insufficiency of nutrition as in the case of the vegetable cell which was proved by *Woods* (*Centralbl. für Bakteriologie* II, Vol. 5, No. 22), or by the poisoning by nitrite.

² The digestive enzymes of *Lepidoptera* are active only in an alkaline solution, and lose their action in an acid solution. *S. Sawamura*, This Bulletin vol. IV, No. 5.

be detected in the tissues and blood of the diseased. It is clear from this fact that flacherie is caused by the propagation of the microbes in the intestinal juice.

By investigating silk-worms reared in spring, summer and autumn, the writer found that the micrococci in the intestinal juice are not of a single species but of many. But as their form is the same they can not be distinguished only by microscopical examination. The most remarkable difference is the color of the colonies on solid media. As in plate-cultures prepared from the intestinal juice of the diseased larvae, colonies of various colors made their appearance, it is beyond question that there exist in the intestinal juice of the diseased not only a single species but many species of micrococci (Fig. I and II). These microbes are present not only in the diseased larvae, but also in the healthy ones, although the number is small. Their presence in the latter case can be detected with a microscope or more easily by preparing plate-cultures.

To know whether the microbes are present in the interior of the eggs of silk-worm, they were washed with 0.1% sublimate solution and then with sterilized water, and crushed in bouillon, in which a micrococcus and a large bacillus propagated after few days. These experiments were repeated many times always with the same results. By examining the properties of the microbes, the large bacillus was found to be *Bacillus megatherium* De Bary and the micrococcus a Sarcina, the properties of which will be described later on. The presence of microbes in the interior of the eggs of insects other than silk-worm was observed by *Bloekmann*, *Korschelt*, and *Zaccharis*.¹

The properties of the microbes found in the intestinal juice of the diseased larvae and in the eggs are as follows ;—

The Large Bacillus.

Form: The cell, when cultured in bouillon for 24 hours, is 0.8 μ . wide and 3—5 μ long. In the intestinal juice it is larger. The extremities of

¹ Central-Blatt für Bakteriologie II. p. 546 and XI. p. 234.

the rod are round; and flagella grow on all sides, and are stained by *Löffler's* method. It exists isolated usually in the intestinal juice, but in nutritive fluid two or more are united.

Spore-formation: Spores are easily formed usually in the middle part of the rod.

Motility: It shows a slow oscillating motion.

Gram's method: Positive.

Oxygen: The growth is better in presence of air.

Bouillon: Propagation is good, and a cloud-like precipitate is formed and a feeble ring on the wall of the tube.

Gelatine streak: Gelatine is quickly liquefied along the inoculated line.

Agar plate: Colony having curl-like appearance, irregularly extending from a light brown point formed in the centre.

Agar streak: A dirty white colony is formed on the whole surface.

Agar stab-culture: Colony is formed straightly along the inoculated line to the bottom, and it propagates on the surface quickly to the wall of the tube.

Potato: An elevated gray colony within 2 days of inoculation at 20°C.

Milk: Milk is coagulated but not with an acid reaction.

Reduction: Nitrate is reduced to nitrite as shown by the iodine-starch and *Griess's* reaction.

Gas-Production: Gas is not evolved by cultivating in a nutritive solution containing glucose.

H₂S: A trace of H₂S is formed by cultivating it in bouillon.

Acid-production: By cultivating for 3 days at room-temperature in a nutritive solution containing 5% of glucose (with Ca CO₃), there was produced 0.153% of acid, calculated from the dissolved CaO, as lactic acid.

By these properties this bacillus is proved to be *Bacillus megatherium* *De Bary*.

The Short Bacillus.

Form: The cell cultured in bouillon for 24 hours is 0.6μ wide and 1.0—1.5μ long. It is isolated both in the intestinal juice and in bouillon,

and very rarely two are united. The extremities of the rod are round. Flagella are colored by *Löffler's* method, and some have them in one end, while the other on all sides.

Spore-formation: Spores are not formed.

Mobility. It moves actively.

Gram's method: It is not colored by *Gram's* method. But some absorb colors especially well in the ends of the cell.

Bouillon: Bouillon becomes turbid and viscous. The precipitate formed can be easily distributed by shaking.

Gelatine plate: A white round colony is formed without liquefying gelatine.

Gelatine stab-culture: Colony is formed straightly along the inoculated line to the bottom, and on the surface a white colony is formed which extends to the wall of the tube. The centre of the colony assumes a light yellow color after some days.

Agar streak: A moist, bright, white colony is formed.

Potato: An elevated yellow colony is formed.

Milk: Milk is coagulated, acid reaction being produced.

Gas-production: Gas is evolved by cultivating it in a nutritive solution containing glucose.

Reduction: It reduces nitrate to nitrite.

Indol reaction: A faint red color is produced in pepton-water culture (for 24 hours at 25°C), when it is warmed with addition of H₂SO₄ or HCl.

Acid: It produces acids in a solution containing glucose.

By these properties this bacillus is proved to be the coli-bacillus.

The Micrococcus I.

Form: The cell cultured in bouillon for 24 hours has a diameter of about 0.8 μ , and appears usually in the form of diplococcus.

Gram's Method: Positive.

Oxygen: Growth is better in presence of air.

Bouillon: A little white precipitate was formed, when cultured for 2 days at 23°C. No scum was formed, although kept for more than 2 days.

Gelatine plate: A yellow, round, sharply defined, moist, bright, homo-

genous and elevated colony, that does not liquefy gelatine, is formed on the surface. By weak magnification the appearance is the same. Deep colony is a white point.

Gelatine streak: Colony is homogenous, and at first white but afterwards turns yellowish brown: It does not liquefy gelatine.

Gelatine stab-culture: Colony is formed straightly along the inoculated line to the bottom.

Agar streak: Colony is elevated, homogenous, moist, and at first white but afterwards assumes a faint brown.

Potato: A white, homogenous colony is produced along the inoculated line in 6 days at 23°C.

Milk: Milk is coagulated, acid reaction being produced.

Gas-production: Gas is not evolved.

H₂S: H₂S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acid are produced when cultivated in a nutritive glucose bouillon.

The Micrococcus II.

Form: The cell cultivated in bouillon for 24 hours is about 0.8 μ in diameter. It occurs usually in the form of diplococcus, but sometimes four are united.

Gram's method: Positive.

Oxygen: Aërobic.

Bouillon: At 15°C on the fourth day of inoculation it becomes turbid, and on the sixth day a precipitate is formed, the supernatant fluid remaining clear. It is the same after 20 day's culture.

Gelatine plate: Surface colony is round, convex, sharply defined, homogenous, moist, bright, white and has a porcelain lustre. Gelatine is not liquefied. Weakly magnified appearance is the same as the above. Deep colony is a white point.

Gelatine stab-culture: Colony is formed straightly to the bottom along the inoculated line.

Gelatine streak: A white, moist, homogeneous, elevated colony is formed along the inoculated line, without liquefying the media.

Agar streak: A white, moist, homogeneous, elevated colony is formed which extends very soon the whole surface.

Potato: A white, homogeneous, moist, elevated colony is formed on the fourth day of inoculation at 30°C.

Milk: Milk is coagulated, acid reaction being produced.

Gas-production: Gas is not evolved.

H₂S: H₂S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acids produced by cultivating in a nutritive glucose solution (with Ca CO₃) for 6 days at 36°C. was found to be 0.12% calculated from the dissolved Ca O as lactic acid.

The Micrococcus III.

Form: The cell cultured in bouillon for 24 hours is about 1 μ in diameter. Usually two are united but sometimes four.

Gram's method: Positive.

Oxygen: Aërobic.

Bouillon: It becomes turbid by two day's cultivation at 23°C. After 20 days a feeble scum is formed, and a yellow precipitate on the bottom, the supernatant fluid remaining still turbid.

Gelatine plate: Surface colony is yellow, round, convex, sharply defined, moist and bright. By weak magnifying power the appearance is the same, granular consistence being visible. Gelatine is liquefied. Deep colony is a white point.

Gelatine stab-culture: Colony is formed straightly to the bottom along the inoculated line, liquefying it in the shape of a nail.

Gelatine streak: A sulphur-yellow colony is formed along the inoculated line, liquefying it completely after a few days.

Agar streak: An elevated moist homogeneous colony is formed, the color of which is white at first, but turns sulphur-yellow after a few days.

Potato: A very elevated, moist, bright, homogeneous colony is formed

along the inoculated line. It is yellow at first, but turns brown after a few days.

Milk: Milk is coagulated, acid reaction being produced.

Gas-production: Gas is not evolved.

H₂S: H₂S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acids were produced by cultivating in pepton-water containing glucose.

In the intestinal juice other micrococci and bacilli are of course present, although their number is commonly less than the above described. But as the colonies formed by those micrococci which are chromogenous, are at first white and assume the proper tint after many days of culture, mistakes are possible by not giving time enough

The Micrococcus present in the Eggs.

Form. The cell cultivated in bouillon for 24 hours is $1\ \mu$ in diameter. It occurs always in packet-form in nutritive fluids. But in the intestinal juice of the larvæ it occurs in the form of diplococcus.

Gram's method: Positive.

Oxygen: Aërobic.

Bouillon: Bouillon becomes turbid little on the seventh day of inoculation, and a light yellow precipitate is formed after 20 day's culture.

Gelatine plate: Surface colony is yellow-moist, bright, elevated, round and sharply defined. By weak magnification it is granular. Deep colony is a yellow point. Gelatine is liquefied.

Glatine streak: A yellow, elevated colony is formed along the inoculated line, gelatine being quickly liquefied.

Gelatine stab-culture: Colonies are formed discontinuously along the inoculated line. Gelatine is liquefied at first in the shape of a nail, but afterwards in the shape of a cylinder.

Agar plate: Surface colony is yellow, moist, bright, non-tenacious, elevated, round, sharply defined, and has a point on the centre. By weak magnification granular consistence is visible. Deep colony is a white point.

Agar streak: An elevated, especially in the central line, moist, homogeneous colony, the color of which is yellow shadowed with black, is formed.

Potato: A yellow, moist, homogeneous colony is formed along the inoculated line on sixth day at 23°C.

Milk: Milk is coagulated with much production of acid.

Gas-production: Gas is not produced.

H₂S: H₂S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acids produced in 14 day's culture in glucose-bouillon at 20°C. was 0,33% calculated as lactic acid.

Yellow pigment of the micrococcus is insoluble in water, alcohol or ether, but soluble in potash solution, which turns pale red by warming with addition of HCl.

By these properties this microbe is recognized as *Sarcina lutea* Flüggé.

Chapter III.

The results of the experiments.

Since in the intestinal juice of the diseased larva, an abundant growth of bacteria takes place, it is certain that this malady is caused by these microorganisms. But as these bacteria make luxuriant growth only in the intestinal juice and never invade considerably the tissues or blood, the pathogenic action will perhaps be due to the production of a certain toxin. Hence some experiments were undertaken to test this suggestion by using a solution, containing toxin, prepared in the usual manner from the culture of the micrococci commonly found abundantly in the diseased larva.

Experiment I.

This experiment was performed in this College in October of 1901. At

this time it was rather cold and since flacherie happens more rarely in cold than in warm weather, the silk-worms used for the experiment were reared in a large box constructed to keep the larva at a somewhat elevated temperature. This box and other apparatus used in the experiment were sterilized with the vapors of formalin.

The material used for this was prepared from *Micrococcus II* cultured in bouillon for 9 days at 36°C. The filtrate was prepared from the above culture by filtering through *Chamberland's* filter; and as in some cases toxin is not secreted from the living bacteria cells, a part of the culture was heated to 65°-70°C. for 30 minutes to kill the bacteria-cells.

Oct. 29. 3 P.M. The original culture, the filtrate and the heated culture were given together with mulberry-leaves to the larvæ of the second day of the fourth age. The number of the larvæ used for each experiment was 20, and the quantity of the materials used was 1,5 cc. to 100 grs. of mulberry-leaves. The larvæ showed a very good appetite, and then they were treated as usual. The temperature in the box was 21°C.

Oct. 30. When they were examined in the morning, there were no diseased larvæ found. Hence the culture, the filtrate and the heated liquid were given again to the larvæ as before, and the temperature was raised to 25°C. and water was besprinkled in order to increase moisture, because high temperature and moisture are favorable to the development of flacherie. But as the arrangement to keep the temperature high was imperfect, it fell too low during the night.

Oct. 31. No symptom of the disease was observed in all the sections.

Nov. 1. All the larvæ, except one in the control experiment that had died, spun healthy cocoons. An excreta of the larvæ fed with the culture of the micrococcus was put into bouillon, in which the micrococci made luxuriant growth after a few days, proving that micrococci had entered and passed the digestive canal of the larva.

Experiment II.

The negative result obtained in the former experiment might have been due to the low temperature. So this experiment was performed to repeat

the former one using higher temperature. The culture used for this was also that of *Micrococcus II* cultured in bouillon for 3 days at 36°C. Filtration and heating were performed as in the former experiment.

Nov. 8. In the afternoon the materials were given twice respectively to 20 of the larvæ of the fourth day of the fourth age in the same manner as in the former. The intestinal juice of the diseased larvæ was also given to 10 larvæ. At 4 P.M. they were put in a thermostat and kept at 27°C. In the thermostat the ventilation was rather poor and the moisture content high, so that moulds grew on the excreta. The larvæ soon got into the stage of ecdysis, and on 10th they ended ecdysis. From this day on death took place.

Nov. 11. The silk-worms were transferred to a room of 21°C.

The number of the dead larvæ will be seen from the following table.

Date.	Control.	The culture.	The filtrate.	The heated culture.	The intestinal juice.
Nov. 10	1	4	1	0	7
11	0	0	0	0	0
12	0	1	0	1	0
13	0	1	0	0	2
14	0		1	0	0
15	0	0	0	0	1
16	0	0	0	0	0
17	1	0	2	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	1	4	0	0	0
Total	3	10	4	1	10

The remainder formed cocoons.

As soon as the larvæ died, their intestinal juice was examined with a

microscope, and according to the microbes present in the juice and also other symptoms, the disease of the dead larvæ was grouped as follows:—

	Control.	Culture.	Filtrate.	Heated culture.	Intestinal juice.
Flacherie! I	0	7	1	0	7
„ II	1	0	0	0	1
Grasserie	0	1	1	0	2
Pebrine	2	2	3	1	0
Total	3	10	5	1	10
Flacherie in % of total larvæ	5	35	5	—	8

Contrary to the former experiment many flacherie-patients were produced in this. It can, therefore, be concluded:—

(I), that flacherie takes place when temperature and moisture are high and ventilation is insufficient, in short, when the conditions are injurious to the health of the silk-worms;

(II) that pathogenic action is not due to the production of toxin.

Experiment III.

This experiment was performed to confirm once more the result of the former ones. The cultures used in this experiment were prepared from *Micrococcus II* cultivated in bouillon for 10 days at 36°C. and from *Micrococcus I* cultivated in bouillon for 34 days at 36°C. The filtrate was however prepared only from the former.

Nov. 14. At noon the cultures and other materials were given to the larvæ on the second day of the fifth age, taking 20 larvæ for each experiment. The temperature of the room was 15°C. and moisture 54. They were kept to the 18th, no diseased one being observed. They were therefore placed in a thermostat and kept at 27°C. and on the 22nd they

! Flacherie I denotes that in which micrococci were abundant, and flacherie II where bacilli were abundant.

were again transferred to the former room, and on the 26th they formed cocoons.

The number of the dead larvæ during the experiment was as follows :—

Date.	Control.	Culture of <i>Micrococcus I.</i>	Culture of <i>Micrococcus II.</i>	The filtrate.
Nov. 19	3	1	2	1
20	1	2	1	3
21	2	2	0	2
22	0	1	0	0
23	0	0	0	0
24	0	0	0	0
25	1	4	0	1
Total	7	10	3	7

The disease was grouped as follows :—

	Control.	<i>Micrococcus I.</i>	<i>Micrococcus II.</i>	Filtrate.
Flacherie I.....	3	5	2	7
„ II.....	2	4	1	0
Grasserie	0	1	0	0
Pebrine	2	0	0	0
Total	7	10	3	7
Flacherie in % of the total larvæ	25	45	15	35

It will be seen from these tables that flacherie was more in the larvæ that were not infected artificially, than in those that received the bacteria. From this fact it can be learned that the bacteria, that cause flacherie, are already present in the vicinity of the larvæ and even in their intestines, waiting for an opportunity for development. Since many patients appeared among the larvæ fed with the filtrate, flacherie would seem to be caused by

some toxins. But this can not be sure, because in the intestinal juice of the diseased larvæ many micrococci were present which certainly had caused the malady.

The results obtained in this and other experiments disprove the infectiousness of flacherie, which is against the belief held by the sericulturists of the present day.

Experiments IV.

This experiment was performed to investigate once more the pathogeny of the micrococci.

1902. May 8. *Micrococcus II* cultured in the decoction of mulberry-leaves for 7 days at 36°C. were given four times to 100 larvæ (*Aohiki* variety) of the first day of the first age. After this they were kept in the usual manner till the fourth age, without observing any symptoms of flacherie.

The number of the larvæ examined on the first day of the fifth age were as follows:—

	Healthy	Dead	Lost
Control	89	6	5
Inoculated	91	3	6

The average temperature and moisture during the experiment were as follows:—

	Temperature	Moisture
First age	19,0°C.	68,4
Second „	21,5	71,4
Third „	22,0	75,3
Fourth „	20,0	78,1

Experiment V.

Since flacherie occurs usually more in old larvæ than in young ones, the negative result obtained in the former experiments might be due to the fact

that the bacteria were fed to young larvæ. Therefore this experiment was repeated, using old larvæ.

The bacteria used for this experiment were *Micrococcus II* isolated this year from a diseased larva and the sarcina¹ isolated from the eggs of silkworm. They were inoculated with the following materials.

- I. The micrococci, cultured on agar, suspended in water.
- II. The filtrate obtained from the decoction of mulberry-leaves cultured for a week at 36°C.
- III. The above culture heated for 30 minutes to 65°C.²
- IV. The same to which formalin was added in the proportion of 1 drop to 10 cc. of the culture.³

May 22. 3 P.M. The materials above described were given together with mulberry-leaves⁴ each to 100 larva (*Akahiki* variety) on the first day of the third age. They were kept in the usual manner till the fifth age without observing any symptoms of the disease.

The average temperature and moisture during the experiment were as follow:—

Date.	Temperature.	Moisture.
May 22	22,5°C.	70,0
23	22,0	78,0
24	22,1	80,0
25	21,1	75,0
26	22,0	82,0
27	22,0	69,0
28	18,5	74,7
29	22,2	78,7

¹ *Sarcina lutea* Flügge.

² Sterilized.

³ Sterilized.

⁴ 1,5 cc. of the materials to 100 gms. of mulberry-leaves.

Experiment VI.

As the result of the former experiments were all negative, it seemed doubtful that the bacteria used in these experiments were not the pathogenic ones. This experiment was therefore performed to observe the infective power of the intestinal juice of a diseased larva.

May 28. 3 P.M. The intestinal juice, obtained respectively from a dead larva whose body was softend and elongated, and from that whose body was contracted, were fed four times together with mulberry-leaves each to 10 larvæ (*Akahiki* variety) of the second day of the fifth age, In the intestinal juice there were of course bacilli and micrococci in great number. They were then fed in the usual manner for a week without observing any symptoms of the disease. The average temperature during the experiment was as follows:—

Date.	Temperature.
May 28	17,0°C.
29	18,0
30	20,0
31	19,5
June 1	21,5
2	19,8
3	20,9

From these experimental results it is clear that silk-worms do not become ill from flacherie when the surrounding conditions are favorable to their health, and they have resistance-power. These results agree with that reported by the Austrian Agricultural Experimental Station.

Experiment VII.

Since the negative results obtained in the former experiments might have been due to the insufficiency of the number of the bacteria, a further

experiment was made by injecting various bacteria directly into the intestines through the anus by means of a syringe, the point of which was carefully rounded off. The bacteria cultured on agar were suspended in water and 0.05 cc. were injected. The species of the bacteria used were as follows:—

Micrococcus I.

„ *II.*

„ *III.*

Coli-bacillus (isolated from the diseased larva).

Bacillus mesentericus vulgatus Flügge.

„ „ *fuscus* „

Bacillus subtilis Cohn.

June 7. 2 P.M. The above materials were injected into the larvæ (*Aohiki*-variety) on the second day of the fifth age. After the silk-worms had recovered the normal state which took about five hours, 10 lively larvæ were selected from each section, and at the same time 100 larvæ were kept as control, among which no disease appeared during the experiment.

According to a previous experiment it was known that flacherie is produced after about three days even by injecting pure water into the intestines through the anus, when the temperature is high, but when bacteria are injected the malady is produced more quickly. A slow development of the disease at a high temperature therefore would give naturally no decisive result. The results of the injection must be observed within 3 or 4 days. The results after three days were as follows:—

1. *Distilled Water.*

The larvæ injected behaved very lively and showed a very good appetite. On the third day two of them died; in their intestinal juice many micrococci were found.

2. *Micrococcus I.*

The silk-worms lost appetite. On the afternoon of the second day four

of them died of flacherie ; in the intestinal juice micrococci and *Bac. megatherium* were found in large number. On the same night one died, in whose intestinal juice only the micrococci were found. On the night of the third day two more died, in which also only the micrococci were found.

3. *Micrococcus II.*

The larvæ lost appetite. On the afternoon of the second day one died of flacherie, in which much of *Bac. megatherium* and little of micrococci were found. On the second night three died, in which a great number of micrococci was found.

4. *Micrococcus III.*

The larvæ lost appetite. On that night two died of flacherie, in one of which the micrococci prevalent, while in the other *Bac. megatherium* exceeded the number of micrococci. On the second day five died of flacherie, in which a great number of micrococci was found.

5. *Coli-bacillus.*

The larvæ lost appetite completely. On the afternoon of the second day six died of flacherie in which coli-bacilli were found in great number. On the second night three died, and on the afternoon of third day one died. In the former only coli-bacilli, while in the latter *Bac. megatherium* was found.

6. *Bacillus mesentericus vulgatus* Flügge.

The larvæ lost appetite completely. On the afternoon of the second day two died, in one of which *Bac. mes. vulgatus* prevailed, while in the other micrococci were more abundant. On the second night seven died, in six of which *Bac. mes. vulgatus*, but in one only micrococci were observed.

7. *Bacillus mesentericus fuscus* Flügge.

The larvæ lost appetite. On the second night seven died of flacherie, in four of which only *Bac. mes. fuscus*, but in three this microbe together with micrococci were found. On the afternoon of the third day one died, in which *Bac. mes. fuscus* alone was observed.

8. *Bacillus subtilis* Cohn.

The larvæ did not lose appetite so completely as the others. In the first night one died of flacherie, in which much *Bac. subtilis* was found. On the forenoon of the third day four, and on the afternoon one died of flacherie; in the former *Bac. subtilis* prevailed, while in the latter micrococci.

The results of the experiments may be summerized in the following table.

	First day.	Second day.	Third day.	in % of the larvæ taken for the test.
Control	0	0	0	0
Water	0	0	2	20
<i>Micrococcus I</i>	0	5	2	70
„ <i>II</i>	0	4	0	40
„ <i>III</i>	2	5	0	70
Coli-bacillus	0	9	1	100
<i>Bac. mes. vulgatus</i>	0	9	0	90
„ „ <i>fuscus</i>	0	7	1	80
<i>Bac. subtilis</i>	1	0	5	60

From the results obtained in this experiment the following conclusions can be drawn:—

1. Many species of bacteria can propagate in the intestinal juice of the larvæ and cause flacherie.

2. Disorder in the digestive organ such as injection of water causes flacherie.
3. From the above facts it is clear that bacteria, that can cause flacherie, are present at all times in the intestinal canal of the larvæ waiting for an opportunity for development.
4. That flacherie caused by the injection of the bacteria is not due merely to the disorder in the digestive canal, is proved by the following facts.
 - a. The bacteria injected into the intestines multiplied therein.
 - b. When bacteria were injected, flacherie was produced more quickly and frequently than when water is injected.

Experiment VIII.

This experiment was performed to test once more for the production of toxin by injection. The materials used for the experiment were *Micrococcus III* cultured in a decoction of mulberry-leaves in absence of air for 7 days at 36°C.

It was filtered through *Chamberlana's* filter and a part of it was neutralized with Na_2CO_3 .

June 9. 2 P.M. 0.05 cc of the original and the neutralized filtrates were injected into the intestines of the larvæ (*Aohiki* variety) on the fourth day of the fifth age. The results were as follows:—

1. *The Original Filtrate.*

Eleven larvæ which received this material remained inactive for two hours. One of them was killed and the intestinal juice was examined, in which *Bac. megatherium* was found in large number. On the next morning eight larvæ died, in two of which micrococci abounded, but little of *Bac. megatherium* was present; and in three others *Bac. megatherium* abounded, while micrococci were few; while in three others only *Bac. megatherium* was found. On the 11th two died, in which *Bac. megatherium* abounded, but few micrococci were found.

2. *The Neutralized Filtrate.*

On the forenoon of the 10th nine out of twelve larvæ used for the operation died, in which *Bac. megatherium* abounded. On the 11th three died, in which both *Bac. megatherium* and micrococci were found.

Experiment IX.

Since some bacteria produce a powerful toxin only when they are mixedly infected, all the bacteria isolated from the diseased larvæ were cultured together in nutritive glucose solution for 24 hours at 36°C. A culture of coli-bacillus also served.

June 10. 11 P.M. 0.1 cc. of the original and neutralized filtrate of the above cultures were injected into the larvæ (*Aohiki* variety) on the fifth day of the fifth age.

The number of the dead was as follows:—

	Number of the larvæ tested.	First day.	Second day.	Third day.	Fourth day.	% of the dead.
Water	10	0	0	2	0	20
Filtrate from coli-bacillus	10	0	6	4	0	100
The same neutralized.	10	0	4	4	2	100
Filtrate from the mixed culture.....	10	0	6	4	0	100
The same neutralized.	10	0	5	2	2	90

According to the kinds of the bacteria found in the intestinal juice they may be grouped as follows:—

	Much <i>Bac. megatherium</i> .	<i>Bac. megatherium</i> + micrococci.	<i>Bac. megatherium</i> + coli-bacillus.	<i>Bac. megatherium</i> + coli-bacillus + micrococci.
Water.....	0	2	0	0
Filtrate from coli-bacillus.	1	7	0	2
The same neutralized ...	0	3	2	5
Filtrate from the mixed culture	4	3	3	0
The same neutralized ...	4	1	0	4

As many died of flacherie in this experiment, it might be supposed that the malady was caused by toxins, but that is very improbable, since water alone might have produced the same result.¹

Moreover flacherie is caused by various kinds of bacteria as was shown in the previous experiments. This makes it very improbable that a specific toxin is the cause of the malady.

Experiment X.

From the results obtained in the previous experiments there is no doubt that flacherie is not caused by a special toxin. But since the malady is caused by the multiplication of bacteria in the intestinal juice, the cause of the disease must be due to some action of bacteria and since many kinds of bacteria can produce this disease, the injurious action must be one common to all these bacteria.

The vital action common to all of them and suspicious of injury to the silk-worm is the formation of acid, because the digestive enzymes of silk-worm are active only in an alkaline solution. The micrococci found in the diseased larvæ and that in the eggs as well as *Bac. megatherium*, *Bac. coli*, *Bac. subtilis*, *Bac. mes. vulgatus* and *fuscus*, all produce acids in a solution containing carbohydrates, which were confirmed by direct experiments. Moreover, since the reaction of the intestinal juice is neutral or faintly alkaline, and the fluid excreted in flacherie is sometimes quite acid, there must exist some relation between flacherie and the formation of acids by the bacteria. Hence the effect of injection of acids was studied.

0.1 cc. of distilled water, 3% normal sodium carbonate solution, 2% acetic acid, 2% lactic acid and 2% butyric acid were respectively injected, as in the former experiments, into the intestinal canal of the larvæ of the fifth age. By this operation some vomited fluid, especially many of those injected with water and sodium carbonate solution. All the larvæ seemed somewhat inactive, but those injected with water and sodium carbonate solution showed very good appetite after a few hours.²

¹ Compare also the above experiment, p. 420.

² The larvæ injected with distilled water did not die within 24 hours.

Those injected with the acids died with vomition and diarrhea after about 10 hours, the dead bodies softened, the third and fourth segments being elongated; in short showing the close resemblance to those died of flacherie.

Those injected with 0,1 cc. of 10% lactic acid died instantly without vomition or diarrhea, the bodies contracting and becoming rather hard. But even in this case the intestinal juice of the dead did not show an acid reaction, but still was alkaline, what shows that the silk-worm even dies when the alkaline reaction of the intestinal juice is a little weakened. By this experimental results it may be explained, why the appearance of the dead bodies of the larvæ in one case is different from that in the other.

Vomition and diarrhea characteristic to flacherie is probably due to the fact, that as the intestinal juice is neutralized by the acids produced by the bacteria, the patient secretes more juice to restore the alkaline reaction on the one hand, while resorption is stopped on the other; hence the quantity of the fluid in the intestinal canal increases so much as to cause vomition and diarrhea.¹

Experiment XI.

But the bacteria seem to produce a certain poison, although it is no toxin. It is a well known fact that the coli-bacillus reduces nitrate to nitrite. But the production of nitrite in the decoction of mulberry-leaves by *Bac. megatherium* and the micrococci were also proved by the writer.²

This experiment was performed therefore to observe the effect of nitrite on the silk-worm.

July 4. 9 A.M. 20 larvæ of the second day of the fifth stage were fed with mulberry-leaves moistened with a 10% solution of sodium nitrite for a day.³ On the next morning a larva died.

¹ In higher animals also the secretion of the intestinal juice is much accelerated by presence of acids. *Bunge*, *Physiol. Chemie*.

² Mulberry-leaves contains often much nitrate.

³ 1,5 cc of the solution to 100 grs. of the leaves. The larvæ did not eat the leaves as usual.

They were then fed with the normal leaves, but on the sixth day two died with vomition and diarrhea, but bacteria were not observed in the intestinal juice as in the case of flacherie.

By injecting 0.1 cc. of 1% solution of sodium nitrite in the usual manner, six out of seven larvæ used for the experiment died instantly, the bodies of which were softened and stretched. Those to which only 0.05 cc. were injected, were, for 10 hours after the operation, in a somnolent condition. Then they became again active, but on the third day five died; the dead bodies becoming softened. With the intestinal juice of the larvæ that died of flacherie, the usual nitrate reactions can sometimes distinctly be obtained. These facts make it clear that nitrite formed by bacteria is one of the injurious products that may contribute to the development of flacherie.

Experiment XII.

Since *Bac. megatherium* or *Bac. coli* are of general occurrence it is no wonder that they propagate also in the intestines of the silk-worms. But as to the micrococci it is different.

As a micrococcus and *Bac. megatherium* exist in the interior of some eggs, they might come from the eggs as Pasteur and Macchiati supposed. But flacherie is usually prevalent after the fourth stage.

Therefore it is very improbable that the micrococcus remains in the digestive canal for so long a time without developing the malady. Moreover the micrococcus found in the eggs was quite different from those usually found in the diseased larvæ.

1902 June 23.¹ Agar-plates were infected with small fragments of a mulberry-leaf.

The colonies formed after two days were as follows:—

The original plate: Colonies of a large bacillus (*Bac. megatherium* or *Bac. subtilis*?)

The second dilution: Numerous colonies of the large bacillus and micrococci.

¹ It rained two days before.

The third dilution: Colonies of the large bacillus and white colonies of micrococcus.

June 24. The former experiment was repeated, as on the previous day there had been a heavy rain.

The results were as follows:—

The original plate: Colonies of the large bacilli and micrococci intermingled.

The second dilution: Colonies of the large bacilli and white and brown colonies of micrococcus.

June 27. The experiment was repeated with mulberry-leaves of *Hara-juku* where silk-worms were never reared before.

The results were as follows:—

The original plate: Colonies of various bacteria covered the whole surface.

The second dilution: Yellow and gray colonies of micrococci besides those of other bacteria.

The third dilution: White and light brown colonies of micrococcus.

To decide whether the micrococci of mulberry-leaves are the same as those of flacherie, it was necessary to observe their action on the silk-worm.

July 7. 9 A.M. The micrococci isolated from mulberry-leaves and those of the diseased larvæ were inoculated in the usual manner into the larvæ of the fifth day of the fifth stage, and as they died, their intestinal juice was examined with a microscope.

The results were as follows:—

	Number of larvæ.	First day.	Second day.	Third day.	Total.	% of the dead.
Water	10	0	1	2	3	30
<i>Micrococcus II</i> of silk-worm	10	0	5	2	7	70
<i>Bac. megatherium</i> from silk-worm.	10	2	8	0	10	100
<i>Micrococcus I</i> from mulberry-leaves ¹	10	0	4	6	10	100
<i>Micrococcus II</i> from mulberry-leaves ²	10	0	5	5	10	100
Control	36	0	0	0	0	—

Micrococcus I from mulberry-leaves formed a white colony, while *Micrococcus II* a light brown colony on agar.

The dead larvæ were grouped according to the species of the bacteria found in the intestines as follows:—

	Micrococcus only.	<i>Bac. megatherium</i> and micrococci.	<i>Bac. megatherium</i> only.	<i>Bac. megatherium</i> and coli-bacillus.	Very few bacteria.
Water	1	1	1	0	0
<i>Micrococcus II</i> of silk-worm.	2	3	1	1	0
<i>Bac. megatherium</i>	0	0	8	0	2
<i>Micrococcus I</i> from mulberry-leaves	3	4	3	0	0
<i>Micrococcus II</i> from mulberry-leaves	9	1	0	0	0

From these results it follows that the micrococci present on the mulberry-leaves can cause flacherie in just the same manner as those from the diseased silk-worms. It is therefore very probable that the micrococci found in the intestinal juice are the same as those found on mulberry-leaves.

Experiment XIII.

1902 October. In order to observe whether the micrococci found in the diseased larvæ exist also on mulberry-leaves or not, micrococci were isolated from these leaves and their properties were examined.¹

No. 1.

Form: The diameter of the cell cultivated in bouillon for 24 hours is 1 μ . Commonly two are united. The cells are colored by *Gram's* method.

Bouillon: At 15°–17°C. bouillon becomes turbid on the second day of inoculation, and on the seventh day a white ring is formed on the wall of the tube and a white precipitate is formed, the supernatant fluid becoming clear. After 20 days a feeble scum appears.

Gelatine plate: Surface colony is white, round, sharply defined, lipped,

¹ The leaves came partly from the College farm in *Komaba*, and partly from a garden in *Tokio* where no silk-worms were kept for 30 years.

moist and has porcelain-like lustre. A point of light brown color is in centre. By weak magnification the appearance is the same, showing a curled consistence. Deep colonies appear as white points.

Gelatine streak: Colony light brown, folded, a film is found along the inoculated line, gelatine being liquefied.

Gelatine stabculture: At 12°C. after 20 day's culture, colonies are formed along the inoculated line, liquefying gelatine.

Agar streak: White, homogeneous, moist, elevated, tenacious colony.

Potato: At 23°C. elevated white colonies are found which on the sixth day turn brown and show granular consistence.

Milk: At 30°C. milk is coagulated in 24 hours, acids being formed.

Oxygen: Growth is better in presence of air.

Gas: Gas is not evolved by cultivating in a nutritive solution containing glucose for 7 days at 15°C.

H²S: H²S is not observed in bouillon cultured for 24 hours.

Reduction: Nitrate is reduced to nitrite.

Acids: Azolithmin is turned red in pepton-water cultures containing 5% of glucose. This micrococcus is therefore probably *Micrococcus coronatus* Flügge.

No. 2.

Form: The diameter of the cell cultured in bouillon for 24 hours is 0.8 μ . Usually two are waited. The microbe is colored by *Gram's* method.

Bouillon: On the fifth day it becomes turbid, and on the seventh day a yellowish brown precipitate is formed. After 20 days a feeble brown scum appears.

Gelatine plate: It did not grow on gelatine within 13 days at room-temperature (winter).

Gelatine streak: Granules of white and light yellowish color are formed intermingled along the inoculated line. Their color changes afterwards respectively to yellow and deep brown. Gelatine is slowly liquefied.

Gelatine stabculture: Thread-like growth to the bottom and liquefaction in the form of a nail.

Agar plate: At 30°C. the surface colony is light brown moist, bright,

round, lipped, sharply defined, and the centre is somewhat elevated. By weak magnification its consistence seems to be homogeneous. Deep colonies appear as white points.

Agar streak: White, moist, granular, non-tenacious colony which assumes after a few days a yellow color.

Potato: On the second day a flat, dry, light brown, granular colony along the inoculated line.

Milk: It is coagulated showing alkaline reaction.

Oxygen: Aërobic.

Gas: Gas is not evolved.

H₂S: H₂S is not formed.

Indol reaction: Faint reaction.

Reduction: Nitrite is formed from nitrate.

Acids: Acids are formed in glucose solution.

This micrococcus is *Micrococcus bicolor*, Zimmermann.

No. 3.

Form: The cell cultured in bouillon for 24 hours has a diameter of 0.8 μ . Two are usually united, but sometimes four, isolated cells are rare. It is colored by *Gram's* method.

Bouillon: At 23°C. on the second day a little white precipitate is formed, the supernatant fluid becoming clear. It is the same after 20 days.

Gelatine plate: Surface colony is dirty white, round, convex, with a white ring. By weak magnification, the centre seems deeply colored and it becomes lighter towards the margin. Gelatine is slowly liquefied.

Gelatine streak: A light brown, homogeneous colony is formed, gelatine being liquefied.

Gelatine stabculture: Thread-like growth to the bottom, liquefying gelatine along the inoculated line.

Agar streak: Moist, homogeneous, tenacious colony which is white at first, but becomes light reddish brown afterwards.

Potato: It did not grow on potato in a week at 30°C.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not formed.

H₂S: H₂S is formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acid is formed in glucose solution.

No. 4.

Form: The cell cultivated in bouillon for 24 hours has a diameter of 1 μ . Two are usually united. It is colored by *Gram's* method.

Bouillon: At 23°C. on the second day a white precipitate is formed. Scum is not formed by 20 days' culture.

Gelatine plate: Surface colony is yellow, moist, bright, round, sharply defined, convex and homogeneous. The appearance is the same by weak magnification. Deep colonies appear as white points.

Gelatine streak: Homogeneous colony along the inoculated line, the color of which is white at first but turns yellowish brown afterwards. Gelatine is not liquefied.

Gelatine stabculture: Thread like growth to the bottom.

Agar streak: Elevated, homogeneous, moist, dirty-white colony.

Potato: At 23°C. on the sixth day of inoculation a white elevated homogeneous colony is formed along the inoculated line.

Milk: Milk is coagulated, turning acid.

Oxygen: Aërobic.

Gas: Gas is not formed.

H₂S: H₂S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acids: Acids are formed in glucose solution.

This micrococcus is the same as *Micrococcus I* of the silk-worm.

No. 5.

Form: The cell of 24 hour's culture in bouillon has a diameter of 1.2 μ . Two or more are united. It is colored by *Gram's* method.

Bouillon: At 23°C. on the second day it becomes turbid and on the fourth day a scum is formed and on the seventh day a white precipitate appears, the middle part being clear. It remains the same after 20 days.

Gelatine plate: Surface colony is yellow, round, sharply defined, granular, moist and bright. It appears alike by weak magnification. Gelatine is liquefied. Deep colony is a yellow point.

Gelatine streak: Sulphur-yellow, moist, homogeneous, elevated colony is formed along the inoculated line. Gelatine is quickly liquefied, yellow precipitate being formed.

Gelatine stabculture: Thread-like growth to the bottom, gelatine being liquefied at first in the form of a funnel but afterwards cylindrically.

Agar streak: Sulphur-yellow, moist, homogeneous, devated colony along the inoculated line.

Potato: At 23°C. dry, flat, white colony, having yellow granules thereon.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not evolved.

H₂S: H₂S is formed.

Reduction. Nitrate is reduced to nitrite.

Acids: Acids one formed in glucose solution.

The yellow pigment is insoluble in water or alcohol, but soluble in potash solution. The pigment dissolved in the latter solution becomes colorless by the addition of HCl, which is restored again to yellow by alkali.

This micrococcus is a variety of *Micrococcus luteus*, *Lehmann et Neumann*.

No. 6.

Form: The cell cultured in bouillon for 24 hours has a diameter of 0.8 μ . Commonly two are united. It is colored by *Gram's* method.

Bouillon: On the third day a precipitate appears, and after 20 days a feeble yellow-brown scum and a yellow-brown precipitate are formed.

Gelatine plate: Surface colony is yellowish brown, moist, bright, round,

sharply defined and elevated. By weak magnification granular consistence is seen. Deep colony is a white point.

Gelatine streak: Light brown, tenacious colony is formed along the inoculated line. Gelatine is liquefied, film being formed.

Gelatine stabculture: Thread-like growth to the bottom, liquefying Gelatine in the form of a funnel.

Agar streak: Yellowish brown, moist, homogeneous, elevated colony.

Potato: At 23°C. on the second day a yellow colony is formed along the inoculated line. It assumes gradually a reddish yellow color and becomes elevated, dry and granular.

Milk: Milk is coagulated, showing an alkaline reaction.

Oxygen: Aërobic.

Gas: No gas is evolved.

H₂S: H₂S is formed.

Reduction: Nitrate is reduced to nitrite.

Indol reaction: A faint reaction.

Acids: 0.024% of acid, calculated from the dissolved Ca O as lactic acid, were formed in pepton-water containing 5% of glucose for a week at 15-20°C.

This micrococcus is probably *Micrococcus pyogenes aureus*, *Lehmann et Neumann*.

No. 7.

Form: The cell cultivated in bouillon for 24 hours has a diameter of 0.8 μ . Usually two are united, but sometimes four. It is colored by *Gram's* method.

Bouillon: On the second day it becomes turbid, and after 20 days a feeble ring on the wall, and a yellow precipitate were formed.

Gelatine plate: Surface colony is yellow, moist, bright, round, convex and sharply defined. By weak magnification it appears granular. Gelatine is liquefied. Deep colonies appear as white points.

Gelatine streak: Sulphur-yellow, homogeneous colony is formed along the inoculated line, gelatine being liquefied very quickly.

Gelatine stick: Thread-like growth to the bottom, liquefying gelatine in the form of a nail.

Agar streak: An elevated homogeneous, moist white colony is formed which gradually turns yellow.

Potato: At room-temperature elevated, yellow, moist bright, homogeneous colonies are formed along the inoculated line.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not formed.

H_2S : H_2S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acids: 0.013% of acid, calculated from the dissolved Ca O as lactic acid, is formed by cultivating in pepton-water containing glucose and Ca CO_3 for 14 days at 15-20°C.

The yellow pigment is insoluble in water or alcohol, but soluble in potash solution. The color is not destroyed by HCl or H_2SO_4 .

This micrococcus is the same as *Micrococcus III* of the silk-worm, and is probably *Streptococcus bombycis* of Macchiati.

No. 8.

Form: The cell cultured in bouillon for 24 hours has a diameter of 1 μ . Commonly two are united but sometimes four. It is colored by Gram's method.

Bouillon: At 15°C. on the fourth day it becomes a little turbid, and on the sixth day a precipitate settles. It remains the same after 20 days.

Gelatine plate: Surface colony is white, round, elevated, sharply defined, and moist. By weak magnification it appears homogeneous. Deep colonies appear as white points.

Gelatine streak: An elevated, white, moist, homogeneous colony is formed. Gelatine is not liquefied.

Gelatine stabculture: Thread-like growth to the bottom.

Agar streak: A moist, bright dirty white, homogeneous colony along the inoculated line.

Potato: An elevated, white, moist, bright, homogeneous colony. On the central line it is more elevated.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not formed.

H₂S: H₂S is formed.

Reduction: Nitrate is reduced to nitrite.

Acids: Acids are formed in glucose solution.

This is the same as *Micrococcus II* of the silk-worm, and is probably the *Streptococcus Pastorianus* of *Krassiltschik*.

No. 9.

Form: The cell cultivated in bouillon for 24 hours is somewhat oblong and 0.8 μ . in the longer diameter. Usually two are united. It is coloured by *Gram's* method.

Bouillon: On the seventh day little white precipitate is formed, the fluid remaining clear. It is the same after 20 days.

Gelatine plate: Surface colony is deep yellow, round, elevated, sharply defined and moist. By weak magnification it appears granular. Deep colonies appear as yellow points. Gelatine is not liquefied.

Gelatine streak: Deep yellow, rather dry, homogeneous colony, which is much elevated on the central line. Gelatine is not liquefied.

Gelatine stabculture: Thread-like growth to the bottom.

Agar streak: It is the same as on gelatine, but the color is fainter.

Potato: At 23°C. on the fourth day flat, light yellow, moist, homogeneous colonies are formed along the inoculated line.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not evolved.

H₂S: H₂S is formed.

Reduction: Nitrate is reduced to nitrite.

Indol reaction: A faint reaction.

Acids: 0.01% of acid, calculated as lactic acid, was produced in pepton-water containing 5% of glucose and some CaCO₃ for 14 days at 15°-20°C.

This micrococcus is *Micrococcus aurantiaca*, Cohn.

No. 10.

The colony of this micrococcus is dark purple. The properties are not yet examined minutely.

Experiment XIV.

Nov. 11. 1902. On 10 A.M. 0.05 cc. of water in which the micrococci above described, cultured on agar, were suspended, were injected into silkworms of the fifth day of the fifth stage, as in the former experiments. As control distilled water was also injected. All the larvæ except those that received water, lost appetite and on the next morning excreted liquid feces. They were kept in the sitting room and after Nov. 19 they were placed near a stove.

The number of the dead larvæ and the temperature during the experiment were as follows:—

	Number of the larvæ.	Temperature (c)†																			Total.	% of the dead.
		11	12	13	14	15	16	17	18	19	20	21	22	23	24	25						
Temperature (c)†		14	11	11	18	16	11	9.5	11	20	13	18	20	20	20	20						
		22	—	—	24	20	13	—	18	—	18	25	20	20	20	20						
Control	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	13			
Water	10	0	0	0	2	2	0	0	0	0	1	1	0	0	0	0	0	6	60			
No. 1	10	0	0	0	3	0	0	0	0	1	0	1	2	0	0	0	7	70				
No. 2	10	0	0	0	0	5																
No. 3	9	0	0	0	1	4	1	1	0	0	0	0	2	0	0	0	9	100				
No. 4	10	0	0	0	1	6	0	1	0	0	0	1	0	0	0	1	10	100				
No. 5	10	0	1	2	1	4	2										10	100				
No. 6	8	0	0	0	2	3	1	0	0	0	0	1	1				8	100				
No. 7	10	0	0	1	1	2	3	1	0	0	2						10	100				
No. 8	10	0	0	0	0	1	3	0	0	0	2	2	2				10	100				
No. 9	10	0	0	0	3	2	4	0	0	0	1						10	100				
Micrococcus from the eggs	10	0	0	0	4	5	1										10	100				

† Temperature after the 19th was very low during the night, as the stove was not used at night.

The conditions of the larvæ in each section of the experiment were as follows :—

Control.

15 silk-worms were kept for control. They were healthy and span cocoons on Nov. 25. During spinning two died of pebrine and flacherie. In the latter case many micrococci were found among green compressed fragments of mulberry-leaves. The average weight of the cocoons was 0.487 gr.

Water.

The larvæ recovered after a few hours.

Nov. 14. On the morning a larva died vomiting a yellow fluid, the body of which was shrunk. In the intestines large bacilli and diplococci were numerous. On the evening another died, fore-part of the body shrunk and back-part expanded. The fragments of mulberry-leaves in the intestines were green; here only large bacilli were found.

Nov. 15. On the morning one died, body softened and stretched out. Large bacilli were numerous, and also some diplococci were found. On the evening another died, body expanded in the middle part. The pieces of mulberry-leaves in the body were brown; streptococci were numerous.

Nov. 20. On the morning one died, body softened, back-part black. No fragments of mulberry-leaves present; some diplococci in the intestines.

Nov. 21. On the morning one died, body softened and stretched out. Some slender bacilli were present.

Nov. 25. The remainder (4) span cocoon, the average weight of which was 0.411 gr.

No. 1.

After the operation the larvæ lost appetite considerably.

Nov. 14. On the morning one died, the third and fourth segments elongated, excretion of soft brown dung of a faint acid reaction. The frag-

ments of mulberry-leaves in the intestines were not compressed; many large bacilli and some diplococci present. On the evening two died, one with shrunk fore-part and green compressed leaf-fragments, and bacilli and diplococci; the other with brown leaf-fragments and numerous bacilli of various size.

Nov. 19. On the evening one died, body softened and expanded in the middle part. Leaf-fragments not compressed; diplococci numerous.

Nov. 21. On the morning one died, body softened and expanded. The intestinal canal was filled only with liquid with numerous diplococci and few bacilli.

Nov. 22. On the morning two died, one with swollen segments and black back-part. In the intestines few leaf-fragments with some diplococci. The other with softened blackened body and green leaf-fragments; diplococci and bacilli present.

Nov. 25. Three remaining larvæ span cocoons, the average weight of which was 0.390 gr.

By preparing a plate-culture from the intestinal juice of the diseased larva many white colonies of the micrococcus inoculated besides various others appeared.

No. 2.

The larvæ lost appetite after the operation.

Nov. 13. On the morning five died. The first of them with some folds on the body; a few brown leaf fragments in the intestines and numerous diplococci.

The second also with folds, brown leaf-fragments and numerous diplococci and some streptococci. The third also with folds, brown leaf fragments, numerous diplococci and some streptococci. The fourth resembled the third; streptococci were more numerous, while with the fifth diplococci again prevailed, all the other conditions being the same.

Nov. 15. The remainder were unfortunately lost by an accident.

By preparing an agar-plate from the intestinal juice of the dead larva many colonies of the micrococcus inoculated were formed.

No. 3.

Nov. 14. On the evening one died, body softened and stretched, faint brown leaf fragments and large bacilli and diplococci in great number in the intestines.

Nov. 15. On larva died, body was faintly yellow, with some folds. Leaf fragments brown; diplococci numerous. On the evening three died, bodies softened and stretched. In the first the leaf fragments green, large bacilli and diplococci present. In the second brown leaf fragments, diplococci numerous. In the third no leaf fragments but a few diplococci were found in the intestines.

Nov. 16. On the morning one died, body stretched and leaf fragments green, diplococci numerous. All the other larvæ excreted liquid feces.

Nov. 17. On the morning one died, fore-part of body somewhat transparent, back-part thin. The few leaf fragments green, few diplococci and pebrine-organisms present.

Nov. 22. On the morning two died, both with softened body and many diplococci, the one with brown leaf fragments; the other with empty intestines, the back part of the latter larva was black.

No. 4.

The larvæ showed poor appetite after the operation.

Nov. 14. On the morning one died, body rather hard, leaf fragments brown and compressed, diplococci numerous.

Nov. 15. On the morning three died, fore-part of bodies shrunk. Leaf fragments brown, numerous diplococci. On the evening three died, bodies softened, leaf fragments green, diplococci numerous.

Nov. 17. On the morning one died, body softened, leaf fragments green, streptococci numerous.

Nov. 21. On the morning one died, leaf fragments compressed; no bacteria were observed.

Nov. 25. One died, body softened. Few fragments of leaves, many diplococci.

By preparing an agar-plate from the dead larva numerous light brown colonies of diplococci were formed.

No. 5.

The larvæ lost all appetite by the operation.

Nov. 12. At noon one died, excreting a brown fluid of a faint acid reaction from the anus. The intestinal canal was full of brown fragments of leaves; numerous diplococci present.

Nov. 13. On the morning two died, excreting a brown fluid of a faintly acid reaction from the anus. One contained many large bacilli and few diplococci; the other few small bacilli and micrococci.

Nov. 14. On the morning one died vomiting a brown fluid, the third and fourth segments were elongated, the middle part of the body expanded, black lines appearing on the fourth and fifth segment, leaf fragments brown and compressed, numerous diplococci and some large bacilli present.

Nov. 15. On the morning four died, bodies stretched and containing brown leaf fragments. In the first the back part of the body black and streptococci, numerous. In the three others streptococci were numerous.

Nov. 16. On the morning two died, bodies softened. Leaf fragments brown, diplococci numerous.

By preparing an agar-plate from the intestinal juice of the dead larva yellow colonies of diplococci were produced in large number.

No. 6.

The larvæ lost completely appetite by the operation.

Nov. 13. On the morning one excreted a light yellow fluid of a faintly acid reaction from the anus.

Nov. 14. On the morning two died. One with stretched and softened body, and green leaf fragments. The second with shrunken body had excreted a yellow fluid of a faintly acid reaction from the anus. Leaf fragments green, numerous diplococci present.

Nov. 15. On the morning two died, bodies faintly yellow. In one of them leaf fragments were few and compressed; diplococci and streptococci numerous. In the other leaf fragments brown, numerous streptococci and some large bacilli present. On the evening one died, the middle part expanded, leaf fragments brown and compressed, diplococci numerous.

Nov. 16. On the morning one died, body was softened, leaf fragments green, diplococci numerous.

Nov. 21. On the morning one died, body shrunk, diplococci present in large number.

Nov. 22. On the morning one died, the fore-part shrunk, and back part blackened. Leaf fragments brown, numerous diplococci present.

No. 7.

Nov. 13. On the morning one died, body shrunk, leaf fragments brown and compressed. There were found a few micrococci.

Nov. 14. On the morning one died, body softened, leaf fragments brown, many large bacilli and few diplococci present.

Nov. 15. On the morning two died, one with softened body and few green leaf fragments, streptococci numerous. In the other leaf fragments were brown and compressed; numerous micrococci and few streptococci present.

Nov. 16. Three died, bodies expanded in the middle part. In the first leaf fragments green, large bacilli present. In the second the leaf fragments brown, streptococci and large bacilli numerous. In the third the leaf fragments brown, diplococci numerous.

Nov. 17. On the morning one died, body softened and leaf fragments brown and compressed, diplococci numerous.

Nov. 20. On the morning two died; one with softened and faintly purple-colored bodies, leaf fragments brown and compressed, bacilli numerous. In the other body softened, the back part blackened, leaf fragments brown. Some *saccharomyces* and large bacilli were found.

No. 8.

Nov. 15. On the morning one died, body expanded in the middle part, leaf fragments green, streptococci numerous.

Nov. 16. On the morning three died. In one the middle part of the faintly yellow colored body expanded, leaf fragments brown with many diplococci. In the second body softened, leaf fragments green and compressed, short bacilli numerous. In the third body softened, leaf fragments green, numerous streptococci present.

Nov. 20. On the morning two died, with softened bodies and brown leaf fragments; diplococci numerous; in one also bacilli present.

Nov. 21. On the morning two died, one with contracted fore-part and a few diplococci; the other with the fore-part elongated and many diplococci.

Nov. 22. On the morning two died during the spinning of cocoon; bodies softened, the back part black, leaf fragments green. In one bacilli were numerous but diplococci few; in the other bacilli and diplococci were equally numerous.

By preparing an agar-plate from the intestinal juice of the dead larva only white colonies of micrococci were formed.

No. 9.

Nov. On the morning three died, two with the body shrunk, and one with the body elongated. One with many bacilli and few diplococci, and brown leaf fragments; the second with numerous diplococci, and green compressed leaf fragments; the third with brown leaf fragments, numerous diplococci and some large bacilli.

Nov. 15. On the morning one died, body black, leaf fragments brown, diplococci numerous but streptococci few. On the evening one died, body softened, leaf fragments green, diplococci numerous.

Nov. 16. On the morning four died, bodies, softened. With three of them small black spots appeared on the body; intestinal contents were

green. In one of these diplococci, streptococci and some pebrine-organisms were observed. With the second large bacilli and diplococci. With the third streptococci. With the fourth green compressed leaf fragments, and streptococci numerous, large bacilli few.

Nov. 20. On the morning one died, body softened with small black spots all over. Leaf fragments brown and compressed, diplococci numerous.

By preparing an agar-plate from the intestinal juice of the dead larva, yellow colonies of diplococci were exclusively formed.

Micrococcus from the eggs.

Nov. 14. On the morning two died, in one the body stretched, the second and third segments yellow, and leaf fragments brown and compressed, few diplococci were observed. In the other the body was shrunk and hard, leaf fragments also brown and compressed; sarcina was found exclusively. On the evening two died, leaf fragments brown and compressed, diplococci present.

Nov. 15. On the morning five died, bodies softened with brown leaf fragments and numerous diplococci in every case.

Nov. 16. One died, body softened, leaf fragments brown, few diplococci.

By preparing an agar-plate from the intestinal juice of the dead larva, yellow colonies of sarcina were exclusively formed.

From the results of these experiments the following conclusions were drawn.

1. The micrococci on the mulberry-leaves can cause flacherie, No. 1 being the least able to multiply in the intestines of the silk-worm.

2. Flacherie can be caused by this micrococcus, what can be proved by the fact that by preparing a plate-culture from the diseased larvæ the colonies of the inoculated micrococcus were formed in greater number or exclusively.

3. Since flacherie is caused by injecting water into the intestines, it is clear that the bacteria that can cause flacherie exist always in the intestinal canal, which fact proves also that the mulberry-leaves are the carriers of the germs.

4. The micrococcus in the eggs can cause flacherie.
5. The decrease of appetite of the silk-worm by the injection of water or bacteria can be observed also from the diminished weight of the cocoons formed.

Average weight of a cocoon.

Control.....	0.487 gr.
Water injected	0.411
<i>Micrococcus</i> No. 1 inoculated	0.390

6. Any constant relation between the bacteria inoculated and the symptoms of the malady was not observed in these experiments.

7. When mulberry-leaves in the intestines are green, the reaction of the juice is alkaline, though weaker than in the healthy animal, while the brown color indicates that the reaction is neutral or faintly alkaline.

8. *Bac. megatherium* seems to multiply usually after the micrococci had developed luxuriantly.

9. At low temperature the bacteria do not bring on flacherie very soon.¹ The cause will probably be due to the slow growth of the bacteria at the low temperature.

General Conclusions.

From the results of the series of the experiments above described the following conclusions were drawn.

I. There is no doubt that flacherie is caused by the growth of bacteria in the intestinal juice.

II. The bacteria usually found in large number in the intestinal juice of the diseased larvæ are various kinds of micrococci and two kinds of bacilli. In most cases micrococci only or together with few large bacilli are found. A short bacillus is also usually found along with the micrococci; however cases in which the short bacillus alone is found are very rare. Besides these microbes there are many other kinds which exist in a small number in the diseased animals.

¹ Compare Experiments VII and XII.

III. The large bacillus was identified with *Bacillus megatherium*, De Bary, and the short one with the coli-bacillus.

IV. There exists in the interior of the eggs of the silk-worm usually a micrococcus and a large bacillus. The former was identified with *Sarcina lutca*, Flügge and the latter with *Bacillus megatherium*, De Bary.

V. Various kinds of micrococci usually adhere to the mulberry-leaves. The writer isolated from mulberry-leaves 10 species of micrococci. Nine of them were used for the experiments, by which it was decided that flacherie is caused by these micrococci and the sarcina isolated from the eggs. The micrococci isolated from the dead larvæ were identified with those isolated from mulberry-leaves.

VI. It is clear that the sources of the bacteria, which multiply in the intestines of the silk-worm and cause flacherie, are the mulberry-leaves serving as food. But when the larvæ are healthy they resist the action of the bacteria. However when silk-worms are reared at high temperature or any disorders are produced in the digestive organs, the microbes multiply and cause the malady. The greater number of micrococci in the intestinal juice is due to their abundance also on mulberry-leaves.

VII. Flacherie is, as above explained, not caused by any special bacteria, hence Macchiatis' and Krassiltschik's assumption can not be confirmed. My observations agree with those of the Austrian Experiment Station that flacherie is not infectious.

VIII. The true cause of the disease is the increase of certain products formed by the undue and rapid multiplication of various microbes. These products are in all probability no toxins, but they may consist of ammonia formed by protein decomposition, or of nitrite formed from nitrate contained in the leaves, or of acids produced from carbohydrates. Very probably these noxious substances are sometimes acting together. I hope to settle this question satisfactorily by further investigations.

The author must express here his sincere thanks to *Prof. Sasaki* who kindly translated Italian articles for him, further to *Prof. Loew* and *Prof. Kozai*, and to *Mr. Honda* and *Mr. Hayashi*, Experts of Tokio Sericultural Institute who furnished him the larvæ and eggs of the silk-worms, and finally to *Mr. Yamasaki*, Assistant of the College.



Zur Physiologie des *Bacillus pyocyaneus*, II.

VOX

O. Loew und Y. Kozai.

In Fortsetzung unserer früherer Versuche,¹ eine möglichst günstige Nährstofflösung für den *Bac. pyocyaneus* zu finden, in welcher trotz lebhafter Vegetation keine Schleimbildung aber reichliche Enzyymbildung statthabe, fanden wir folgende Lösung diesen Bedingungen entsprechend:

Pepton.	0.5 %
Glycerin.	0.1 „
Magnesiumsulfat.	0.01 „
Dikaliumphosphat.	0.1 „
Natriumbicarbonat.	0.1 „
Chlornatrium.	0.4 „

Das Magnesiumsulfat wurde sterilisirt bei der Infection zugesetzt. Wir vaviirten in dieser Lösung die einzelnen Bestandteile mehrfach und jedesmal war das Resultat entweder eine langsamere Vegetation oder eine Verzögerung der Wiederauflösung der Massen.² In dieser Lösung läuft die Vegetation in 18–20 Tagen bei 25–28°C. ab, wenn die Kolben *nur zur Hälfte* voll sind was behufs reichlicher Enzymproduction nötig ist und jeden Tag kräftig ungeschüttelt wird, wobei unter Sauerstoffabsorption die gelbe Lösung tief grün wird. Erst vom 13. Tage ab hört die Reduction des grünen Farbstoffs auf. Die anfänglich reichlichen Massen lösen sich bis auf einen geringen Bodensatz allmählig wieder auf.

¹ Siche diese Bulletins, Bd. 4, No. 4 und No. 5.

² Wir erhöhten z. B. das Pepton auf 1%, das Dikaliumphosphat auf 0.4%, wir eliminirten das Natriumbicarbonat und setzten endlich die Chlornatriummenge auf 0.2 und 0.1% herab; auch versuchten wir dieses durch Natriumsulfat zu ersetzen.

Diese zuerst von *Enimerich* und *Lowe* beobachtete Wiederauflösung wurde von *Conradi* als eine Autolyse aufgefasst, was aber wohl nicht dem wirklichen Vorgange entspricht; denn Autolyse¹ ist die Gesamtheit der in einem Organ (oder Organismus) nach dem Tode stattfindenden fermentativen Vorgänge, es wird also als charakteristisch angesehen, dass *irgend eine vorherige Secernirung von Enzym nicht stattfindet*. Andernfalls ist der Vorgang eben lediglich eine gewöhnliche Verdauung; denn es ist doch z. B. ganz und gar irrelevant, ob ein Magensecret den Magen verdaut, der es abgesondert hat, oder einen anderen Magen. Bei der Wiederauflösung der gewachsenen Bacterienmassen durch die secernirte Pyocyranase muss erst eine gewisse Anhäufung der letzteren in der Culturflüssigkeit erreicht worden sein. Dann erst kann der Angriff auf die Nucleoproteide² der Bacterienleiber Erfolg haben und wird dann auch das weitere Wachstum eingeschränkt und endlich ganz verhindert.³ Wenn aber die Bacillen auf festem Nährboden (Glycerin-Agar) cultivirt werden, so bleibt jedenfalls das Enzym in den Zellen, wenigstens grossenteils. Dafür spricht die Beobachtung von *Krause*,⁴ dass der Presssaft des *B. pyocyranus* milzbrandheilend wirkt.⁵ Die Bacillen waren auf Agarplatten cultivirt und die Vegetation nach 48 Stunden mit Platinspatel abgenommen worden. Nebenbei bemerkt muss dieser Presssaft kaum Toxin und auch nur wenig Pyocyranolysin enthalten haben; denn 3 cc. waren nach *Krause* einem Kaninchen nicht schädlich.

Bei dem Interesse, welches sich an die Pyocyranase knüpft, suchten

¹ *Theobald Smith* hat bereits i. J. 1894 die verdauende Wirkung in sterilen Geweben von Thieren beobachtet; später haben *Sidlowsky*, *Jacobi*, *Magnus-Lery*, diese Erscheinung weiter verfolgt. Besonders interessant sind die Resultate *Conradi's*.

² *Kischkow* (*Hilfensteiner Beiträge* I, 530) hat *Pyocyranus*-Zellen mit verdünnten Natron extrahirt, mit Essigsäure die Lösung gefällt und nach dem Reinigen das Nucleoprotein analysirt. Er fand darin: C 52,73%; H 6,91%; N 16,50%; P 2,11%; S 1,0%. In den Membranen fand er C 46,2%; H 6,7%; N 8,8. Dieser Stickstoffgehalt deutet auf eine chitinartige Substanz, was auch von *Ersmann* für die Membranen des *Bac. fluorescens liquefaciens* vermutet wird (*Ber. Chem. Ges.*, 35, 702).

³ Nach *Siegwart* (*C. Bakt.*, 30, 573) werden die Nucleoproteide der Bacterien auch von Pepsin verdaut, aber erst nachdem die Bacillen gekeimt sind, was jedenfalls auffallend ist.

⁴ *Centrbl. f. Bakt.*, 37, No. 14.

⁵ Typhus konnte beim Meerschweinchen damit nicht geheilt werden.

wir nach einer Methode, welche bei grosser Einfachheit doch ein reineres Product liefert, als bisher möglich war. Unser Ziel ist noch nicht erreicht worden, doch mögen immerhin einige Beobachtungen der Mittheilung wert sein. Vor einigen Jahren hat *F. Neuenberg*¹ über erfolgreiche Behandlung der Staphylomykosis mit der Pyocyranase (Rohfermentlösung) berichtet. Derselbe stellte, wie *K. Vaerst*² in seinen erfolgreichen Versuchen der Mitzbrandbehandlung mit Pyocyranase, dieselbe in etwas verschiedener Weise dar, wie *Emmerich* und *Loew*, nämlich durch Aussalzen nach Erhitzen auf 58° (6 Stunden). Auf 1 L. der sechswöchentlichen Bouilloncultur wurden 500 g. Ammonsulfat gegeben,³ nach 24 Stunden das Ausgeschiedene einer mehrtägigen Dialyse überlassen und dann die Lösung im Vacuum zur Trockne gebracht. Die alkalische Lösung wurde also nicht erst neutralisirt, und in der That haben uns vergleichende Versuche gezeigt, dass dieses vorzuziehen ist. Beim Versetzen mit Essigsäure⁴ wird Kohlensäure frei, welche beim nachfolgenden Aussalzen in Blasen festgehalten wird, so dass eine sehr schaumige Masse erhalten wird, welche schwer weiter zu behandeln ist. *Neuenberg* sowohl wie *Vaerst* verwendeten Bouillonculturen. Diese liefern aber eine sehr schleimige Flüssigkeit,⁵ welche beim Aussalzen auch den Schleim ausscheidet, der nun einen grösseren oder geringeren Theil der Pyocyranase mit sich reisst. Wird nun diese Ausscheidung der Dialyse unterworfen, um das Ammonsulfat zu entfernen, so bemerkt man eine auffallende Abnahme des Schleims, so dass man zur Vermutung kommt, es habe ein mit ausgeschiedenes Enzym (wegen nun grösserer Concentration) den Schleim durch Hydrolyse in nicht schleimige Producte verwandelt. Wenn die dialysirte Lösung dann im Vacuum eingedampft wird, so wirkt kein Schleim mehr störend, beim Lösen, resp. Injiciren des Products. Wir haben aus 1 Liter Bouilloncultur

¹ Habilitationsschrift, Bern 1900.

² Centrbl. f. Bakt., 31, No. 7.

³ Eine mässige Vermehrung des Salzes bringt nur noch eine geringe Mehrausscheidung zu Wege.

⁴ Es ist nahe zu 1 promille Essigsäure behufs Neutralisation nötig.

Diese Schleimbildung beruht vielleicht auf der Gegenwart milchsaurer Salze. Auch essigsäure Salze und Asparagin liefern schleimige Culturen, Pepton aber nicht.

nur 1,5 g. des Rohferments erhalten. Prof. Nitta beobachtete, nach Darreichung von 0,1 g. desselben *per os*, bei einem Meerschweinchen keine Spur eine Temperaturerhöhung oder irgend welchen andern Effect, es waren also keine Substanzen vorhanden, die *per os* hätten schädlich wirken können, was von einigem Interesse sein mag, falls einmal dieses Rohferment zur Bekämpfung von Bacillen (Cholera) im Darm zur Verwendung kommen sollte.

Wir haben nun die Aussalzmethode auch bei Culturen angewandt, welche *nicht schleimig* werden, speciell bei der eingangs erwähnten Culturelösung. Zehn Liter der 18 tägigen Cultur wurden zunächst mit Chloroform versetzt und einen Tag stehen gelassen, um etwa noch vorhandene lebende Zellen abzutöten. Am folgenden Tage zeigte die Flüssigkeit einen intensiven *Geruch nach Isonitrit*, es musste also ein primäres Amin in der Cultur gebildet worden sein. Die klare Loesung wurde abgegossen, der letzte Theil filtrirt und in die Gesamtmenge der alkalisch reagirenden Flüssigkeit (10 L.) sechs Kilo Ammonsulfat eingetragen und unter häufigen Umrühren bei 6—8° stehen gelassen. Es schied sich nach einiger Zeit eine flockige Masse an der Oberfläche ab, welche abgenommen und durch Filtration und Pressen von der anhängenden Ammonsulfatloesung so gut wie möglich getrennt wurde. Durch dreitägige Dialyse wurde der Rest des Ammonsulfats entfernt. Schon beim Anrühren mit Wasser wurde bemerkt, dass sich ein grosser Teil nicht wieder löste, trotzdem wurde die Gesamtmasse in den Dialysirschlauch¹ gegeben. Der unlösliche Theil war von einer melaninartigen Substanz schwarz gefärbt, und enthielt neben blaugrünen Pyocyaneusfarbstoff noch einen geringen Anteil höherer Fettsäuren, und etwas Proteinsubstanz. Das Filtrat wurde zunächst auf verdauende Wirkung geprüft, aber nur eine äusserst schwache Wirkung auf gequollenes Blutfibrin beobachtet, selbst als noch 0,2% Soda zugesetzt wurde. Daraus durfte wohl der Schluss gezogen werden, dass die Pyocyanaase keine Albumosenatur besitzt,² sonst wäre sie mit ausgesalzen worden. Bei den Versuchen von *Neuenberg* und von *Faerst* musste wohl die volumi-

¹ Zu antiseptischem Zwecke wurde auch etwas Chloroform zugesetzt.

² Wahrscheinlich ähnelt sie den Peptonen.

nöse Schleimmasse, die ausgesalzen wurde, viel Enzym mit niedergerissen haben.

Es ist desshalb wohl der Schluss gerechtfertigt, dass bei nicht schleimigen Culturen die Abdampfmethode (im Vacuum) der Aussalzmethode vorzuziehen ist, da sie sicher die Gesamtmenge des Enzyms liefert.





Über den Kalkgehalt der Milchdrüse.

VON

M. Toyonaga.

Ich habe in meiner früheren Arbeit über den Kalkgehalt der grauen und weissen Hirnsubstanz darauf hingewiesen, dass die Drüsen im Verhältnis zur Magnesia viel mehr Kalk enthalten als andere Gewebe des Tierkörpers, was jedenfalls mit der grösseren Zellkernmasse zusammenhängt. Es war in dieser Beziehung natürlich von Interesse diese Untersuchungen fortzusetzen, insbesondere weil in Bezug auf die verschiedenen Organe des Tierkörpers auffallend wenige Aschen-Analysen vorliegen, während in Bezug auf den Pflanzenkörper diese äusserst zahlreich sind.

Ich habe zunächst die Milchdrüse in Betracht gezogen, welche insbesondere deshalb Beachtung verdient, weil ihr Secret in Bezug auf Mineralbestandtheile ganz ausserordentlich von dem Blute differiert; so fand *Bunge*:—

100 Theile Asche enthalten :	Hundemilch,	Hundeblut,
K_2O	10,7	3,1
Na_2O	6,1	45,6
$Ca O$	34,4	0,9
$Mg O$	1,5	0,4
Fe_2O_3	0,14	9,4
$P_2 O_5$	37,5	13,3
Cl	12,4	35,6

Wir erschen hieraus in Bezug auf den Kalkgehalt ganz enorme Unterschiede. In der Hundemilch berechnet sich das Verhältnis

$$MgO : CaO = 1 : 22,93$$

in Hundeblute

$$MgO : CaO = 1 : 2,25$$

Ich beschränkte mich bei der Analyse auf die Bestimmung des Kalks und der Magnesia, da besonders dieses Verhältnis für die verschiedenen Gewebe sehr charakteristisch, und die Asche der Milchdrüse überhaupt noch nicht untersucht ist.

Ich trennte bei der Milchdrüse einer Kuh so gut als möglich das Bindegewebe von der eigentlichen Drüsensubstanz ab und bestimmte zunächst den Wassergehalt, derselbe betrug 66,7%.

Nun wurden 88,641 g Trockensubstanz mit 5 g wasserfreiem Natriumcarbonat gemischt und verascht wobei das Weissbrennen wie gewöhnlich sehr lange dauerte. Die Masse wurde zunächst mit Wasser extrahiert und nach Entfernung des kohlensauren- und phosphorsauren Natrons der ausgewaschene Rückstand mit Salzsäure gelöst, wobei eine Minimalmenge Kieselsäure ungelöst blieb hierauf die Lösung mit Ammoniak bis zu alkalischer Reaktion versetzt und dann mit Essigsäure bis zu schwach saurer Reaktion vermischt.

Hierbei bleibt ein geringer flockiger Niederschlag von phosphorsaurem Eisen ungelöst. Aus dem Filtrat wurde nun der Kalk mit oxalsaurem Ammoniak gefällt und das eingeeengte Filtrat vom Kalkniederschlag zur Magnesiabestimmung verwendet.

Es wurde erhalten:

CaCO_3	= 0,3995 g	= 0,2231 g CaO
$\text{Mg}_2\text{P}_2\text{O}_7$	= 0,1562 g	= 0,0566 g MgO
Hieraus berechnet sich		100 Teile der Trockensubstanz:
für 1000 Teile frischer Drüse:		
CaO	= 0,8401 Teile	0,2517 Teile
MgO	= 0,2131 „	0,0630 „

Vergleichen wir das sich hieraus ergebende Verhältnis $\frac{\text{Ca}}{\text{Mg}}$ mit den für Milz und Niere von Aloy¹ gefundenen Zahlen und mit den Zahlen für das Muskelfleisch von Säugetieren, so ergibt sich:

	Milchdrüse,	Milz,	Pankreas,	Niere,	Säugetier-Muskel,
$\frac{\text{Ca}}{\text{Mg}}$	4,67	6,79	4,05	1,84	0,34

Es ist somit auch bei der Milchdrüse wie bei der anderen Drüsen der

¹ Jahresbericht L. Tierchemie 30, S. 392.

Calciumgehalt grösser als der Magnesiumgehalt, während für das Muskelgewebe der Warmblüter umgekehrt der Magnesiumgehalt grösser ist als der Calciumgehalt.

Vergleichen wir noch die Mengen von Ca und Mg im Muskel mit denen in Milchdrüse und Milz, so hat man für die organische Trockensubstanz:

	Säugethier <i>Muskel</i> (Katz).	<i>Milchdrüse.</i>	<i>Milz</i> (Rilaut)
Ca	— 0.033%	0.173%	0.141%
Mg	— 0.109 „	0.038 „	0.056 „

Es ergibt sich somit, dass nicht nur der Calciumgehalt absolut grösser ist in der Drüse wie im Muskel, sondern auch dass der Magnesiumgehalt dort weit geringer ist als hier. Ich werde meine Untersuchungen fortsetzen.



Der Erntequotient.

VON

Oscar Loew.

In allen vollständigen Ernteberichten aus der Praxis sowohl, wie den Versuchs-Stationen, wird ausser dem wesentlichen Erntebestandteil, wie Knollen, Wurzeln, Früchten auch noch die Menge des Krautes oder Strohes angegeben. Aus diesen Zahlen ersieht man aber nicht sofort, ob sich das Verhältniss zwischen diesen Bestandteilen dem Mittel oder einem Optimum nähert. Behufs einer sofortigen Beurteilung dieses Verhältnisses möchte ich den Begriff des *Erntequotienten* einzuführen vorschlagen. Er gestattet sofort zu ersehen, ob unter den gegebenen Bedingungen (Boden, Düngung, Wetter, etc.) ein mittleres oder optimales Verhältniss erzielt wurde. Er gibt die Hauptleistung der Blätter, der wichtigsten Producenten organischer Materie, in vergleichbaren Zahlen an, er zeigt, ob diese Organe ihre Aufgabe voll und ganz erfüllt haben. Dieser Erntequotient

$$q = \frac{k}{s} \cdot 100$$

drückt die Ernte des wesentlichsten Bestandteils k = Körner, Knollen, Wurzeln, in Procenten der Blattsubstanz, des Stroh's, s , aus. Man kann für die Zwecke der Praxis die Gewichte des lufttrocknen Krautes oder Strohs zu Grunde legen, während für rein wissenschaftliche Zwecke das Gewicht der absoluten Trockensubstanz zu dienen hätte. Es wäre von einigem Vorteil, den absoluten Erntewerten pro ha auch den Erntequotienten, der sich bei normalen Pflanzen oft zwischen genau bestimmten Grenzen bewegt, beizufügen. So beträgt derselbe im Mittel bei Gerste 73, während er im Optimum, wie es wohl in der Praxis nicht erreicht wird, 100 betragen kann.¹ Bei Bohnen liegt er in der Regel weit über 100, bei Erbsen häufig über 200.

¹ *Hellriegel* teilt mit, dass er unter sehr günstigen Bedingungen im Glashaus bei Gerste gleiche Gewichte Stroh und Körner geerntet habe. Andererseits beschreibt *E. Wolff* Feldversuche, welche auf 100 Stroh nur 60 Thl. Körner gaben und bei Weizen gar nur 40.

Ferner würde er sich im Mittel aus zahlreichen Daten ergeben für

Weizen	53
Hafer	66
Mais	80
Senf	52
Buchweizen	54

Wohl haben schon verschiedene Forscher den Körnerertrag hier und da auf 100 Theile Stroh bezogen, aber es ist systematisch weder der mittlere noch der optimale „*Erntequotient*“ bestimmt worden. Besonders war es *P. Wagner*, welcher seine Resultate mit Cerealien in dieser Form ausdrückte. So fand er z. B. dass bei verschieden starker Stickstoffdüngung auf 100 Thl. Stroh resultiren können bei Hafer 51-87 Thl. Körner, bei Roggen 46-53, bei Weizen 33-63. Ferner hat er bei Hafer auf 100 Thl. Stroh 52 Thl. Körner erhalten, als er Chilesalpeter bei der Einsaat gab, aber 64 Thl. Körner, wenn er diesen bei beginnendem Schossen zufügte.¹

Gewisse Verhältnisse führen zu einem Uebermass von Blattproduction, andere wieder ermöglichen den Blättern, die von ihnen bereiteten organischen Nährstoffe in ausgiebigster Weise der Ausbildung der Früchte zukommen zu lassen. Diese Arbeit mit einer Zahl auszudrücken, beabsichtigt der *Erntequotient*.

¹ Die Stickstoffdüngung der landwirtschaftlichen Culturpflanzen, 1892, S. 164.



Ueber die physiologische Wirkung des Chlorrybidiums auf Phanerogamen.

VON

Oscar Loew.

Versuche mit Buchweizen hatten mir früher gezeigt,¹ dass eine physiologische Vertretung von Kalium durch das ihm so nahe stehende Rubidium nicht möglich ist. Zu diesem Schlusse zwar schon vor mir *Birner* und *Lucanus*² gekommen, allein ich constatirte immerhin einen grossen Unterschied zwischen der Wirkung von Rubidiumnitrat und Rubidiumchlorid. Mit Nitrat ergaben sich pathologische Stärkeanschoppungen, eine Verdickung und Torsion des Stengels, Sistirung des Längenwachstums, Einrollen und Fleischigwerden der Blätter und schliesslich erfolgte der Tod, bevor eine Blüte entwickelt war. Wurde gleichzeitig ein Chlorid (Salmiak) zugesetzt oder Rubidium nicht als Nitrat, sondern als Chlorid verwendet, so streckten sich die Pflanzen und gelangten nach Erreichung einer weit bedeutenderen Höhe bis zur Blütenbildung was deutlich für den Einfluss von Chloriden auf den Stärketransport spricht. Erst nach der Blütenbildung traten Hemmungserscheinungen ein, es fand eine Anhäufung von Zucker und Veränderungen des Chlorophylls statt und die Pflanzen verfielen einem langsamen Siechtum, ohne einen Samen producirt zu haben. Weiter gelangten Pflanzen, denen Kalium und Rubidium zugleich gegeben wurde, indem die Hälfte des in der Controlloesung verwendeten Chlorkaliums durch Chlorrybidium ersetzt war. Indessen auch hier wurde die Höhe der Controlpflanzen nicht erreicht und kein reifer Same gebildet, die Pflanzen starben nach der Blütenperiode ab. Trotz der pathologischen Wirkungen ergab sich immerhin

¹ Landw. Vers. Stat. 21, S. 389.

² Hbbl. 7, S. 263.

für das Rubidium ein physiologischer Nutzen, den das Natrium nicht besass, denn die Pflanzen producirten weit mehr Trockensubstanz, was vielleicht nur auf einer Unterstützung der Wirkung der im Samen gespeicherten Kaliumsalze beruhen mag. Von Interesse ist hier die Beobachtung von *Molisch*,¹ dass Algen in einer Culturloesung sich gar nicht entwickeln, wenn darin statt der Kaliumsalze Rubidiums Salze vorhanden sind. Ist es hier die Zelltheilung oder die Assimilation des Kohlenstoffs, oder die Eiweissbildung oder sind es diese drei wichtigsten Vorgänge zusammen, welche mit Rubidium statt des Kaliums nicht ausgeführt werden können? Diese Frage konnten vielleicht Versuche mit Pilzen entscheiden. Hier beobachtete ich nun, dass Bierhefe und der gemeine Pinselschimmel sich sogar noch besser entwickeln können, wenn bei Zucker als organischer Kohlenstoffquelle Rubidium statt des Kaliums dargeboten wird. Beide Elemente kamen als Tartrate zur Verwendung. Werden jedoch weniger gute Kohlenstoffquellen, wie Natriumacetat, verwendet, so stösst man auf einen bedeutenden Unterschied zu Gunsten des Kaliums. Da nun verschiedene Pilze eine verschiedene Wachstumsgeschwindigkeit besitzen, somit wahrscheinlich der Eiweissbildungsprocess mit ungleicher Fertigkeit ausgeführt werden dürfte, liess sich vermuten, dass die Verwendbarkeit des Rubidiums bei diesem Process auch nicht stets mit derselben Leichtigkeit vor sich gieng. In der That hat Günther² beobachtet, dass während der Pilz *Botrytis cinerea* Rubidiums Salze physiologisch verwerten kann, dieses bei *Rhizopus nigricans* nicht der Fall ist. Ich beobachtete eine Vertretbarkeit bei *Bacterium coli* und, wenn auch in weit geringerem Grade, bei *B. pyocyaneus*, während bei *Cladothrix odorifera* selbst bei Zucker als Nährstoff eine Vertretung sich als unmöglich erwies.

Rubidiums Salze zeigen somit in physiologischer Beziehung ein eigenartiges Verhalten. Die oben erwähnten pathologischen Effecte beim Buchweizen einerseits, die günstigen Effecte bei Hefe und Schimmel andererseits veranlassten mich, die Versuche mit Phanerogamen in modificirter Form wieder aufzunehmen. Ich versuchte die Wirkung kleiner Dosen Rubidiumchlorids bei Pflanzen unter normalen Ernährungsbedingungen.

¹ Wien, Akad. Ber. 1896.

² Inauguraldissertation, Erlangen 1897.

Versuch mit Brassica chinensis.

Drei Töpfe mit je 1 Kg. Boden wurden gedüngt mit: 1 g. Kaliumnitrat, 0.5 g. Ammonsulfat, und 0.5 g. Monokaliumphosphat. Ausserdem erhielt Topf a, 10 Milligramm Rubidiumchlorid.

„ b, 50 „ „
 „ c. diente zur Controlle.

Der Chlorgehalt des Bodens entsprach nahe zu 0.05 g. Na Cl per Kilo, er war ein lehmiger Boden, zum Teil aus vulkanischer Asche bestehend.

Am 21. October wurden 10 Samen pro Topf ausgesät und am 5. November die jungen Pflanzen auf je drei möglichst gleich grosse, 6-7 cm. hohe, reducirt. Gegen Mitte November ergab sich, mit Ausnahme einer Pflanze in b. für die Rubidiumpflanzen ein besseres Wachstum als für die Controlpflanzen, ein Unterschied, der mit der weiteren Entwicklung immer bedeutender wurde. Am 17. December ergaben die Messungen für das längste Blatt jeder Pflanze Folgendes:—

a	b.	c. Controlpflanzen.
21.0 cm.	14.0	16.5
22.4 „	19.1	17.0
28.2 „	25.5	21.1

Am 22. Dec. wurden die Pflanzen ausgezogen, die Wurzeln gereinigt und mit Fliesspapier gut abgetrocknet, und die ganzen Pflanzen gewogen im frischen Zustande, mit folgendem Ergebniss:—

a	b.	c.
14.3 g.	6.1	10.1
16.7 „	14.0	10.2
18.8 „	25.2	15.0
Mittel: 16.6	15.1	11.8

Es war somit ein stimulirender Effect des Rubidiumchlorids zweifellos, doch war dieser bei Erhöhung von 10 Milligramm auf 50 pro Kg. Boden nicht vermehrt worden, die Pflanzenmasse war im Gegenteile in letzterem Falle etwas kleiner als im ersteren.

Versuch mit Gerste.

Zwei Töpfe mit je 1 Kg. lufttrocknem Boden erhielten als Grunddüngung je 1.5 g. Ammoniumsulfat, 0.5 g. Monokaliumphosphat, 1.5 g. Calciumsuperphosphat, 1.0 g. Kaliumcarbonat¹ und 0.5 g. Natriumnitrat. Einer erhielt ausserdem noch 0.2 g. Rubidiumchlorid, der andere die äquivalente Menge Natriumchlorid. In jeden Topf wurden am 14 October 10 vorher gequollene Samen ausgesät und die Entwicklung im Glashause wie beim vorigen Versuch beobachtet. Am 21. October wurden die Pflanzen auf 4 pro Topf reducirt, so dass alle von möglichst der gleichen Höhe waren. Gegen Ende November zeigte sich ein deutlicher Höhen-Unterschied zu Gunsten der Rubidiumpflanzen, der stets zunahm. Die Messung am 17. December ergab:—

Rb- Pflanzen.	Control- Pflanzen.
44.6 cm.	39.1 cm.
46.5 „	46.0 „
46.8 „	47.0 „
54.3 „	49.5 „

Die Höhen-Unterschiede nahmen zu, wie die am 19. Januar angenommene photographie (Tafel XXV) gut erkennen lässt. Dabei waren die Rubidiumpflanzen vollständig normal.² Wegen Auftretens von Pilzen wurden die Pflanzen schon bald nach der Blütenperiode geschnitten. Das Gewicht betrug:

¹ Das Kaliumcarbonat wurde später separat dem Boden einverleibt

² Ob Buchweizen und andere Pflanzen hierbei ebenfalls normal bleiben, soll noch geprüft werden.

Ueber die physiologische Wirkung des Chlorrubidiums auf Phanerogamen. 465

	Rubidiumpflanzen,	Controlpflanzen,
Aehren, Frischgewicht;	6.1 g.	3.7 g.
Lebende Blätter, frisch;	81.3 "	53.3 "
Abgestorbene Blätter, lufttrocken;	5.2 "	4.8 "

Ein dritter Versuch wurde mit *Spinacca oleracea* angestellt. Alle Verhältnisse waren hier die gleichen wie oben bei *Brassica*. Bei den Rubidiumpflanzen erhielt der Boden 50 Milligramm Rubidiumchlorid per Kilo. Als der Samen reif war, wurde geschnitten und die besten Exemplare frisch gewogen.

	Rubidiumpflanzen,	Controlpflanzen,
Gewicht der grössten Pflanze, Varietät I	18.2 g.	12.0 g.
Die zwei grössten Pflanzen der Varietät II ...	16.5 g.	13.2 g.

Es hatte somit in allen diesen Fällen ein stimulirender Effect des Rubidiumchlorids stattgefunden, was wohl von beträchtlichem theoretischen Interesse ist. Für die Zwecke der Praxis jedoch ist eine Anwendung des Salzes ausgeschlossen, da dessen Preis ein zu hoher ist.¹

¹ Es kosten 100 g. Rb Cl = 12 Mark (ca. 6 Yen).





On the Stimulating Action of Manganese upon Rice.

BY

M. Nagaoka.

In our last Bulletin the observation was communicated that small doses of manganese administered as sulphate had a very favorable action on the development of various plants. This made it very desirable to carry on a field experiment with rice which is the most important agricultural plant in Japan.

Thirty six wooden frames each representing an area of 0.826 square Meter were placed, three feet apart, into the paddy field of our College farm to a depth of 60 cm., leaving 6 cm. above the ground. The soil had not received any manure the previous three years' and was now manured¹ in the ratio

of 100 Kg. N per ha, as ammonium sulphate
" " " K₂O " " as potassium carbonate
" " " P₂O₅ " " as double superphosphate.

The potassium carbonate was separately applied (June 23) and the other two salts four days later.

On June 29 manganese was applied as manganosulphate in such quantities that the amount of manganic oxid corresponded to the following proportions, three series being observed in each case:—

¹ Before manuring the soil was sifted, and all remnants of former vegetation removed.

No of wooden frames,			Mn ₂ O ₃ per ha, Kg.	Mn ₂ O ₃ per frame, Gram.
1	13	25	0	0
2	14	26	0	0
3	15	27	10	0.833
4	16	28	15	1.250
5	17	29	20	1.666
6	18	30	25	2.083
7	19	31	30	2.499
8	20	32	35	2.916
9	21	33	40	3.332
10	22	34	45	3.749
11	23	35	50	4.165
12	24	36	55	4.582

On July 7 the young rice plants (55 days old) from the seedbed, were transplanted into the frames, each receiving 16 bundles of twelve healthy individuals of equal size.¹ The treatment (irrigation, etc.) did not differ, from that usually observed with the rice fields in Japan. The weather conditions were not favorable this year for this crop in the whole Empire of Japan, but the relatively low summer temperature diminished on the other hand the dangers from fungi and insect pests with this crop. Our frames remained free from such pests. The crop was harvested on November 29 with the following result, obtained by weighing in the air day condition.

¹ The variety was the *Satsunagi*, characterized by its resistance power and medium duration of vegetation.

On the Stimulating Action of Manganese upon Rice.

No. of frames,	Mn ₂ O ₃ per ha, kg.	Full grains, gr.	Empty grains, gr.	Straw, gr.	AVERAGE.			
					Full grains,	Empty grains,	Straw,	Total,
1	no manure	151.6	3.2	193.0				
13	and no Mn ₂ O ₃	142.6	3.0	171.0	150.3	3.0	185.0	338.3
25		156.5	2.7	191.0				
2	no Mn ₂ O ₃	177.0	4.6	242.0				
14		227.8	6.3	312.8	202.5	5.4	260.6	477.5
26		202.6	5.2	254.0				
3		250.6	6.8	319.6				
15	10	239.6	5.8	285.6	247.3	7.0	308.7	564.0
27		251.8	8.4	321.0				
4		249.9	6.3	401.6				
16	15	257.6	4.8	307.5	256.7	4.4	329.1	500.2
28		262.6	5.1	278.0				
5		277.5	5.3	354.6				
17	20	269.4	7.8	341.6	264.3	6.3	327.7	508.3
29		245.6	5.9	287.0				
6		279.0	10.3	330.6				
18	25	264.5	5.1	335.0	272.1	6.8	348.5	627.4
30		272.7	5.2	326.0				
7		270.0	5.8	374.7				
19	30	256.5	4.4	316.0	267.7	4.2	340.0	612.8
31		276.8	5.3	332.0				
8		264.6	8.2	325.8				
20	35	267.8	4.4	334.0	267.3	6.0	322.0	506.2
32		269.4	5.5	309.0				

No. of frames.	Mn ₂ O ₃ per ha. kg.	Full grains. gr.	Empty grains. gr.	Straw, gr.	AVERAGE.			
					Full grains.	Empty grains.	Straw.	Total.
9		261.8	8.0	364.6				
21	40	269.3	5.6	368.0	272.3	6.0	338.5	617.7
33		285.9	7.0	343.0				
10		256.5	9.0	340.6				
22	45	256.5	7.1	338.0	271.9	7.1	334.9	613.9
34		272.8	5.3	326.0				
11		? (198.6)	(9.0)	312.5				
23	50	270.6	6.7	399.0	278.1	6.6	359.5	645.2
35		287.6	6.5	367.0				
12		254.5	6.7	331.4				
24	55	279.4	5.2	364.1	272.6	4.1	345.2	621.9
36		283.9	6.4	340.0				

The application of manganese had therefore a considerable influence upon the yield, which will be noticed more conveniently by the following table, in which we take the yield in grains of the manured plot without manganese as a unit:

Mn ₃ O ₄ , Kg. per ha.	Harvest of full grains. (Average)
none.....	1.00
15	1.26
20	1.30
25	1.34
30	1.32
35	1.32
40	1.34
45	1.34
50	1.37
55	1.34

It will be noticed from these figures, that a moderate dose of 25 Kilo Mn_2O_3 per ha led to an increase of the harvest of one third and that higher doses of Mn_2O_3 did not influence essentially this result under the given conditions.

It is further of some interest to examine whether the average ratio between the weight of grain and straw is affected to any extent by the influence of manganese. The quotient of yield¹ $\frac{K. 100}{S}$ which expresses the percentage of grain relatively to straw is for the different cases:—

MANURED PLOTS.	
Amount of Mn_2O_3 per ha.	Quotient of Yield.
No manganese	75
10 Kg.	80
15 "	78
20 "	82
25 "	78
30 "	78
35 "	82
40 "	80
45 "	81
50 "	77
55 "	79

Average 79.5

The application of manganese had therefore—*ceteris paribus*—a favorable influence on the quotient of yield.

Let us now determine by calculation whether the application of mangano-sulfate would be profitable for the farmer. The price of 100 Kilo of pure crystallized manganosulphate is according to the latest pricelist of Theodor Schuchardt = 110 Mark or 53 yen.²

The average production *per ha.*, of grains of rice with husk is = 3525 Kilo and of airdry straw = 5250 Kilo.

¹ On the *Quotient of Yield* (Erntequotient) see the article of O. Löwe in this Bulletin.

² 1 Mark = 38.75 sen, latest quotation.

The wholesale price of crude rice grains is 9.9 sen per Kg., of airdry straw = 1.2 sen, per Kg. hence the average yield per ha. has a value of 349 yen in grains and 63 yen in straw = 412 yen.

An increase of one third would have an additional value = 137.33 yen while the cost of the mangano-sulphate required would be = 30 yen. Hence the application of this salt on soils poor in manganese would be of advantage. The impure manganous chlorid of commerce would fulfill the same purpose and would cost less than 10 yen in the above case.

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On the Physiological Action of Iodine and Fluorine Compounds on Agricultural Plants.

BY

S. Suzuki and K. Aso.

A. On the Influence of Potassium Iodid on Oats.

By S. Suzuki.

I have demonstrated in a former article¹, that potassium iodid in exceedingly high dilution can exert a stimulant action on plant growth. The pea had served for that experiment. I had, however, at the same time commenced an experiment with oats, the result of which are described in the following lines.

Soil and manure were exactly the same as in the former case: each pot contained 2300g. air dry soil and was manured with 3g. Na NO₃, 3g. K₂CO₃ and 4.6g. common superphosphate. The seeds were sown (15 in each pot) on Feb. 21 and the young shoots reduced on March 7, to five per pot of equal height. Pot No. I. received on March 11 and 25, April 14, 21 and 28 and May 6, each time 0.01g. potassium iodid dissolved in 100 c.c. water; further pot No. II. 0.001g. and No. III. 0.0001g. of that salt, while No. IV. served as control. Those quantities of potassium iodid expressed in percentage of soil are:

No. I.	=	0.00 2609	%
No. II.	=	0.000 2609	%
No. III.	=	0.00002609	%

In the beginning of May, the tips of the leaves of No. I. turned reddish yellow and further growth was retarded, but an increase of shoots made up for the loss in height, leading finally to an increase in the yield compared with the control plants (compare the photograph, Plate XXVI). The plants were irrigated almost daily with 300 c.c water until the flowering stage was reached, after that with 500 c.c. The flowering period was over on May 16. The plants were cut on July 6. The straw and the grains, unhusked, were weighed in the air dry state with the following result :

	I.	II.	III.	IV.
Number of Stalks,	15	14	14	9
Weight of grains, un- husked, g.	24.8	25.5	27.2	21.4
Weight of straw, g.	48.5	56.6	58.4	45.2

The result undoubtedly proves a stimulant action of iodine, even if present in such a small quantity as 2. 6g KI in 10000 Kilogram of soil as in No. III. The increase however, is with oats not so large as with the pea (These Bulletins, vol. V p. 199).

I had mentioned already in my former article, the experiment of *A. Væleker* who soaked seeds of wheat and barley for a short time in a 1% solution of sodium iodid and observed with such seeds, an increase of yield. The quantity of sodium iodid that penetrated into those seeds must then have been exceedingly minute, otherwise a poisonous effect would have shown itself. I have repeated that experiment with oats. The seeds were soaked for 24 hours in a 1% potassium iodid solution, washed and then sown in two pots, 15 seeds in each. Later on the young shoots were reduced to five of equal height. After a few weeks, it became clear that the plants did not so well develop as the control plants. This may be due to more iodid having entered into the grains than in the case described by *Væleker*. This difference is probably caused by the prolonged soaking in my case. Also differences of temperature during the soaking process can influence the result. The plants were cut on July 13, and the straw and grains, unhusked, weighed in the air dry state with the following result :

	I.	II.	Control.
Weight of grains, unhusked,	18.1 g.	16.8 g.	21.4 g.
Weight of straw,	24.4 „	22.5 „	45.2 „

This shows that the amount of KI absorbed in the soaking was large enough as to cause a retarding influence, which was much greater, however, in regard to the production of straw than in that of grains.

A field experiment further was made with oats. On 3 plots, each measuring 20 square meters, an equal amount of oats grains previously soaked for two days in water was sown on March 21. On April 15, the young plants had reached 3—4 cm. and were treated now the first time with potassium iodid solution¹. The treatment was repeated on Apr. 22, May 7 and 22, and June 10. The total quantity of potassium iodid applied to the plot No. I. was 0.25g., to the plot No. II. 0.025g. On June 26, flowering commenced, on July 16, some spots of rust became visible. On August 6, the plants were cut, but owing to several storms, some loss of grains had occurred; hence the final weight is somewhat below the actual production. The straw and grains, unhusked, were weighed in the air dry state with the following result:

	I.	II.	Control.
Weight of straw and grains,	6.96 Kg.	6.08 Kg.	6.05 Kg.
Weight of grains.	0.99 „	0.78 „	0.83 „

A small increase of yield had therefore taken place by the application of 0.25 g. KI for 20 □ Meters, while 0.025g had no influence.

¹ The solution was highly diluted, each dose of potassium iodid being dissolved in 10 litres of water.

*B. On the influence of potassium iodid on radish**By S. Suzuki.*

The same plots¹ which had served for the culture of oats just mentioned served for this experiment with radish. One plot received 0.5 g. potassium iodid in one dose that is double the quantity of that of the last experiment with oats, the next plot received 0.05 g. potassium iodid in one dose (also double of the last experiment). The radish seeds were sown Oct. 1 and the young plants were thinned out on Nov. 4. After four weeks a considerable difference in favor of the iodine plants was noticed. On each plot (20 square meters) were grown 60 plants, which were harvested on Dec. 24. The results are as follows:—

	0.5 KJ.	0.05 KJ.	Control.	
Large plants, Average periphery of roots = 9.5 c.m.	Number.	19	23	10
	Weight.	5440 g.	7500 g.	2770 g.
	Weight of roots.	2370 „	3360 „	1440 „
Middle size plants, Average periphery of roots = 7.5 c.m.	Number.	24	20	15
	Weight.	4020 g.	4220 g.	3020 g.
	Weight of roots.	1370 „	1540 „	1010 „
Small plants, Periphery of roots 4.7 c.m. and less.	Number.	17	17	35
	Weight.	1960 g.	1980 g.	3110 g.
	Weight of roots.	520 „	510 „	790 „
Total weight of plants.	11420 g.	13700 g.	8900 g.	
„ „ „ root.	4260 g.	5410 g.	3240 g.	

¹ Each plot was manured with 200 g. double superphosphate, 312.5 g. $(\text{NH}_4)_2\text{S}^0_4$, and 312.5 g. wood ash, the latter being given in a highly diluted state ten days later.

This result shows a very favorable influence of potassium iodid in small quantities on the yield with radish. A calculation as to the outlay and profit is of some interest.

KI applied for 20 square meter	= 0.05 g.
Corresponding for 1 ha	= 25 g.
its value	= 0.6 yen
The increase in harvest per 20 sq.m.	= 2170 g. root,
Corresponding per ha	= 1085000 g. „
	= 289 Kwamme.
its value	= 2.89 yen

Hence it would certainly be profitable to apply small doses of potassium iodid to the field; the costs would be however very trifling, if we would substitute the crude ash of seaweeds for the purified potassium iodid¹. It might be here also called attention to the interesting fact that the farmers along the coast of Japan apply sea weeds as a green manure with very much success, which very probably is not only due to the small quantities of potassa, nitrogen and phosphoric acid, but also to some extent to the small doses of iodine present. Finally I might point out that it might not be advisable to make an application of iodine compounds every year on the same field, since the iodine might gradually be increased to a point where the stimulating action ceases and a noxious action commences. An application on only every second or third year might therefore be preferable.

C. On the influence of sodium fluorid on oats.

By K. Aso.

In a former article was shown that fluorine in the form of sodium fluorid applied in exceedingly high dilution on barley, wheat, rice, soy-bean

¹ Since this ash contains about 5 per mille iodine, 5 Kilo of it would suffice to supply the necessary quantity per ha.

² Bul. College of Agriculture, Tokyo, Vol. 5, No. 2, p. 181.

and pea plants, can exert a stimulant action.² In the following lines another experiment with oats will be described. All the conditions in regard to soil, manuring, time of sowing, kind of seed, the number of shoots, watering and harvesting were exactly the same as in the above described pot-experiment made with potassium iodid *by S. Suzuki*; hence the reader, is referred in this regard to the introductory remarks of the above communication.

Pot No. I received on five days 0.01g. sodium fluorid in 100.c.c. water, No. II. 0.001g and No. III. 0.0001g, while No. IV served as control. The applications of the highly diluted solutions of sodium fluorid were made on March 11, April 14, 21 and 28, and May 6. On May 20, it was noticed that the plants of No. II. developed best, then followed those of No. I. There was hardly noticed any difference between the plants of No. III, and No. IV. The color of the leaves of No. I. was a little paler than that of the control plants. On May 29, the number of ears was :

No. I.	3
No. II.	4
No. III.	4
No. IV. (control).	2

The plants were cut on July 6. The straw and grains, unhusked, were weighed in the air dry state with the following result :

	I.	II.	III.	IV.
Number of stalks.	8	9	10	9
Weight of grains, unhusked, g.,	23.0	24.2	25.5	21.4
Weight of straw, g.	50.1	45.6	48.6	45.2

This result undoubtedly shows a stimulant action of fluorine in the proportion of 2.17g. in 10000 kilo soil as in No III, although the differences are here not so large as in the case of the pea, described in a former Bulletin.

² Although the presence of fluorine has to be assumed almost in every soil, it is of special interest that it occurs naturally in wines from certain countries (Holzman).

¹ Cf. Bul. V, No. 2.

D. On the influence of sodium fluorid on radish.

By K. Aso.

Two plots, each measuring 10 square metre, had received during the summer, 0.6g and 0.06g NaF., and again shortly before sowing the seeds of radish, they received 0.8g. and 0.08g. sodium fluorid respectively. The manure was the same as mentioned in the above field experiment with potassium iodid. The radish was sown on October 1, and the young plants thinned out on Nov. 4. Towards middle of December a difference in development between these plants and control plants was very plain. The plants were harvested on Dec. 24 with the following results:—

	a. (0.14 g. NaF)	b. (1.4 g. NaF)	control.
Total weight	7970g.	6490g.	3814g.
Weight of the ten largest roots.	2050g.	1470g.	617

A stimulating action of considerable magnitude is therefore quite evident and it is of special interest that the smaller quantity, 0.14g. sodium fluorid has produced a better result than the ten times larger quantity. The cost of production of the increased amount of radish is to be seen from the following calculation:

NaF applied for 10 square metres	= 0.14 g.
„ corresponding to 1 ha.	= 140 g.
Its cost	= 8.4 sen.
Increase of harvest per 10 sq.m.	= 4.156 Kg.
„ corresponding to 1 ha.	= 4156 Kg.
Its value	= 110.6 yen.



On the Chemical Nature of the Oxidases.

BY

K. Aso.

The oxidases are considered generally as kinds of enzymes and indeed various of their properties are in favor of this view. Also the observation of *Slotzoff*¹ and of *Epstein*² seem to fully establish the enzym-nature of the oxidases. Recently, however, *J. H. Kastle* and *A. S. Loewenhardt*³ described experiments which seem to indicate a certain analogy between the behavior of oxidases and that of organic peroxids towards certain antiseptics and poisons. These authors conclude therefore: "the oxidizing ferments are peroxids, formed when autoxidizable substances come in contact with air and these peroxids give up a part of their oxygen to other less-oxidizable substances present in the cell." "In other words, that the process of rendering oxygen active by the living cell, is probably brought about in essentially the same way that this is accomplished by phosphorus, benzaldehyd and other oxygen carriers, viz, as one phase of autoxidation." Further, these authors hold that "the function of hydrogen peroxid in the guaiacum hydrogen peroxid reaction, is to react with some one or more of the organic substances present in the plant or animal extract to form an organic peroxid."

¹ Z. Physiol. Chem. 31. *Slotzoff* observed that the action of laccase is proportional to the square-root of its quantity. He considers the laccase as a peculiar protein substance which is not changed by pepsin or pancreatin. In the pure state, it is killed already at 50°C, in presence of mineral substance however between 65-70°C.

² Arch. Hyg. 36. p. 140. *Epstein* observed that the presence of hydrocyanic acid in small quantities prevents the action of oxidase and that after the removal of hydrocyanic acid, the activity of oxidase is restored.

³ Americ. Chem. Journ. XXXVI. No. 6. Dec. 1901.

These authors based their view principally on the following special observations:

1. Benzoyl-phthalyl- and succinyl peroxids give directly guaiacum blue with tincture of guaiac. Hydrogen peroxid alone gives a faint blue color and that only on heating with guaiacum tincture. The authors mentioned further, that lead dioxid and manganese dioxid give the blue color. But from these facts certainly does not follow that every substance which can produce a blue color with guaiacum must be of the nature of a peroxid. Indeed lead dioxid and manganese dioxid are no genuine peroxids like barium peroxid is and further we find that not only every weak oxidizing agency (nitrous acid, ferric chlorid, potassium ferricyanid), but also oxid of silver and further quinone may produce the blue guaiacum reaction at once.¹ Some other observations of these authors are however of considerable interest, namely the bluing of the guaiacum tincture by benzoylperoxid can be prevented by hydroxylamine, and phenylhydrazine. In the same way, also the oxidizing action of the potato juice on guaiaconic acid can be inhibited. However, it might be objected that hydroxylamine and phenylhydrazine might merely destroy the guaiacum blue, while they do not counteract the oxidizing activity itself. Indeed, I have already observed some time ago that the blue color produced with guaiacum tincture by the action of oxidase disappears, when a little free hydroxylamine is added. Also phenylhydrazine (0.5%) decolorized the freshly formed guaiacum blue. The inhibitive effect of hydrocyanic acid on the action of oxidase was observed again by *Kastle* and *Loevenhart*² after *Epstein* and also myself had made the same observations.

A further observation of those authors relates to the inhibiting action of sodium thiosulfate. When to 2c.c. of a potato extract 0.5c.c. of $\frac{N}{100}$ solution of the thiosulfate was added, the blue color with guaiacum was prevented. Since other enzymes are not injured by sodium thiosulfate, it was inferred that the oxidase is no enzym proper. But here it might be objected

¹ Quinone produces a blue color also with the tetra paper of Wurster. The common quinone is probably a diketone and not a peroxid as formerly believed (Fittig).

² They observed that "9 parts prussic acid in 10 million parts of juice very nearly mark the limit of the poisonous effect of that acid on the oxidizing substances in the potato.

that an oxidizing enzyme must naturally contain differently constituted active atomic groups than the other merely hydrolyzing enzymes, devoid of any oxidizing power;¹ further it might be objected that the oxidase caused the oxidation of thiosulfate sooner than that of guaiaconic acid.

I had observed a year since that the guaiacum blue may easily be decolorized by certain compounds present in some plant juices as, e.g., in that of radish and it requires often a certain excess of guaiacum tincture to preserve the blue guaiacum reaction for some time. Also tannin not only prevents the usual guaiac reaction of peroxidase, but in certain quantities can also bleach out again the blue color after it has made its appearance.² Such facts might also apply to the observation of *Kastle* and *Loewenhardt* that the onion bulb gives no guaiac blue reaction. I can confirm this statement, but if we precipitate the enzymes of the onion with alcohol first and thus remove compounds that interfere (allylsulfid?), the guaiacum reactions for oxidase and peroxidase, can be obtained with the aqueous solutions of these enzymes, although these reactions set in here more slowly than in other cases. I might further add that the onion juice shows an unusually strong acid reaction and that after neutralization also the guaiacum blue reaction can be slowly produced with the juice itself.

Recently *Bach* and *Chodat* observed that the juice of *Lathyrus squamaria* yielded on addition of some diluted baryta water, a precipitate which after treating with dilute sulphuric acid produced at once an intense blue color on paper impregnated with starch paste containing potassium iodid.³ Their conclusion is, "Die sofortige Jodausscheidung aus Jodkalium konnte daher nur von einem acylirten Hydroperoxid herrühren."

¹ A similar observation was made by the writer in regard to the behavior of enzymes toward sodium fluorid; while most enzymes are not injured by it, the oxidase proper (laccase) is killed easily.

² Compare my article, "On the Rôle of Oxidase in the preparation of Commercial Tea; Bul. College of Agric. Tokyo, Vol. IV, No. 4, p. 256.

³ Schönbein had observed as early as 1864 (*Journ. für prakt. Chem.*, Bd. 55, 3, 460) that certain aqueous extracts of plants give a blue reaction with acidulated iodid of potassium starch, which reaction he supposed to be due to nitrous acid. Many plant juices however yielded that reaction only after standing for a series of days. In the latter case, nitrite might have been produced from the nitrates, frequently present in plants, by bacterial action.

But we must take into consideration that iodine can be very easily liberated from potassium iodid by the most different oxidizing influences, in presence of an acid reaction. These authors also observed that some plant juices will lose the property of liberating iodine within a few minutes. If this is so, we have already a clear proof before us that this oxidizing principle is not identic with the oxidase characterized by the guaiacum blue reaction, since this can still be easily observed in a plantjuice after a few days, although the reaction will then be weaker. Also the further interesting observation of these authors that in the wilting of a plant the iodine reaction disappears first, militates against the identity of this oxidizing principle with the common oxidase (Iaccase).

I have made a series of tests with the juices of potato tubers and the root of radish, which yield the guaiacum reactions for oxidase and peroxidase very well. But, with these juices, I could not observe the iodine reaction. As I supposed that these juices might contain some substance which interfered with the formation of iodine starch, or absorb the iodine immediately after being liberated I have treated those juices with an excess of absolute alcohol and after washing the precipitates, containing the oxidizing enzyms, with alcohol they were dissolved again in some water. These solutions also yielded the guaiacum reactions upon oxidase and peroxidase very well, but not a trace of the iodine reaction. I applied for one volume of this solution $\frac{1}{3} - \frac{1}{2}$ volume of a 2% starch paste to which 1% potassium iodid and 0.5 % acetic acid was added. These mixtures yielded even after twenty four hours standing in darkness, no trace of any blue reaction, while the guaiacum blue reaction even in absence of hydrogen peroxid was still obtained with great intensity.¹

Bach and *Chodat* recommend to add some mangano-sulfate in those cases in which the iodine reaction with plant juices fails. But in the above mentioned cases with the juices of potato and radish, this sulfate did not

¹ In one case I had applied intentionally a potassium iodid solution not freshly prepared, but one which had been exposed in presence of air for a few days to sunlight. In this case, a blue reaction was gradually observed, evidently due to slight traces of free iodine formed in this solution.

change the result. However after such mixtures were left for a series of hours to themselves a weak reaction set in. But also in some control tests without plant juices, I observed that mangano-sulfate alone in presence of some acetic acid can gradually cause the liberation of some iodine.

In order to decide whether the oxidizing enzymes are really organic peroxids, I have made the following experiments relating to the special oxidizing enzym, which produces a red color with a 1% guaiacol solution of weak acid reaction. The juice of the leaves of radish contains besides oxidase and common peroxidase, also a peculiar oxidizing enzym which produces the red reaction just mentioned.¹ This juice was mixed with $\frac{1}{10}$ of its volume of a hydrogen peroxid of about 2% and of a faint acid reaction. After five minutes standing, about four times the bulk of absolute alcohol was added and the precipitate very well washed with alcohol. This precipitate was then dissolved in some water and tested with guaiacol. but *no reaction whatever was taking place*. If *Kastle and Loewenhardt's* view was correct, then the supposed organic peroxid would be formed almost instantaneously when hydrogen peroxid comes in contact with the proper organic material in the juice. This supposed organic peroxid would consequently be also present in the alcoholic precipitate containing all the oxidizing enzymes, hence the aqueous solution of this precipitate ought to give now without the further aid of hydrogen peroxid, the red guaiacol reaction, but the fact was: *no reaction in absence*, but an intense reaction in presence of hydrogen peroxid. What is true for this kind of peroxidase (β -guaiacolase) is very probably also true for the common peroxidase characterized by the blue coloration with guaiacum tincture and hydrogen-peroxid,² but thus far I was not able to prove it in the way just mentioned.

¹ Since *Bouquetot* observed in the fungus *Russula*, an oxidizing enzym which produces a red color with guaiacol even in absence of hydrogen peroxid, I propose to distinguish this peculiar enzym as α -guaiacolase, from the above-mentioned enzym which I call β -guaiacolase. About this reaction compare also my article, 'On Oxidizing Enzymes in the Vegetable Body' *Bull. College of Agric. Tokyo*, Vol. V, No. 2, p. 207—235.

² On heating the solution of the enzym precipitate above mentioned for 5 minutes to 75°, the oxidase and the common peroxidase are killed, while the guaiacol-hydrogenperoxid reaction was still obtained although weaker.

since I encountered some difficulty in the preparation of a peroxidase precipitate sufficiently pure. It cannot be denied that a transient formation of an organic peroxid takes place when the oxidase causes the oxidation of a certain other compound. *Such peroxids are then the first products of an oxidation caused by the oxidizing enzym and this opinion seems to be also that of Bach and Chodat, and differs essentially from the hypothesis of Kastle and Loewenhart, according to which oxidases themselves are the peroxids.*

It must be remembered, moreover, that the liberation of iodine from potassium iodid not only may be due to different oxidizing influences but also that on the other hand, it is not a specific property of all organic peroxids. Thus neither diethylperoxid nor dibenzoylperoxids will liberate iodine, but *benzoylhydroperoxid can do so*. But it is a very striking fact that this peroxid can also liberate iodine from potassium iodid in the presence of sodium bicarbonate and not only in presence of free acid. Such *hydroperoxids* as can liberate iodine are exceedingly powerful compounds,¹ resembling hypochlorites in their actions;² hence the amount of such poisons the cells can only be exceedingly minute.

Since I have now proved that the iodine reaction does not go parallel to the blue guaiac reaction and since further there exists no proof that organic peroxids are the cause of the iodine reaction in many vegetable objects, it was important to decide the nature of the iodine liberating substance. Two suppositions seemed to deserve some consideration, either there might exist certain organic ferric compounds in some objects or traces of nitrites. The following lines will doubtless prove of some interest in regard to this question.

¹ Recently, *R. H. Page* (Amer. Pat. 717016 of 30. Dec. 1902) described acetylhydroperoxid $\text{CH}_3-\overset{\text{O}}{\text{C}}-\text{O}-\text{C}-\text{H}$ which has a strong odor after hypochlorous acid and has a powerful bactericida action.

² Compare in regard to the data here mentioned, the articles of *Bayer* and *Tilliger* in *Berichte der Deutschen Chemischen Gesellschaft*, 1899 and 1900, especially in the latter volume, page 1578.

Experiments with Buds.

Sections of potato-buds yielded directly the iodine reaction in presence of some acetic acid.¹ Also the blue guaiac reaction was directly produced. The cold prepared extract behaved alike. In a second case, with buds from other potatoes, however, the iodine reaction failed, although oxidase, peroxidase and β -guaiacolase² were present. Of considerable interest were the observations made with the tubers and buds of *Sagittaria sagittifolia*.

The cold prepared aqueous extract of the bulb gave no iodine reaction, but it gave the blue guaiac reaction, while the extract of the buds yielded directly both reactions. Eight buds of *Sagittaria* were extracted with 100c.c. water; a portion was tested directly and another after boiling for $\frac{1}{2}$ minute. In the former case, oxidase, peroxidase and β -guaiacolase were easily recognized by their reactions, while in the latter case, no trace of this reaction was more obtained. But very different were the phenomena in regard to the iodine reaction. *Not only the unboiled, but also the boiled juice yielded this reaction with great intensity after addition of some acetic acid.*³ Even boiling for 2 minutes did not alter this result.⁴ It is therefore undeniable that hereby another proof is furnished that the substance which gives the guaiac reaction for oxidase is not identic with the substance that give the iodine reaction. It was of interest to me to decide whether in the *bulbs* a compound would be present that can prevent the iodine reaction which so easily and intensely is obtained in the *buds*. Hence four bulbs were crushed after removing the skins and macerated with 100c.c. water. The filtrate was mixed with alcohol, whereby a considerable precipitate

¹ The tubers did not give this reaction, as was mentioned above.

² See above p. 485.

³ A blind control test with acetic acid and potassium iodid-starch paste showed no reaction whatever.

⁴ *Bach* and *Chodat* mention that heating to 80°C prevents the iodine reaction. This is, however, probably only the case when the acidity of the juice is more marked than in the case of the *Sagittaria* buds. It can then be very easily explained that nitrons acid set free reacts upon amido-compounds and is destroyed with development of nitrogen.

was obtained. This was filtered off, the residue washed and after well pressing between filter paper and evaporation of the alcohol at common temperature, extracted with water and tested again. The iodine reaction failed however while the guaiac reaction was obtained. Further tests convinced me that among other substances soluble albumin as well as pepton can prevent the appearance of the iodine reaction which very easily can be understood, since these compounds can bind some iodine, thus rendering formation of iodine starch impossible. Since, the juice of the bulb contained some soluble albumin, it was not surprising to find that the juice of the *bulb* was capable to prevent the iodine reaction with the juice of the *bud*, and further that the *boiled* juice of the bulb did not prevent any more that iodine reaction with the juice of the bud.

It was further tested whether the juice of the bulb itself would yield the iodine reaction after removing the soluble albumin. But after a few seconds boiling whereby the albumen separated in flocculi, no iodine reaction was obtained in the filtrate, although short boiling does not destroy the active compound as I have mentioned above.

The resistance of the active principle towards boiling heat suggested to make a careful test for nitrites and indeed to my great surprise the reaction of *Griess* for nitrites yielded at once a very decisive result. Hence the liberation of iodine is due not to any enzym nor to any peroxid, but to nitrites.

It is very strange that the occurrence of nitrite in plants thus far was overlooked. It is true that *Schönbein* more than thirty years ago had supposed the existence of nitrite in plant juices and further that *Berthelot* had assumed the formation of nitrate in leaves and shoots from ammonia. But some authors did not agree with these observations. The occurrence of nitrite in plants is indeed surprising, since we know that nitrites are very poisonous for plants with an acid plant juice.¹ But in this regard we must not overlook that the quantity of nitrite present in these shoots is only very small, and that nitrous acid can here not exist in the free state since the acidity of these shoots is exceedingly weak.

¹ O. Loew, *Natürliches System der Giftwirkungen*, p. 61 and p. 109.

Since the question is of some interest whether this nitrite is formed by reduction of nitrate or by oxidation of ammonium salts, I have tested the bulbs with diphenylamine, but no reaction was obtained. The boiled juice of the buds was also poured carefully on the surface of diphenylamine solution in concentrated sulphuric acid, and here *soon observed a blue ring*, probably due to the small quantity of nitrites present.¹ A strong reaction for nitrites could not be expected in this manner, since we know that nitrites are in presence of some strong acids and amido-compounds very quickly destroyed with evolution of nitrogen. We can therefore infer that nitrous acid in the buds in analogy to nitrification process is formed by oxidation of ammonia.

Summary.

It is very improbable that the oxidase and peroxidase of plant juices are organic peroxids. The liberation of iodine by plant juices was proved in one case to be due to traces of nitrite and it is probable that these are present sometimes also in other plant juices. The iodine and the guaiac reactions do not show any parallelism.

¹ The reaction of Griess sometimes may be prevented by the presence of certain benzene compounds, like tannins.



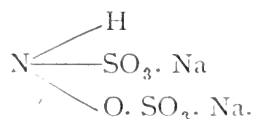
Can Sulfo-derivatives of Hydroxylamine Serve as a Source of Nitrogen for Plants ?

BY

S. Suzuki.

It is well known that hydroxylamine as well as diamidogen are not only incapable of furnishing nitrogen to phænogams but they are directly poisonous.¹ But thus far no tests have been made with the sulfoderivatives of these compounds. It seemed to me of some interest to test one of such derivatives in this line. I selected the sodiumsalt of α - β hydroxylamine-disulfonic acid which preparation was kindly furnished me by Prof. T. Haga.²

This compound has the formula.



I prepared the following solutions :—

- | | | |
|----|-------------|--|
| a) | 1 per mille | Calcium acetate. |
| | 1 „ „ | Magnesium sulfate (anhyd.) |
| | 1 „ „ | Potassium chlorid. |
| | 1 „ „ | Ferrous sulfate (anhyd.) |
| b) | 2 per mille | Dipotassiumphosphate. |
| | 1 „ „ | Sodium α - β . disulfhydroxylamate. |

and for the control plants :

- c) same as a).

¹ Loew, Ein natürliches System der Giftwirkungen. 1893, p. 41.

² This interesting salt was prepared by Prof. Haga from oximido-sulphonate of sodium by a complicated process which will be published later by that author.

- d) 2 per mille Dipotassium phosphate.
 0.28 „ Ammonium sulfate.¹

The application of two different solutions instead of a single one containing all mineral nutrients was necessary for the following reason. The above named derivative contains two sulpho-groups which on being set free by a decomposition would probably form an acid salt which in itself would be injurious. Hence I applied the secondary potassium phosphate in place of the usually applied monopotassium phosphate. But since that phosphate would precipitate the lime and magnesia of the nourishing solution, it had to be applied separately together with the above named derivative. Barley shoots (about 13 c.m. high) were placed on Nov. 21. in the solutions a) and c), on the following day in the solutions b) and d). This manipulation was repeated in this way for nearly 7 weeks. Two series with two shoots in each flask were observed. On Dec. 15 the plants were transferred to larger flasks and all solutions renewed. At this time it was evident, that the control plants showed a much better development. From then to Jan. 10th, a decided starvation was noticeable; all the old leaves died off, while young leaves developed but remained of very small size. The experiment was closed on Jan. 10th, since there was no further growth observed and only very few leaves had remained green, while in the control case the plants looked vigorous and healthy. It seems further that the plants can not regenerate hydroxylamine to any noticeable degree from the salt, since a very poisonous action would have soon become noticeable. The state of affairs at the close of experiment is seen in the following table:—

	Number of stalks,	Number of leaves, living	Number of leaves, dead	Length of the longest leaves,	Weight of the fresh plants,
Solution of a and b series.					
A	1	1	3	6	13 c.m.
	2	1	3	6	11 „
					2.68 g.
B	1	1	2	6	12 „
	2	1	2	5	11 „
					2.00 „

¹ This amount of ammonium sulfate corresponds to that of the sodium salt of α β . hydroxylamine disulfonic acid, in the solution (b), in regard to the amount of nitrogen.

Control plants.

C	{	1	5	12	5	17.5	c.m.	}	7.02 g.
		2	4	7	4	17.5	..		
D	{	1	8	13	4	17.5	..	}	8.42 ..
		2	4	9	4	16.5	..		

Experiment with fungi.

A culture solution was prepared, consisting of

- 200 c.c. Water.
- 2 g. Canesugar.
- 0.4 .. Hydroxylamine disulfonate of sodium.
- 0.2 .. KH_2PO_4 .
- 0.01 .. Mg SO_4 .

Two flasks, a and b, each containing 100 c.c. of this solution were after sterilisation infected with:

- a) *Penicillium glaucum*.
- b) *Bac. methylicus*.

An infection in bouillon from the same source served as control. After 14 days there was no trace of development noticed in the main flasks, while the control flasks showed luxuriant growth.

My general conclusions are therefore:—

1. The α . β . hydroxylamine disulphonic acid is no direct poison for the barley but it is incapable to furnish nitrogen and hence the plants undergo starvation when nitrogen is supplied in this form.¹
2. Development of fungi is impossible, when in the culture solutions the nitrogen is offered in the form of α - β -hydroxylamine disulfonic acid.

¹ It is of some interest to compare with this result the poisonous character of amido-sulfonic acid, these Bulletins, vol. II, page 487 (1897).



On the Influence of a Certain Ratio Between Lime and Magnesia on the Growth of the Mulberry-tree.

BY

K. Aso.

Since sericulture is one of the most important agricultural industries in Japan, much attention is paid to the cultivation of the mulberry-tree, and various investigations have been published in relation to it. A short review of some of these may be not out of place.

The composition of the ashes of healthy mulberry-leaves was found in average, as follows:¹

$P_2 O_5$	12.02%
$K_2 O$	31.47%
$Na_2 O$	3.14%
$Ca O$	33.75%
$Mg O$	12.48%
$S O_3$	4.64%
Cl	0.06%
$Si O_2$	1.45%
$Fe_2 O_3$	1.59%

On analysis of the bark of the healthy mulberry-roots (var. *Nezumigae-shi*) collected on Dec. 4., I have obtained the following result:

In 100 parts of the crude ash,

$P_2 O_5$	18.48
$K_2 O$	9.39
$Ca O$	35.50
$Mg O$	7.34

¹ Nagaoka: Chemical Tables for Daily Use, p. 84.

S O ₃	1.38
Si O ₂	2.40
Fe ₂ O ₃	8.73

U. Suzuki¹ has determined the lime and magnesia content of healthy and dwarf-diseased leaves without however observing great differences; the diseased leaves contained, like the healthy, from 2 to 4 times as much lime as magnesia, although in most cases, the ratio between these was somewhat greater in healthy than in diseased leaves.

Maeno² observed in mulberry-leaves after liming the soil, a moderate decrease of the percentage of woody fibre and increase of the non-nitrogenous extract; further by applying lime, sodium nitrate and calcium sulphate, not only some increase of the non-nitrogenous extract, but also of the protein and fat.

Since the so-called dwarf-disease (Schrumpf-Krankheit) causes an immense damage to the mulberry plantations in Japan, it seemed to me of interest to look also into the composition of such soils as seemed especially favorable for the development of the disease, that is, causing such a condition of the plant as would render it more susceptible for that disease. I restricted myself to the determination of those quantities of lime and magnesia which are available to the root, and for this purpose I have treated the soil with cold hydrochloric acid (10%) for 48 hours. Our experiments with other plants had sufficiently shown that the ratio between lime and magnesia in the soil has a most powerful influence on the development. My analyses, indeed, have shown that the amount of magnesia predominated over that of lime, which is a very unfavorable condition.

In 100 parts of dry soil,

LOCALITY.	Ca O	Mg O
Okakaramura, (Aichiken)	0.232	0.332
Jōtan Sericultural School, (Kyōtofu).....	0.115	0.259
Angamura (Kyōtofu)	0.150	0.388

¹ Bul. College of Agriculture, Vol. IV, No. 3.

² Ibid. Vol. II, No. 7, p. 495.

The following experiments will prove, indeed, that a normal and good development of this plant depends to a great extent on the ratio of lime and magnesia offered to the roots.

Experiment with Water Culture.

Three young mulberry plants (var. Takasuke) with stems about 15 cm. high were placed June 9, in glass vessels of 3 litres capacity containing the following solutions:—

	I.	II.	III.
Ca (NO ₃) ₂	0.5%	0.3%	0.1%
Mg (NO ₃) ₂	0.1%	0.3%	0.5%
KHP ₂ O ₄	0.1%	0.1%	0.1%
(NH ₄) ₂ SO ₄	0.1%	0.1%	0.1%
FeSO ₄	trace	trace	trace

On July 11, there was not yet any other difference observed except in the number of rootlets that had developed. There were very numerous rootlets in I and II, while none in III.

On July 25, it could be clearly noticed that in solution III, the leaves developed were very small and of pale green color. On August 8, these small leaves had withered while those developed in the other two solutions appeared healthy and of dark green color, but it was further noticeable that the leaves in I were darker green and smaller than those in solution II. Further, while no rootlet were developed in solution III, numerous rootlets had appeared in I and II. This experiment shows that an excess of magnesia over lime is very injurious for the mulberry tree.

Experiment with Soil Culture.

Each pot contained about 3.8 K. dry soil of an unmanured field from Nishigahara, Tokyo. The content, in the fine earth, of lime and magnesia soluble in hot concentrated hydrochloric acid was as follows:—

In 100 parts of dry soil,

Ca O	1.5
Mg O	1.8

As the development of mulberry-roots is very vigorous, it could be assumed that these amounts of lime and magnesia might be assimilable for this tree. I altered the ratio between lime and magnesia in this soil by mixing calcium carbonate or magnesium carbonate with it to reach the following ratios:—

Pots	Ca O	:	Mg O
a	1	:	3
b	1	:	2
c	1	:	1 (original)
d	2	:	1
e	3	:	1
f	4	:	1

The surface of the pots measured 0.0495□ m. As general manure served:

7.5 g. N in the form of ammonium sulfate and
5.6 g. P₂ O₅ in the form of potassium phosphate for each pot.

These salts were applied in solution. Young mulberry plants (var. Shihōzaki) of equal size, weighing 976.7 g.—1014.3 g. and of stem-length of about 30 cm. were planted on April 21.

On June 6, and Sept. 19, the following observation was made:—

Date.	I.	II.	III.	IV.	V.	VI.
June 6.	Leaves very small,	Leaves small,	Control,	Developed best,		
Sept. 19.	One plant died. With another the leaves are very small,	Leaves developed to some extent,		„	Developed very well nearly, same as IV.	Less well developed than V.

On Oct. 1. A photograph was taken (plate XXVII) which exhibits the great difference of development at once. On Oct. 2 the following observations were made :—

No. of pot.	$\frac{\text{Ca O}}{\text{Mg O}}$	Number of leaves.	Fresh weight of total leaves, g.	Average weight of one leaf.	Number of branches.	Remarks.
I.	0.33	8	1.5	0.19	3	Branches were very small.
II.	0.5	16	6.8	0.41	4	
III.	1	21	9.8	0.45	7	
IV.	2	30	31.9	1.05	7	One branch was longest of all.
V.	3	38	36.4	0.98	8	Average development of branches was here better than in IV.
VI.	4	20	13.5	0.68	5	

Taking now in consideration, that the plant with the lime factor 2 had the longest branch all that other branches were smaller than with the lime factor 3, which latter had also one branch more, it will be safest to conclude that the best ratio Ca O : Mg O for the mulberry tree lies between 2 and 3. It follows further that an excess of magnesia over lime depresses the growth considerably; the leaves become smaller, but true symptoms of dwarf-disease are not observed.

—•••—



On the Influence of Different Ratios between Lime and Magnesia upon the Development of Phaseolus.

BY

G. Daikuhara.

The knowledge of the physiological functions of lime and magnesia is not only of theoretical but also of practical value, as shown by the recent publications of *Loew*, *May*, *Aso* and *Furuta*. *O. Loew* has named the ratio of $\frac{\text{Ca O}}{\text{Mg O}}$ most favorable for plant development the lime factor, taking the absolute quantity of the available magnesia as the unit. Thus it was found that the lime factor for buckwheat is 3, for cabbage 2, for oats 1.

I have sought to determine this limefactor for Phaseolus and also to observe whether an increase of the absolute quantities of those bases would have any modifying influence upon the result.

Thirty small zinkpots of about two Liters capacity served for this experiment. Each received 2,5 Kilo pure quartzsand, mixed with the carbonates of lime and magnesia in the following quantities and ratios:—

Total quantities of Ca CO ₃ + Mg CO ₃ for the air dry sand :		A. 0.05%	B. 0.1%	C. 0.2%
Limefactor : $\frac{\text{Ca O}}{\text{Mg O}}$	I	$\frac{3}{1}$	$\frac{3}{1}$	$\frac{3}{1}$
	II	$\frac{2}{1}$	$\frac{2}{1}$	$\frac{2}{1}$
	III	$\frac{1}{1}$	$\frac{1}{1}$	$\frac{1}{1}$
	IV	$\frac{0.5}{1}$	$\frac{0.5}{1}$	$\frac{0.5}{1}$
	V	$\frac{0.33}{1}$	$\frac{0.33}{1}$	$\frac{0.33}{1}$

As general manure for each pot served :—

K_2HPO_4	0.1%
KH_2PO_4	0.1%
KNO_3	0.2%
$(NH_4)_2SO_4$	0.1%
$FeSO_4$	0.001%

On Sept. 9th small plants of Phaseolus, grown in sand, and of equal size, were planted into these pots, two in each. After three weeks a considerable difference was noticed. In the three series the development was best, where the ratio $\frac{CaO}{MgO}$ was = 2.

The following table gives the measurements taken at this time :—

	$\frac{CaO}{MgO}$	A.	B.	C.
Length of stem, cm.	3	13.5	11.5	13.5
	2	18.5	17.0	15.5
	1	14.0	14.0	11.5
	0.50	13.0	15.0	10.0
	0.33	13.0	—	—
Length of the largest leaf, cm.	3	8.0	6.0	5.0
	2	9.0	7.3	5.5
	1	7.5	6.0	5.5
	0.50	6.0	6.5	5.0
	0.33	6.5	—	—
Breadth of the largest leaf, cm.	3	3.9	4.0	3.0
	2	4.0	3.8	3.2
	1	4.0	3.0	3.0
	0.50	2.8	3.5	2.5
	0.33	3.5	—	—

This table shows clearly not only an *influence of the ratio of $\frac{\text{Ca O}}{\text{Mg O}}$ upon the height of the plant but also on the size of the leaves.* The best ratio is here 2 : 1, at least before the fruiting stage of this plant. The plants No. V of B and C had died at the time the measurements were made, very probably from the excess of magnesium carbonate.

Unfortunately the experiment had to be terminated soon afterwards on account of fungi making their appearance on the leaves.





On the Behavior of the Phosphoric Acid in the Soils Towards Different Organic Acids.

BY

G. Daikuhara.

Many investigations have been carried out to determine how much phosphoric acid in a soil is available for the plant roots. Nearest to the truth came *B. Dyer* who published an elaborate investigation on "The Analytical Determination of probably available Mineral Plant Food in Soils," and proposed to apply a solution of 1% citric acid to determine whether a soil is in need of phosphatic manure. He suggested that "when a soil is found to contain as little as about 0.01 percent of phosphoric acid soluble in a 1 percent solution of citric acid, it would be justifiable to assume that it stand in immediate need of phosphatic manure."

I believed to be of some interest to compare citric acid with other acids in this regard and also to compare different soils.

I. *Application of organic acids in 1 per cent solution.*

The samples of soil were taken from the experimental paddy field (sandy loam) of the Kinai Branch of the Imperial Agricultural Experiment Station at Kashiwara, Osaka, which were manured every crop with different quantities of phosphoric acid during three years as follows:—

Upland Soil.	{	No. I.	No. P ₂ O ₅
		No. II.	37.5 Kg P ₂ O ₅ as Superphosphate per ha.
		No. III.	93.75 Kg P ₂ O ₅ " " " "
Paddy Soil.	{	No. I.	No. P ₂ O ₅ .
		No. II.	37.5 Kg P ₂ O ₅ as Superphosphate per ha.
		No. III.	112.5 Kg P ₂ O ₅ " " " "

¹ Journ. of the Chem. Soc., London, 65, 115 (1894).

Each plot had received moreover 112.5 Kilo N as NH_4Cl and 93.75 Kilo K_2O as K_2CO_3 per ha.

The extractions were carried out according to B. Dyer's method. The following table shows the result of analysis in the percentage of dry fine earth:—

		P_2O_5 Soluble in				
			1% acetic a.	1% tartaric a.	1% citric a.	1% oxalic a.
A.	Upland Soil.	No. I. No. P_2O_5	0.00832	0.04190	0.08381	0.12955
		No. II. 375 Kg P_2O_5	—	0.04798	0.09596	0.16929
		No. III. 93.45 Kg P_2O_5	0.01184	0.06078	0.09916	0.18001
B.	Paddy Soil.	No. I. No. P_2O_5	0.00096	0.00928	0.01823	0.04606
		No. II. 37.5 Kg P_2O_5	0.00128	0.01408	0.02495	0.05342
		No. III. 112.5 Kg P_2O_5	0.00192	0.01823	0.04526	0.09660

The weakest acid was therefore acetic, the strongest oxalic acid. In other cases, however, tartaric and citric acids extracted a little more than oxalic, as seen from the following table:—

SOILS OF Exper. Stations	Geological formation,	Character,	P ₂ O ₅ Soluble in hot H Cl.	P ₂ O ₅ Soluble in 1%			
				Acetic acid,	Tartaric acid,	Citric acid,	Oxalic acid,
Tokyo Central Station.	Diluvium.	Clayey.	0.37%	trace,	0.0068%	0.0115%	0.0061%
Kinai Branch ¹ (Upland soil)	Alluvium.	Sandy.	0.20%	0.0355%	0.0495%	0.0948%	0.1768%
Kinai Branch ² (Paddy soil)	"	Sandy loam,	0.68%	very little,	0.0170%	0.0248%	0.0562%
Fuso Branch,	"	Clayey,	0.10%	trace,	0.0133%	0.0245%	0.0414%
Sanyo Branch,	"	Sandy loam,	0.18%	0.0066%	0.0332%	0.0880%	0.1075%
Shikoku Branch,	"	Loam,	0.16%	0.0058%	0.0249%	0.0475%	0.1442%
Kin-hu Branch,	Diluvium.	Clayey.	0.33%	trace,	very little,	0.0075%	0.0054%

¹ Upland field soil containing 31.54% fine earth.

² Paddy field soil containing 71.98% fine earth.

II. *Extraction of Soils with Organic Acids of Different Strength.*

The soil serving for these experiments contained 1.635% of hygroscopic water and 0.1727% of P_2O_5 soluble in boiling hydrochloric acid (sp. gr. 1.25). The method of extraction was also here that of Dyer.

The following table shows the results:—

Of the acids.	Acetic acid.	Tartaric acid.	Citric acid.	Oxalic acid.
0.25%	—	—	—	0.1326%
0.50%	—	0.0403%	0.0855%	0.1609%
1.00%	0.0355%	0.0495%	0.0948%	0.1798%
2.00%	0.0499%	0.0704%	0.1029%	0.1954%
5.00%	0.0586%	0.0918%	0.1173%	0.1964%

In this case the extractive power of oxalic acid for P_2O_5 in the soil was strongest, next in order came citric and tartaric acids, and finally acetic acid.

Can Boric Acid in High Dilution Exert a Stimulant Action on Plants ?

BY

M. Nakamura.

It has been shown by various authors that the soil of certain districts contains small quantities of borates, hence also the plants grown on such soils contained some boric acid. *E. Hotter*¹ proved the presence of boric acid in many plants by extracting their ashes with water and transforming the boric acid into its methylic ether which was distilled off. It is especially the fruits in which the boric acid accumulates; in 10000 parts of the dry matter of fruits the amount of boric acid was found to vary from 2.2 to 12.8 parts.

*Callison*² made similar observations. Of some interest is also the observation of *Crampton*³ that boric acid occurs in grapes grown in California. *A. Herzfeld* and *E. v. Lippmann* have made further observations on the occurrence of not insignificant traces of boric acid in lemons and other fruits. *F. Schaffer*⁴ observed recently its normal occurrence in wines. *Hotter* determined to which extent boric acid and borates will exert a poisonous action on plants. When borates are added to the amount of one per mille to culture solutions, the growth was very much injured and the plants died after 20 days. Even an amount of 10 milligram boric acid per liter can exert some noxious action; some difference in resistance power was, however, noticeable with various plants.⁵ The source of boric acid in the soils is probably turmalin which mineral contains about 10% of boric acid

¹ Jahresbericht f. Agricultur Chemie, 1890, p. 203, also Zeitschrift für Nahrungsmittel, etc. 1895.

² Jahresbericht f. Agricultur Chemie 1890.

³ Jahresbericht f. Agricultur Chemie 1889.

⁴ Schweiz. Wochenschr. Chem, Pharm. [1902,] 40, p. 478.

⁵ In regard to algae (*Spirogyra*, *Vaucheria*) *Loew* mentions in *Flora*, 1892, p. 374 that they are not injured within several weeks by adding 0.2 per mille boric acid to the culture water.

and frequently occurs in crystalline rocks and granular limestone. Since poisons exert a less powerful action in the absorbed condition in the soil than in the dissolved state in a culture solution, and moreover since poisons in small doses can exert a stimulant action, I observed cultures of barley in soil to which I added 10 milligr. and 50 milligrams respectively of borax per Kilo. These pots were manured with 1g. NaNO_3 , 1gr. K_2CO_3 and 1.2 g. double superphosphate. Ten young barley shoots were planted, October 24, into each pot and after the young shoots had reached about 15 cm they were reduced to 4 of nearly equal height. The pots were kept in the glass house in which even in the late autumn the temperature on bright days reached sometimes 25°C . Measurements of the shoots to the tip of the longest leaves were made on Nov. 9, Dec. 1 and Dec. 12 with the following results:

		Measurements Cm.		
		Nov. 9	Dec. 1	Dec. 12
50 mg Borax	1.....	31	39,8	45
	2.....	27	35,5	42
	3.....	26	34	41
	4.....	28	38,5	46
	Average	28	36,8	43,5
Control	1.....	30	41	51
	2.....	24	37	45
	3.....	33	43	51
	4.....	28	37	40
	Average	29	39,5	46,75

The percentage of increase was therefore from Nov. 9 to Dec. 12 the following.

$$\left. \begin{array}{l}
 1) \quad 31\% \\
 2) \quad 35,7\% \\
 3) \quad 36,5\% \\
 4) \quad 39,0\%
 \end{array} \right\} \text{average} = 35,5\%$$

$$\text{Control.....} \left\{ \begin{array}{l} 1) \quad 41,2\% \\ 2) \quad 46,6\% \\ 3) \quad 35,3\% \\ 4) \quad 30,0\% \end{array} \right\} \text{average} = 38,5\%$$

A photograph was taken on February 15. It is reproduced on Plate XXVIII and shows that 50 milligrams of borax acted very injuriously on the development of barley, and even as little as 10 milligrams per kilo soil did some damage. On February 16 were added 0,5 g. ammoniumsulphate in high dilution to each pot. On March 3 the control plants showed development of three ears, while even 8 days later there was no sign of ears observed with the borax plants.

On April 23 the plants were harvested with the following results, showing an injurious action of even the small amount of 10 milligrams borax per kilo soil.

	Total wt.	Number of grains	Number of branches	Average length of branches
Control	45,0 g	132	8	63,5 cm
10 mg. Borax	28,2 „	30	4	58,0 „
50 mg. Borax	17,3 „	24	4	46,0 „

In the following experiments, commenced February 1, with pea and spinach the amount of borax was reduced to 5mg. and 1mg. per kilo soil respectively. 10 seeds were planted into each pot and the young shoots were reduced to 4 per pot in the case of the pea. On April 24 the following results were observed :

Pea		
	Average length	Number of flowers
5mg. Borax per kilo soil.....	62 cm	2
1mg. Borax per kilo soil.....	86,25 „	6
Control.....	69,5 „	3

Spinach		
	Average wt. of plants	Average length of leaves
5mg. Borax per kilo soil.....	10,35 g.	38,2 cm
Control.....	7,2 g.	34,0 "

It will be observed that one milligram of borax per kilo soil exerted some stimulant action with the pea plants and 5mg. also with spinach plants.

The high degree of poisonous qualities of borax, which even injures plants in doses of 10 milligrams per kilo soil are certainly unexpected. This is of especial interest at present as, a discussion is now carried on as to the admissibility of borax for purposes of preservation of articles of food. In this discussion the remarkable poisonous character of borax for animals is pointed out. *F. Hofmann*¹ inferred from his experiments with dogs and rabbits that boric acid is "ein starkes Zellgift." *Rost*² observed that the body weight decreases continuously by the use of borax and vomition and diarrhea may result. *E. Kister*³ and also *G. Merkel*⁴ observed that 1—2 grams of boric acid can produce injuries of the stomach and diarrhea. Such an opinion was also expressed by *H. Mayer*.⁵ On the other hand *Liebreich* and *Gerlach* deny the injurious character of borax and boric acid in small doses. However, when we take the highly poisonous character of borax for plants into consideration, we must admit also the dangerous character of borax for animals and man.

¹ D. Med. Wochenschr. 1902, No. 46.

² Ibid. 1903, February.

³ Zeitschr. Hyg. 1901.

⁴ M. Med. Wochenschr. 1903, No. 50, p. 100.

⁵ Hyg. Rundschau 12, 1230.

It must also be mentioned here that *Doane* and *Price* reported that calves fed with milk containing borax lost their hairs—Marryland Agric. Exper. Station Report No. 86.

On the Action of Vanadin Compounds on Plants.

BY

S. Suzuki.

Although vanadin compounds occur very rarely in nature, vanadin was nevertheless discovered in the ash of the sugar beet by *Ed. O. v. Lippmann*.¹ Observations on the action of vanadin compounds on plants have not been made to my knowledge. It was, however, very probable that in moderate concentration they would act poisonously. Since poisons, however, often exert a stimulant action when applied in very high dilution, I have instituted a series of experiments.

In order to observe at first the degree of poisonous action, shoots of barley (20 c.m. high) were placed in solutions of vanadin sulphate of 1% ; 0.1% and 0.01 per cent. After 5 days the shoots in the solution of 1% were dead, while in that of 0.1% the leaves wilted. But the shoots in the 0.01% solution were still healthy even after 12 days.

Water cultures in Knop's solution² were also started to which 1.0 and 0.1 per mille of that sulphate was added. A third experiment was made with a soil culture, 10 mg. of the hypovanadic sulphate being added per kilo soil in one pot, 50 mg. per kilo in a second. A third pot served as control. Each pot contained 10 kilo soil³ and was sown on Dec. 13 with winter barley, 15 seeds in each, which were reduced to 7 of equal size in each pot on Jan. 20.

¹ Berl. Ber. Vol. 21, p. 3492.

² I applied the bluish green sulphate of commerce, the so called hypovanadic sulphate, $V_2O_5(SO_4)_2$. This salt has a strong acid reaction.

³ Only the amount of magnesium sulphate was a little increased.

⁴ Each pot was manured with ammonium sulphate 2.3 g., sodium nitrate 2 g., potassium sulphate 2 g., sodium phosphate 2.5 g. and sodium chlorid 1 g.

Water culture. Barley shoots (16-17 cm. high) were placed (Dec. 4) in 6 flasks.¹

a and a₁ received 0.1 per mille vanadin sulphate.

b and b₁ 0.01

c and c₁ served as control.

The observations made on Jan. 20 were as follows:—

	Length of the longest leaves,	Number of stalks		Number of leaves		Length of the roots,	Remarks.
		thick	thin	living	dead		
a	16.0 c.m.	1	5	4	9	2.0 c.m.	No root hairs visible; Development stopped; fresh leaves appear but the old leaves die off.
a ₁	15.5 "	1	3	5	8	1.5 "	
average	15.8 "	1	4	5	9	1.8 "	
b	21.0 c.m.	5	3	22	2	20.0 c.m.	Normal.
b ₁	21.0 "	5	1	17	3	20.0 "	
average	21.0 "	5	2	20	3	20.0 "	
c	18.0 c.m.	6	2	21	3	17.5 c.m.	Normal
c ₁	23.0 "	4	2	15	3	10.0 "	
average	20.5 "	5	2	18	3	13.8 "	

A very weak stimulant action on the roots seemed to have taken place in the case b and b₁. But in the cases a and a₁ the poisonous action was so decisive that the plants were no longer observed. The final observations were made on Feb. 27 with the following results:—

	Length of the longest leaves,	Number of thick thin stalks,		Number of living dead leaves,		Length of the roots,	Weight in a fresh roots upper portion state,	
b	32.5 c.m.	21	1	76	10	25	39.7 g.	27.4 g.
b ₁	30.0 "	18	2	70	8	25	37.7 "	28.3 "
average	31.3 "	20	1.5	73	9	25	38.7 "	27.9 "
c	34.0 c.m.	21	1	70	13	30	37.7 g.	25.9 g.
c ₁	33.0 "	18	1	69	10	23.5	42.7 "	28.2 "
average	33.5 "	20	1	71	12	27	40.2 "	27.1 "

This experiment proves that in a normal water culture barley is very much injured by the addition of 0.1 per mille vanadin sulphate, and further

¹The solutions were renewed on Dec. 20, Jan. 8, 20, 31 and Feb. 21.

that when applied in the further dilution of 0.01 per mille no decisive stimulation takes place, although no injurious action is any more exerted.

Soil culture. The shoots above mentioned were measured several times. The observations were as follows:—

	Average length of the longest leaves,			Average number of stalks,	
	Jan. 23.	March 27.	April 10.	March 27.	April 10.
Pot I. (0.1 g. vanadin sulphate per 10 kilo soil.)	c.m. 8.6	c.m. 26.4	c.m. 57.7	3	3
Pot II. (1 g. " ")	8.9	30.3	59.1	3	3
Pot III. (Control)	9.1	37.2	62.8	4	4
Pot IV. (")	9.3	33.1	63.5	4	4

This experiment plainly shows that vanadin sulphate even in a very small quantities has no stimulating action on barley.

—•••—



Can Potassium Ferrocyanid Exert any Stimulant Action in the Soil on Plant Growth ?

BY

S. Suzuki.

In a former article I have shown that potassium ferrocyanid even in a very high dilution acts poisonously on plants in water culture.¹ The question, however, seemed to be of some interest whether this compound could exert a stimulant action when incorporated in a small quantity into the soil. Four Wagner's pots each containing 10 kilo soil served for the experiment. Each pot received as manure :—

Ammonium sulphate	2.3 g.
Sodium nitrate.....	2.0 ..
Potassium sulphate	1.9 ..
Sodium phosphate (cryst.)	2.5 ..

Two pots served as control while one pot received 0.1 g. potassium-ferrocyanid and another 1 g. Fifteen seeds of barley were sown in each pot on Dec. 13, and the young shoots reduced to 7 of equal size on Jan. 20. After a few weeks a decided difference was noticed in favor of the pot that received 1 g. potassium ferrocyanid. Measurements were made on March 7 and 27 with the following results :—

¹ These Bul. Vol. V, No. 2.

	Average length of the longest leaves,		Average number of stalks,	
	March 7.	March 27.	March 7.	March 27.
Pot I. (0.1 g. $K_4 Fe Cy_6$).	14.4 c.m.	31.2 c.m.	5	5
Pot II. (1 g. $K_4 Fe Cy_6$).	26.8 "	44.6 "	4	5
Pot III. (Control).	12.8 "	37.2 "	4	4
Pot IV. (").	15.6 "	33.1 "	4	4

The question arised whether the favorable effect in pot II was due to the potassium ferrocyanid as such or to the nutritive action of its decomposition products. It was possible that the soil bacteria decomposed the salt, whereby the iron was liberated as ferric hydrate,¹ nitrogen as ammonia and potassium as carbonate. In order to decide this question 20 g. of the soil of the pot II were extracted on March 17 with water and the filtrate tested with ferric chlorid, but only an exceedingly feeble reaction was obtained. In a second test diluted hydrochloric acid served for the extraction, but with no better result. A control test with 100 g. unmanured soil moistened with a dilute solution of 10 m.g. potassium ferrocyanid showed further that this small quantity is entirely absorbed. It remains therefore for the present undecided whether in the case above mentioned the potassium ferrocyanid acted favorably as a stimulant or by the products of its decomposition as a source of nutrients.

¹ Previous experiments had shown that a small addition of ferrous sulphate to the soil in question increases the yield of rice and of oats.

Are Soluble Iodids Absorbed by the Soil ?

BY

S. Suzuki.

My experiments on the stimulating action of potassium iodid on agricultural crops¹ made it desirable to know whether the soil can retain iodids in a certain measure by absorption. In regard to chlorids, absorption by adhesion has been observed by various authors. The interesting experiments by *B. Dyer*² on the field of *Rothamsted*, e. g., have shown that chlorids are to a certain degree retained by clay soils. He writes: "Now the average quantity of chlorine which falls annually in the rainfall at Rothamsted, as calculated on observations for 22 harvest years, 1877-1878 to 1898-99, was 14.75 pounds." "Yet we see that the soil of plat 5 in the Broadbalk wheat-field retains, on the average, within each depth of 9 inches down as far as 90 inches, a quantity of chlorine equivalent to that which falls upon its surface each year in the form of rain."³ In other words, down to a depth of 90 inches the soil, though continually subjected to the washing influence of the rain, contains a quantity of chlorids equivalent to that falls upon it during ten years, neglecting the very few pounds annually supplied to it as impurities in the mauures." "It would seem that the clay enters into some sort of combination with the chlorids from which they are only dislodged by a very free application of water." "The difficulty of

¹ These Bulletins Vol. V, No. 2 and p. 474 in this number.

² Office of Experiment Stations, Bul. No. 106, U. S. Depart. of Agric. p. 82 and 83.

³ These quantities are in certain countries comparatively large. Thus in *Barbados* were found by *Albuquerque* per million parts of rain water from 6 to 38.5 parts of chlorine, while the nitrogen as ammonia varied between 0.015 to 0.212. (Report of the Agric. work in Barbados, Government Exp. Station, 1902.)

removing chlorids from the soil by percolation except when a relatively very large quantity of water was used, was demonstrated in some experiments described in the paper on the rain and drainage waters at Rothamsted.¹

In my experiments with potassium iodid I compared the behavior of this salt in the soil with that of potassium chlorid, 1 per mille solutions of both these salts serving for the filtration through the soil. As reagent for iodine served starch paste to which freshly neutralized hydrogen peroxid and a trace of ferrous sulphate was added. By this reaction of *Schönbein* very small traces of iodine can be discovered, in the form of the blue iodine starch. First test:—

The stratum of soil was 8.5 c.m. high and 5.8 c.m. wide. 200 cc. of each solution were poured gradually on the surface of the soil contained in a cylindrical vessel. After 35 minutes the first drops appeared at the lower end. While now in the case of potassium chlorid already the first 2 cc. showed a moderate and the second 2 cc. a considerable reaction for chlorine¹ with silver nitrate, there was no iodine reaction obtained in the first 25 cc. of the filtrate. After this a moderate reaction appeared in the next 6 cc. and a strong reaction in the following 2 cc.

Second test:—Here the column of the soil was higher, namely 15 c.m., but the diameter was smaller than in the former case, namely 3 c.m. While the weight of the fine soil used in the first case was 200 g., it was here only 84 g. The solutions were added in this case in such a manner that the surface of the soil was constantly covered by it in a height of 2 c.m. The total quantity of solution added was 100 cc. After about one hour the first drop appeared at the lower end, and while the chlorine reaction was obtained with the first 2 cc. there was no iodine reaction noticed in the first 15 cc. After this the next 3 cc. showed a weak and the following 3 cc. a strong reaction for iodine. Both tests proved decisively that an iodid is much better absorbed in the soil than a chlorid. The calculation shows for

¹ A control test was made with a distilled water free from chlorine. The first few cc. of this filtrate showed a weak reaction for chlorine owing to the chlorid already present in the soil, but the turbidity was much lighter than in the case of potassium chlorid solution.

the first experiment that 100 g. soil absorbed 0.0125 g. potassium iodid and in the second case 0.018 g. The effect depends naturally much on the height of the soil stratum.





Fig I



Fig II

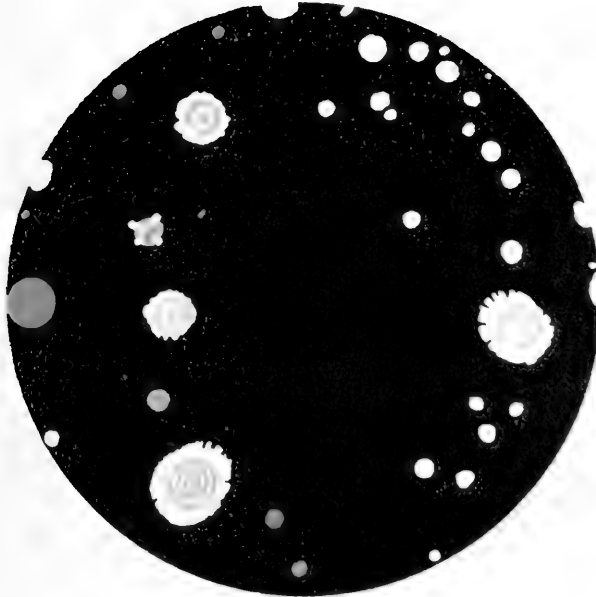


Fig. I. Agar-plate from the digestive juice of a silk worm, 30 days at room temperature. Original plate.

Fig. II. The same. Second dilution.





I

II

Plate showing the stimulating action of rubidium chlorid upon barley.
I Rubidium plants. II Control plants. To page 464.





I
II
III
IV
Plate showing the influence of iodid of potassium on oats. I, II and III, Iodine plants; IV, Control plants.
To page 474.



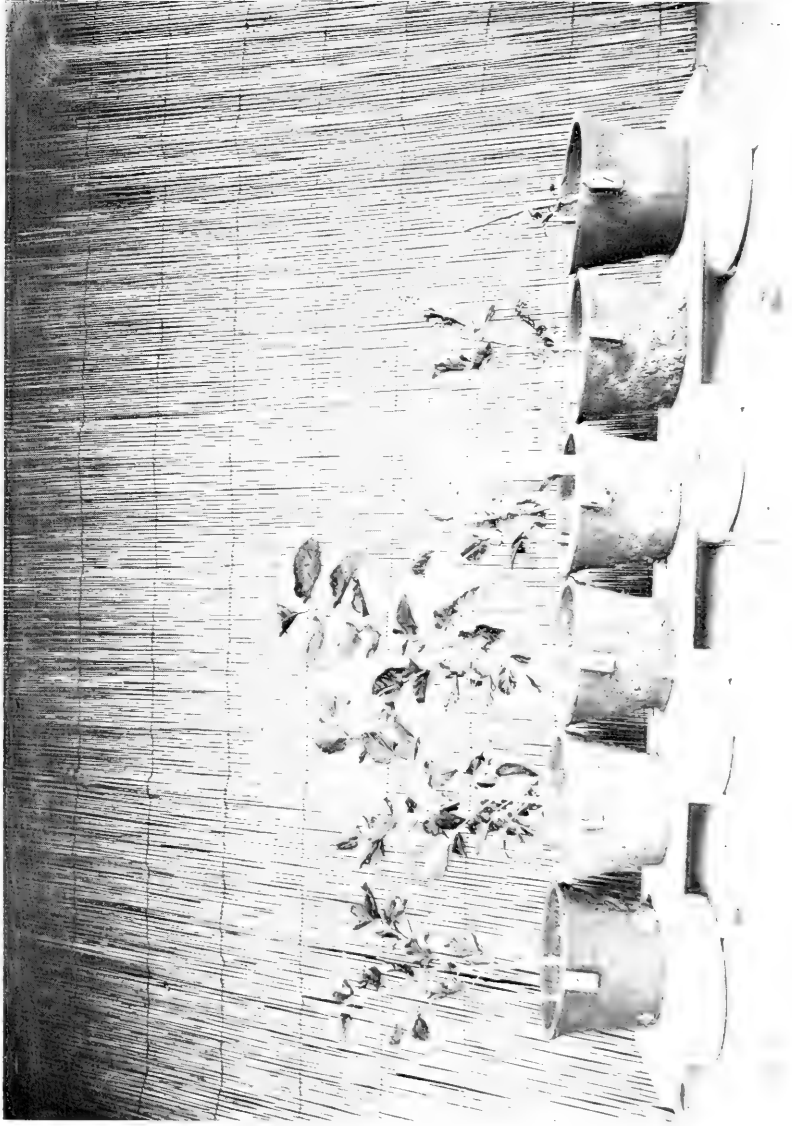


Plate showing the influence of different ratios of lime and magnesia upon mulberry plants. To page 499.





I

II

III

Plate showing the injurious action of borax on barley. I, 0.05 g borax per Kilo soil.
II, 0.01 g borax. III, Control. To page 511.



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THE

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On the Wax-producing Coccid, *Ericerus pe-la*, Westwood.

BY

Prof. C. Sasaki, *Rigakuhakushi*.

Agricultural College, Imperial University, Tokyo, Japan.

WITH PLATES I.—II.

The waxy product secreted by the coccid insect, which is well known among us, is vulgarly called "Chiuhakuro" (Insect's white wax), and is economically employed for certain purposes. It is usually found on two sorts of trees, viz:—*Ligustrum Ibot*, Sieb. (Jap. Ibotanoki), and *Fraxinus pubinervis*, Bl. (Jap. Toneriko); but Ranzan Ono mentions in his celebrated work,¹ that it may also not rarely be found on *Ligustrum Japonicum*, Thunb. (Jap. Nedzumimochi).

Mr. Stanislas Julien² mentions in his "Nouveaux renseignements sur la cire d'arbre et sur les insectes que la produisent, etc. Extraits des Auteurs chinois," the three kinds of trees (*Rhus succedaneum*, *Ligustrum glabrum*, and *Hibiscus syriacus*), on which the Chinese rear the wax-insects. Further, from the article "Insectes à cire" of the same author,³ I quote the following lines:—"Les insectes à cire sont d'abord gros comme des lentes. Après l'époque appelée Mang-Tchong (après le 5 Juin), ils grimpent aux branches de l'arbre, se nourrissent de son suc et laissent échapper une sorte de Salive. Cette liquer s'attache aux branches et se change en une grasse blanche que se condense et forme la cire d'arbre. Elle a l'apparence du givre. Après l'époque appelée Tchouchou (après le 23 Aout, on l'enlève en râclant et on l'appelle alors la-tcha, c'est-à-dire sédiment de cire." "Après l'époque appelée pe-lou (après le 7 Septembre), cette cire se trouve agglutinée si fortement à l'arbre qu'il serait fort difficile de l'enlever. On fait fondre cette matière, et on la purifie en la passant dans une sorte de filtre en étoffe. Quelques personnes la liquéfient à la vapeur et la font découler dans un Vase. Lorsqu'elle est figée et réunie en masse, elle forme

ce qu'on appelle la cire d'arbre." "Quand les insectes sont petits (C'est-à-dire viennent de naître) ils sont de couleur blanche. Lorsqu'ils ont produit de la cire et qu'ils sont atteints leur vieillesse, leur couleur est rongée et noire. Ils se rapprochent entre eux et s'attachent par paquets aux branches des arbres. Dans le commencement ils sont gros comme des grains de millet et de riz, dès que le printemps est venu, ils croissent peu à peu et deviennent gros comme des œufs de poule. Ils sont de couleur violette et rouge. Ils se tiennent par grappes et enveloppent les branches; on dirait que ce sont les fruits de l'arbre.

"Lorsque cet insecte est sur le point de poudrer, il se forme une coque (littéralement une maison) qui ressemble aux loges des mantes qu'on voit sur les Muriers. Cette coque s'appelle communément la tch'ong (cire graine), ou La-tseu (cire fils). L'intérieur est rempli d'œufs blancs qui ressemblent à de petites lentes. On les trouve réunis par paquets qui en renferment plusieurs centaines. A l'époque appelée li hia (le 6 de Mai), on recueille ces œufs, on les enveloppe dans les feuilles de gingembre, et on les suspend à différentes distances aux branches de l'arbre à cire....."

The work of Mr. V. Signoret⁴ has proved a great convenience to my study of this interesting subject; but the descriptions of the female coccid as well as its life history appears to me imperfect on certain points.

In 1876 Mr. Daniel Hanbury⁵ made the following statement on the coccid:

"Chung-pih-lah; Chinese insect wax; pun-tsan, Fig. 837 secreted by *Coccus pela*, Westw. upon the branches of *Fraxinus chinensis*, Roxb., which is cultivated for the purpose, and possibly upon other trees, some accounts of the habits of the insect by a competent observer are much required, the Chinese statements on the subject being extremely obscure."

Further Mr. S. Uyeno⁶ has published some account of the coccid, chiefly extracted from the Report of the English consul at Tchong-King-Fou in China. A brief extract with regards to the coccid is as follows:—

"The wax secreted by the coccid is collected in June, and from the mixture of the former with the fat of the ox, the Chinese prepare candles."

Mr. K. Minemura, who has travelled in Sichuen in China, last year, has brought me some specimens of the coccid and its food plants as well as the

transporting sacs for the insect used for the purpose of propagation by the Chinese. On comparing the specimens with those of our indigenous species, we could not find any difference between the two; but the food plant in China is *Fraxinus chinensis*, Roxb., while in our country, both *Fraxinus pubinervis* and *Ligustrum Ibot*, Sieb. are known to serve as the food plants. It is said that the Chinese cultivate the plant specially for the purpose of breeding the coccid, and at a proper season, they carry the female to various localities, where the plants are either cultivated or growing wild, for the purpose of collecting the wax. The wax harvested in a year, amounts to 600,000 Chin (100 chin=nearly 88 yen*).

For the last four years, I have devoted some time to the study of this interesting wax-producing coccid (*Ericerus Pe-la* Westwood), specimens of which have been collected by my friends Messrs. M. Shimidzu, Y. Miyoshi, T. Tsuchida and also by myself, at various localities of our main island as well as in the island of Shikoku.

In the following lines, I shall state the results of my study and observations made on the coccid collected from the stems or branches of *Fraxinus pubinervis*, Bl. planted on the ridges separating rice fields in a village named Okudomura in Chibaken, not far from the city of Tokyo.

Female coccid:—The full grown female coccid is pretty large, nearly globular in form and either found solitary or in aggregations; in the latter case, it is more or less deformed naturally by mutual pressure (Fig. 1. Pl. I.). The diameter of the largest specimens measures about 11 mm. and the height about 9 mm. (Fig. 2. Pl. I.). The dorsal part which forms the larger portion of the body, is dark reddish brown in color, while the flattened ventral surface, by which the insect is firmly attached either to the stems or branches, is yellowish white. The posterior end of the globular body is marked with a deep incision. The dorsal surface is marked with a number of lightly colored transverse ridges, which indicate the abdominal segments. Besides these ridges, there may be found, all over the surface, blackish patches of variable size, and irregular outlines (Fig. 3. Pl. I.). Close to the smaller patches, there opens a very fine

* One yen equals to 2.5 francs nearly.

pore, from which is secreted a very fine light greyish yellow filament, a large number of the filaments accumulate on the body and forms a loosely interwoven filamentous covering.

Just beneath the pore lies a large oval glandular cell, which may be seen through the skin. The larger blackish patches, which are less numerous than the smaller, bear some light orange yellow roundish spots, and very often, two of these spots are united leaving only a slight constriction between them, or in other cases, two of them are united together by a shorter or longer streak running between them. These light orange yellow roundish spots are the seat of secretion of a sticky transparent mucous fluid. Generally the secretion, accumulating into a light orange yellow drop, hangs down from the body of the insect, and finally they drop down on the ground (Fig. 1, a. Pl. I.). The covering of such secretion on the body seems to protect the latter from other insect enemies. The sticky secretion bears a peculiar odor, which is very similar to that of the cedar oil.

The ventral flattened surface of the insect is almost oval in shape, but its large central portion becomes gradually concave as the eggs are deposited, and finally this concavity becomes deeper and deeper so as to form a large hollow space, wide enough to protect many thousand eggs. If we remove the insect from the stems or branches the eggs may freely fall off. The scar left after the removal of the insects from the stems or branches, is an oval greyish yellow ring, whose central oval depression is occupied with a white cottony secretion. The eggs are elongated oval, light yellow, with the diameters of 0.432 mm. and 0.216 mm. (Fig. 4. Pl. I.). Male coccid:—Cylindrical, somewhat tapering towards the two extremities (Fig. 5. Pl. I.). Head nearly triangular, light orange yellow in color; the dorsal surface is marked with a broad greyish brown median band, whose posterior end, becoming broader, reaches the front edge of the occiput. On either side of this broader end are again some large patches of the same color. There are five pairs of blackish simple eyes, which can be seen when one looks at the vertex of the head. A pair of large round ocelli lies on the dorsal, and also a pair of large oval ones on the ventral side, while the remaining three pairs of smaller roundish ones, lie at the

sides of the vertex between the dorsal and ventral larger ocelli (Fig. 6. Pl. I.). The antennæ are long and composed of ten segments, and covered with long hairs. The segments are long except the two basal ones, which are shorter and stouter than the rest. The last or terminal segment bears at its tip three digitules. The thorax is large, elongated and broader than the head. It is of the same color as the head; but the mesothorax bears two dark reddish brown broad bands, which lie close to the lateral sides so as to enclose a nearly hexagonal light orange yellow area. The meta-thorax is marked on its side with a dark brownish oblique streak. The meso-sternum, which occupies the larger ventral surface of the thorax is dark brown and hexagonal in shape. Legs are comparatively long, light greyish brown, and covered with long greyish hairs. The first pair of legs lie far apart from the remaining two pairs. The tibia of each leg is nearly twice as long as the femur, and bears a single spine at its distal end. Two pairs of digitules are present near the insertion of the claw on the tarsus. Fore wings are long oval, nearly transparent, but the costal margin is light brown. The posterior edge bears a single small lobe very close to the insertion of the wing. Balancers are long and stout, of a brownish color, and have each three long slender hooks at the tip. Abdomen is of nearly equal length to the thorax, and its anterior segment is closely attached to the thorax by its entire breadth. It is of a light greyish green color, the abdominal spike or the sheath of penis is rather short and pointed. From the sides of the last abdominal segment grow out two long slender snowy white filaments, which are much longer than the body. The length of the body is 3 mm. Expansion of wings is 5 mm.

The male insect appears from the end of September to the beginning of October. They fly about the young female coccid, which is already attached to the stems or branches, and copulation is effected by projecting the abdominal spike beneath the body of the female from outside. After copulation, the male soon dies.

Metamorphosis of the Coccid.

The female coccid begins to lay eggs from the first part of May, and the young larvæ begin to hatch out at the beginning of June.

The newly hatched larva is light orange yellow, long oval and depressed (Fig. 7 and 8. Pl. I.). It is about 0.61 mm. in length and 0.37 mm. in breadth. The body is composed of eleven segments. The antennæ are short and stout, and composed of eight segments instead of six as described by Mr. V. Signoret⁴, and all the segments except the single basal one, bear a few long fine hairs. The first and second segments are short and stout, the third is about twice as long as the second; the fourth and fifth are nearly equal in length, and exceed not more than one half the length of the third segment. The remaining three segments, that is the sixth, seventh, and eighth, are much smaller than the others, and the seventh segment possesses an excessively long hair. Eyes simple, roundish, brownish red. The rostral setæ, forming an elongated loop on the ventral side of the body, lie beneath the epidermis of the larva, through which the setæ can be seen very distinctly. The thoracic as well as the abdominal segments are distinct, but the boundary line between the throat and abdomen is indistinct.

The three pairs of legs are of moderate size, and nearly equal in length. The coxa is somewhat stout and long. The femur and tibia are nearly equal in length; but the former is stouter than the latter. A large and long tarsus bears a single claw at its end. At the insertion of the claw on the tibia, there lie two pairs of digitules. The segments of the legs except the trochanter and coxa, bear a few simple hairs. The last abdominal segment is marked with a wide indentation, within which lies, dorsally at each side, a membranous blunt swelling bearing a single long filament. Between these swellings, opens the anus around which are six stout hairs while beneath the indentation, there lies a small semicircular plate, which seems to be the rudiment of the last abdominal segment.

The larvæ distribute by crawling about nearly every young branch, and after moulting passes to the 2nd stage of growth. The distinction of the sexes probably appears during the first stage; but I have failed to recognize it.

Male Larva of 2nd stage:—Body oval, depressed, pale greyish brown, with a wide indentation at the posterior end (Fig. 9. Pl. I.). It is about 0.70 mm. in length, and 0.42 mm. in breadth. The dorsal surface of the

body is densely covered with snowy white, entangled filaments secreted by the dermal glands (Fig. 10, a. Pl. I.), while the periphery of the body is provided with a row of sharply pointed transparent spines of variable length. Within the indentation or cleft at the posterior end of the body, lies a fleshy protuberance on which the anus opens. Dorsally at the base of this fleshy protuberance, lies a pair of nipple-like appendages each having two small spines at the tip. The roundish, dark brownish red eyes lie ventrally close to the lateral margin of the head. The rather short antennæ, which are composed of three segments, are provided with a few long hairs, and lie also ventrally on the head just in the wide space between the eyes. The rostral setæ, which have now become free and long thread-like in form, are deeply thrust into the bark of the host plant. The legs are all rudimentary, and lie close to the ventral surface of the body by their entire length. The first pair of legs, which lie far anteriorly close to the insertion of the rostral setæ, is wide apart from the remaining two pairs, which lie very close to each other.

In the last part of August, the male larvæ (of the 2nd stage) is completely imprisoned within an oval cocoon formed by snowy white filaments (Fig. 10, b. Pl. I.) secreted by the dermal glands as mentioned before. Usually a large number of the oval flattened cocoons lie in irregular masses or completely surrounding the stems or branches, thus forming snowy white patches or a sort of broad girdle (Fig. 12. Pl. II). These white masses of cocoons are again covered with white long and flossy filaments. Within the cocoons, the larvæ undergo the second moulting, and pass to the third stage.

Male larva of the 3rd stage:—Body oval, depressed, light yellow, with a shallow indentation or cleft at the posterior end (Fig. 13, 14; 14, a. and 14, b. Pl. II.). Dorsally the segment lines of the body are conspicuous, and along either side of the median line, runs longitudinally a dark purplish brown wavy band, which meets its fellow at both ends. Anteriorly the united band soon divides into two broad branches, which again subdividing into two, run far forwards to the anterior margin of the body. Eyes small, greyish, and lie wide apart at the front edge of the body. Antennæ lie ventrally at the front end of the body, and is composed of nine more or

less stout segments, bearing sparsely some long hairs, there being four or five of them on the terminal segment. The rostral setæ are long, filamentous, and of a dark brownish color. Legs moderately long and are composed of five segments. The trochanter small, nearly triangular, and closely attached to the side of the proximal end of the femur. At the insertion of the claw on the tarsus, there are a pair of long digitules. The first pair of legs lie far in front nearly at the sides of the insertion of the rostrum, while the second and third pairs, lying close to each other, are widely separated from the first.

About the beginning of October, the larvæ of the third stage change into an elongated, dull greyish brown pupa, with a light colored abdomen the ventral surface of which is light yellowish green. Antennæ, wings, and legs are all free. The length of the body is 2.2 mm.

A few days after remaining in the pupa state, the winged insect appears through a slit-like opening at the free edge of the cocoon. It usually comes out at the posterior end, which bears two long snowy white filaments (Fig. 15. Pl. II.), a large number of the males coming out at the same time. The aggregation of the cocoons give the appearance of their being covered all over with long white filaments.

Female larva of the 2nd stage:—I have failed to examine exactly the larva of this stage; but it is probably very similar to the male larva of the same stage.

About the end of August, there may be found many young female coccids, lying in groups on the stems or branches, more or less apart from the groups of the male cocoons (Fig. 12. Pl. II). The female larva probably undergoes only two moultings before attaining the final stage.

Young female coccid:—Body oval, dorsally conical, and ventrally flattened. The longer diameter of the body is 1.5 mm. and the shorter 1.35 mm. (Fig. 16. Pl. II.). The dorsal surface is light greenish yellow, with more or less depressed punctuations. The exuviae lie excentrically on the dorsal surface in the form of an elongated narrow ridge. The posterior end of the body is marked with a deep narrow cleft. The caudal lobes lying on either side of the cleft are long oval and crimson red in color. The margins of the body are thickened, and are provided with a series

of long transparent spines, whose base is supported by a short, stout, chitinous, greenish yellow process, while the tip ends with a transparent pointed cone (Fig. 17. Pl. II.). The transparent spines, thus projecting radially from the margins of the body, firmly attach the insect to the host plant. The ventral surface of the body is much lighter in color than the dorsal. Close to the front margin of the same surface lie two small blackish eyes wide apart from each other. The central portion of the ventral surface is occupied with an oval depression, in which lies a broad longitudinal swelling, whose sides are symmetrically constricted so as to form several paired blunt processes, which indicate probably the rudimentary segments of the body. The rostral setæ are borne on an elevation lying at the front portion of the central depression. The antennæ (Fig. 18. Pl. II.) lie somewhat apart from each other on the flattened ventral surface just above the front edge of the central depression. They are each composed of eight segments, of which the 1st and 2nd are short and stout, the 3rd much elongated, and the remaining ones are gradually reduced in size towards the end, only the three terminal segments bearing a few long hairs. Legs are small, nearly equal in size, and almost rudimentary (Fig. 18, a. Pl. II.). The first pair of legs, lying at the sides of the anterior swelling, where the rostral setæ are inserted, are far apart from the remaining two pairs. The tarsus is single, and ends with a blunt claw, at the insertion of which are two pairs of digitules. Two pairs of spiracular depressions lie on the broad even ventral area lying between the central depression and the periphery. They have the appearance of a milky white streak.

In January, the female coccid grows in size, but still retains an oval shape, with an elevated dorsal and flattened ventral surface (Fig. 19. Pl. II.). The longer diameter of the body is 5 mm., the shorter 3.3 mm. and the height 1.32 mm. The dorsal surface of the body is now light greyish orange yellow, and is covered all over with dark punctuations as well as sparsely scattered short spines (Fig. 19, a. Pl. II.) while its ventral surface is provided with small secretory pores. The abdominal segments are indicated by obscure segment lines. The cleft at the posterior end of the body is deep and narrow, and the caudal lobes are spindle-shaped

and of a dark brownish color. The periphery of the body is much thickened, of a hard and brittle nature, and bears a single row of transparent spines (Fig. 20. Pl. II.), which are longer than in the younger stage. The dorsal skin is provided with scattered short spines, and the ventral with loosely arranged minute pores, whose snowy white dusty secretion forms an oval ventral scale on the bark on which the coccid lies. This scale is marked thickly with radial striations, and also by some dark broader streaks, indicating the position of the spiracular depression as well as the deep cleft at the posterior end of the body (Fig. 21. Pl. II.).

About the beginning of May, the female coccid grows larger, and is now light green, with blackish punctuations over the surface. The body is almost conical with a round base. The summit of the cone is tinged with yellow, and from it four or more yellowish lines run towards the base (Fig. 11. Pl. I.). Later, the coccid becomes mature as shown in the Figs. 1 and 2. Pl. I., and begins to deposit eggs, as mentioned before.

Product of the coccids:—The wax of the coccids collected by the Chinese is nothing else than the white cocoons formed by the male larvæ of the coccid, and the Chinese is said to employ it as a material for preparing candles and several ornamental images. Our people also collect the cocoons at some localities for certain limited purposes. In China, the coccid and the host plants are cultivated largely for the purpose of procuring the wax. In order to breed the coccid, the Chinese collect the mature female in April, and they pack them up in a triangular sac made of a leaf of *Sterculia* sp? (Fig. 22. Pl. II.). Each two of these sacs are tied together by the petioles of the leaves or some other material, and are thus transported to some localities where the breeding is carried on. Some years ago, I have also tried the transportation of the coccid by keeping them in wooden or tin boxes, and was successful. The female as well as the male pupæ of the coccid are largely infested by a parasitic chalcid fly, apparently of the genus *Encyrtis* (Fig. 23. Pl. II.). The fly appears in the latter part of August. The females are larger, and are about 2 mm. in length. The body is somewhat long and depressed, and of a dull brown. The head is vertically depressed, and its breadth is nearly equal to that


of the thorax. Eyes oval, dark reddish brown. Ocelli light yellow, and arranged on the occiput widely separated from each other. Antennæ are rather long, eleven segmented, light brown and lie very close to the upper portion of the mouth. The basal segment is long and cylindrical, the remaining ones are smaller, but gradually increase in size towards the end. The 7th and 8th segments are white, the 9th, 10th and 11th segments dark brown. The thorax is nearly equal in length to the abdomen, and stout. The scutellum is large and nearly triangular. The fore wings are large and broad in the outer half, the marginal and submarginal veins dull yellowish brown. They are covered thickly with cilia except for a smaller portion of the inner area. A single clear hairless line runs from the naked inner area towards the outer margin of the wing. The larger hair bearing portion of the wing is marked with three dusky brown patches, which run from the front to the hinder margin of the same. The hind wings are clear transparent, and covered sparsely with cilia. The fringes are somewhat longer than those of the front wing. The distal end of the marginal vein ends at a slight projection on the front margin of the wing, on which are three hooks. Legs are greyish brown and nearly equal in length, but a spine on the distal end of the tibia of the second pair of legs exceeds much in size those of the other pairs

Abdomen is sessile, subcylindrical, of nearly equal breadth to the thorax, and light yellowish brown in color. The end of the abdomen is abruptly pointed, and the ovipositor appears hardly beyond it. Spiracular hairs on the abdomen are of variable length. The length of the body is 2 mm.; the expansion of wings 4 mm. Males are very similar in general aspect to the other sex; but somewhat smaller in size, and differ from the latter in the following points:—The body is dark bluish black, antennæ colored uniformly light brown; the front wings lack the light brownish patches. The male genitalia are pretty long and project beyond the end of the abdomen. Length of the body is about 1.2 mm.; expansion of wings about 3 mm.

As the results of my study on this interesting coccid insect, I may conclude that it is the native not only of China, but also of Japan, where it is widely distributed. Further, the food plants differ in the two countries;

Ligustrum Ibot, Sieb., and *Fraxinus pubinervis*, Bl. in Japan, and *Fraxinus chinensis*, Roxb. in China.

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Explanation of Plate I.

- Fig. 1. Aggregation of Female coccid on the branch of *Fraxinus pubinervis*, Bl.; a, oil drop. 1/1.
Fig. 2. Mature female coccid; a, top view; b, side view; c, looked from behind. 1/1.
Fig. 3. Dorsal view of ditto. Zeiss B/I.
Fig. 4. Eggs. Zeiss A/I.
Fig. 5. Male coccid. Zeiss aa/I.
Fig. 6. Head of ditto. Side view; Fig. 6, a. Top view. Zeiss B/I.
Fig. 7. Newly hatched Larva. Dorsal view. Zeiss B/I.
Fig. 8. Ditto. Ventral view. Zeiss B/I.
Fig. 9. Larva of the 2nd Stage. Dorsal view. Zeiss B/I.
Fig. 10. Section of the dermal gland of male larva of 2nd Stage. Fig. 10, a. filaments secreted by the gland. Zeiss D/4.
Fig. 11. Femal coccid collected at the beginning of May. 1/1.
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Explanation of Plate II.

- Fig. 12. Branch of *Fraxinus pubinervis*, Bl. with the group of cocoons, and with young and mature female coccids. 1/1.
- Fig. 13. Male Larva of the 3rd Stage. Dorsal view. Zeiss A/I.
- Fig. 14. Ditto. Ventral view. Zeiss A/I.
- Fig. 14, a. Antenna; Fig. 14, b. Leg. Highly mag.
- Fig. 15. Winged males getting out of cocoons. 2/1.
- Fig. 16. Young female coccid. Dorsal view. Fig. 16, a. Side view. Fig. 16, b. Ventral view. Zeiss. aa/1.
- Fig. 17. Long transparent spines along the periphery of ditto. D/I.
- Fig. 18. Antenna. Fig. 18, a. Left fore leg. D/I.
- Fig. 19. Female coccid collected in January. Dorsal view. 10/1. Fig. 19, a. Small spines on the dorsal surface. Zeiss F/I.
- Fig. 20. Spines on the periphery of ditto. Zeiss D/I.
- Fig. 21. Ventral scale left on the branch after the removal of coccid. 10/I.
- Fig. 22. Triangular sac for transporting mature female coccids. 1/2.
- Fig. 23. Parasitic chalcid fly (*Encyrtis* sp.?) 20/1.
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Fig. 1.

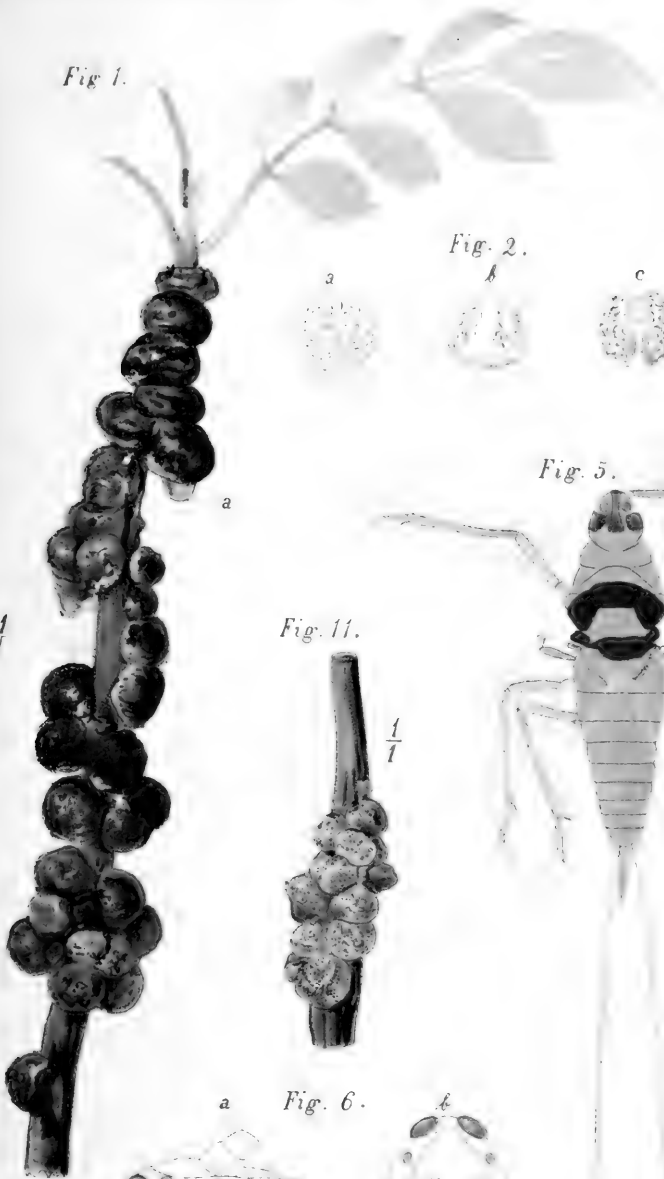


Fig. 2.



Fig. 3.

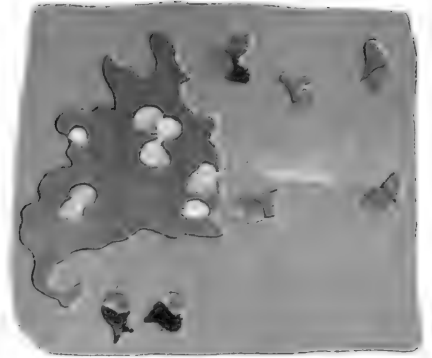


Fig. 5.



Fig. 11.



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Fig. 6.



Fig. 10.

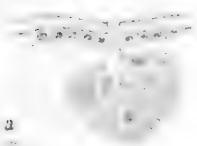


Fig. 10a



Fig. 9.



Fig. 7.



Fig. 8.

Fig. 8.



Fig. 14, a



Fig. 14, b



Fig. 17, c



Fig. 13.

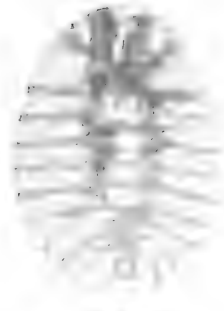


Fig. 15.



Fig. 16, b



Fig. 16, a

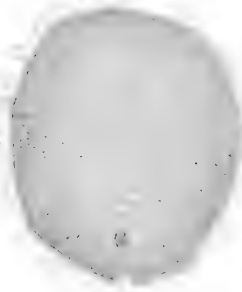


Fig. 20.



Fig. 16, c



Fig. 18, a

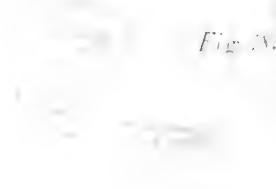


Fig. 18, b

Fig. 18, c



Fig. 22.



Fig. 21.



Fig. 19, a

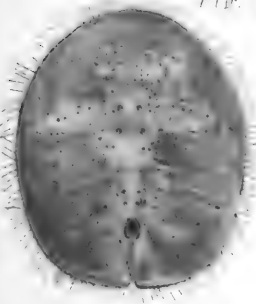


Fig. 19, b





On the Feeding of Silkworms with the Leaves of *Cudrania triloba*, Hance.

BY

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August 1903.

(With Plates III and IV.)

Some years ago, I tried several times to feed our native silkworms (Race Awobiki) with the leaves of *Cudrania triloba*, Hance (Figs 1. 2. Pl. III and IV.) which has been cultivated in the farms of our college since its introduction from China, but my attempts were then unsuccessful, as no cocoons could be procured. My friend, Mr. Minemura, who travelled in the central part of China last year, has collected for me some eggs of silkworms largely cultivated in Si-chuen in China, where the Chinese feed them partly with *Cudrania* and partly with mulberry leaves.

This year, I have succeeded to rear the Chinese race by the careful aid of my special student, Mr. Y. Tsuji, and was able to procure cocoons. The results obtained by rearing it with *Cudrania*, are as follows:—

First stage: The larvæ or silkworms of the *Cudrania* race¹ came forth on May 5th at the temperature of 65°—68° F. They were fed with finely chopped leaves of *Cudrania*, regularly six times a day. The average length of ten largest worms at the end of this age was about 1 cm. Moulting began on May 12th, and ended on 13th.

2nd stage: On May 14th, all the worms completed the 1st moulting. The leaves of *Cudrania* were chopped a little larger than in the previous age, and given regularly six times a day. Average length of ten largest worms at the end of this stage was about 2.1 cm. On the 22nd May, the worms began to moult.

3rd stage: On May 22nd, all the worms completed the 2nd moulting. The leaves of *Cudrania* were chopped still larger than in the previous stage.

1 I have named the Chinese race feeding on *Cudrania triloba*, Hance "Cudrania race."

and given regularly six times a day. The average length of ten largest worms at the end of this age was about 4 cm. Moulting began on the 29th.

4th stage: On the 31st, all the worms accomplished the 3rd moulting or casting. From the beginning of this age, the worms were separated into two groups A and B. To A, the leaves of *Cudrania* (chopped or entire) as usual, and to B, those of the mulberry (chopped or entire) were given regularly five times a day. On June 6th, the worms of the two groups attained maturity, and commenced to spin cocoons. The average length of ten largest worms fed with mulberry leaves was 7.1 cm., and their average weight 2.6 grms., while that of the same number of the worms fed with *Cudrania*, was 7.7 cm. and their average weight 3.4 grms.

In each of these two groups of worms, one fed only with *Cudrania* and the other with *Cudrania* and mulberry leaves, there appeared two sorts of worms differing in coloration of the body as well as of the cocoons.

The first sort of worms, which are more numerous than the 2nd and which are taken as the materials for my experiments, is white tinged more or less with a yellowish shade. The markings on the anterior three body segments are light greyish yellow with a pair of dark greyish spots on either side of the median dorsal line of the 2nd segment. A pair of hook shaped markings of a light purplish hue lies on the 5th and 8th segments. The ventral side of the body as well as the legs are light yellow (Fig. 3. Pl. IV.).

The second sort of worms, which are less numerous than the first is white, with a faint bluish color; but no trace of yellowish shade. The markings on the anterior two body segments are dark grey. The two pairs of spots on the 2nd body segment, are conspicuously black. The 3rd body segment bears no markings. The markings on the 5th and 8th segments are small and indistinct, but are still of a pale purple. The ventral side of the body as well as the legs are not yellowish (Fig. 4. Pl. IV.).

The worms of this race, without passing the 4th moulting or casting, which is usually the case in other silkworms, became mature on June 6th, and spun cocoons.

On the 23rd and 24th of June, the moths issued from the cocoons and laid eggs as usual.

The cocoons are long spindle shaped or elongated oval in shape (Fig. 5.

6. 7. Pl. IV.). Their length varies from 37 to 43 mm. and their breadth is 15 mm. on the average. The coloration is of two sorts—white and orange yellow.

The whitish cocoons (Fig. 5. Pl. IV.) are formed by the worms, which have no trace of yellowish color on the body and legs; and the orange yellow ones, by those having a yellowish color in their body and legs (Fig. 6. 7. Pl. IV.). The cocoons are destitute of a constriction or depression at the middle, which is present nearly in all of our native races of silkworms. The wrinkles on the surface of the cocoons are moderate. The tissue of the cocoons is more or less hard and compact, but in some cases, they are soft and thin. The average weight of 20 empty cocoons (pupa removed) formed by the worms fed throughout with *Cudrania* is 0.2135 grms., while that of 20 empty cocoons formed by the worms fed with mulberry from the beginning of the 4th stage, that is, after the 3rd moulting is 0.2065 grms. Thus the different meals given to the silkworms after the 3rd moulting, does not affect their health as well as their growth to any larger extent, and moreover, we see from the above that the empty cocoons of the worms fed with *Cudrania* are sensibly heavier than those partly fed with mulberry.

If we compare the duration of the larval period of the *Cudrania* race of China with that of our native race *Awobiki*, there is not any great difference between them, although the former becomes mature and commences to spin cocoons already after the 3rd moulting, instead of the 4th, which our native races generally pass through.

The following shows the duration of each age of the two races of silkworms :—

	Cudrania race (fed only with <i>Cudrania</i> , and with it and mulberry)	<i>Awobiki</i> race
1st stage	8 days	7 days
2nd stage.....	8 "	5 "
3rd stage	9 "	6 "
4th stage	7 "	7 "
5th stage	— "	8 "
	32 days	33 days

From the above table, we see that although the *Cudrania* race passes through only three moulting, that is, one moulting less than our native races, the duration of each age is more or less longer than the latter, and thus the number of days of the larval period is nearly similar in the two. Accordingly the quantity of the meals they consume is nearly similar in both.

The qualities of the average ten filaments of cocoons taken from the two sorts of silkworms fed throughout with *Cudrania*, and with *Cudrania* and mulberry are as follows :—

	Physical nature of the filaments reeled from the cocoons of silkworms fed with <i>Cudrania</i> only.	Physical nature of the filaments reeled from the cocoons of silkworms fed with <i>Cudrania</i> and mulberry.
Aver. length of ten filaments.....	516 aunes (613,04 metre)	527 aunes (627,13 metre)
Aver. weight of ditto at the length of 400 aunes	0,147 grms.	0,146 grms.
Aver. titre of ditto	1,96 denier	1,96 denier
Aver. number of duvets of ditto	1,1	0,5
Aver. number of ruptures of ditto ...	0,2	0,3

It is very interesting to observe that the parasitic maggot (*Larva* of *Ugimyia sericariae*, Rond., which does terrible harm to our silkworms) is entirely absent in the *Cudrania* race fed absolutely with *Cudrania triloba*, Hance, while that which is fed with the same plant and later with mulberry trees, is more or less infested by this pest. Consequently if we feed the *Cudrania* race exclusively with *Cudrania triloba*, Hance, it will be entirely free from the parasite, and the crops will be superior than if fed exclusively or partly with mulberry.

The results obtained from the foregoing experiments may be summarized as follows :—

1st. The *Cudrania* race of silkworms, which passes only four stages instead of five, has nearly the same length of larval period with our native races, and the consumption of the meals is also similar to the latter.

2nd. The quality and quantity of the filaments reeled from the cocoons of the *Cudrania* race, are never inferior to our native races.

3rd. If the *Cudrania* race is fed exclusively with *C. triloba*, Hance, it is entirely free from the parasitic maggot, which does great harm to our native silkworms.



Explanation of Plates.

Plate III.

Fig. 1. Branch of *Cudrania triloba*, Hance 1/2.

Plate IV.

Fig. 2. Largest leaf of *Cudrania triloba*, Hance 1/1.

Fig. 3. Mature silkworm forming a yellowish cocoon 1/1.

Fig. 4. " " " a whitish cocoon 1/1.

Fig. 5. White cocoon 1/1.

Fig. 6. Yellow cocoon 1/1.

Fig. 7. Ditto of varied coloration. 1/1.







Fig. 1.

Yokoyama del.





Figs. 3-7. Yokoyama del.



Corean Race of Silkworms.

BY

Prof. C. Sasaki, *Rigakuhakushi*.

Agricultural College, Imperial University, Tokyo, Ja .

June 1903.

(With Plate V.)

For the last two years, I have tried to rear the Corean race of silkworms, which I have procured from a Corean friend in Japan. The Corean cartons on which the eggs are laid, are soft nearly rectangular pieces of paper of about 24 cm. by 17 cm. The surface of the cartons on which the eggs lie, is evenly covered with the ashes of mulberry leaves. The covering of the eggs with the ashes, according to the Coreans, protects spontaneously the coming forth of the silkworms during the summer months. In this condition, the cartons are kept during winter, and early in spring, some days before the hatching of silkworms, the ashes are washed away from the cartons and hatching then takes place.

The worms came forth at the beginning of May, and after passing the 3rd moult, they become mature and commence to spin the cocoons, thus they lack the 4th moult, which most other races of silkworms generally undergo.

In rearing the Corean race, I employed the temperature of 70° to 75° F. in the breeding chamber. The length of each of their ages and number of meals given them each day are as follows:—

Number of days taken for breeding.	Date.	Ages.	Number of meals given per day.
1	7 V. 1903	1.	2
2	8 " "	"	7
3	9 " "	"	7
4	10 " "	"	7

Number of day taken for breeding.	Date.	Ages.	Number of meals given per day.
5	11 V. 1903	I.	7
6	12 " "	"	7
7	13 " "	"	1
8	14 " "	"	0
9	15 " "	II.	2
10	16 " "	"	6
11	17 " "	"	6
12	18 " "	"	6
13	19 " "	"	6
14	20 " "	"	6
15	21 " "	"	5
16	22 " "	III.	3
17	23 " "	"	5
18	24 " "	"	5
19	25 " "	"	5
20	26 " "	"	5
21	27 " "	"	5
22	28 " "	"	0
23	29 " "	"	0
24	30 " "	IV.	4
25	31 " "	"	4
26	1 VI. "	"	4
27	2 " "	"	4
28	3 " "	"	4
29	4 " "	"	4
30	5 " "	"	4
31	6 " "	became mature	
	20 " "	imago appeared	

From the above table, we see that the Korean race, though it passes through only four ages instead of five, which is usually the case with our silkworms, takes nearly the same length to complete larval life,

and consumes nearly the same quantity of leaves as our silkworms.

From the Corean race, I have been able to separate five varieties according to coloration or markings, which shows that the Coreans are satisfied with breeding such a mixed race, and do not care to select the best varieties from them.

The five varieties I have obtained so far are:—

1st. Body whitish with a pale bluish shade at the junction of the body segments. Head dull greyish brown; the dorsal shield of the 1st segment light pinkish grey; no particular markings on the 2nd and 3rd segments. The markings on the 5th and 8th segments are pale bluish purple, and those on the former are imperfectly horse-shoe shaped, while those on the latter are simply curved lines. The tip of the anal horn on the 11th segment as well as the free end of the 12th and the free edges of the last abdominal legs are faintly tinged with a light brownish yellow. The length of the mature silkworms varies from 6.7 cm. to 6.9 cm. (Fig. 1. Pl. V.). Cocoons are all white. Their shape is very variable; but the normal ones are long oval, spindle shaped, or perfectly globular. The long oval ones have rarely a slight constriction at the middle of their length. Besides these, there are found many double cocoons, which are mostly larger and very irregular and variable in shape, and they contain often more than two chrysalids.

2nd. Body whitish with a pale bluish shade all over its surface. Head and the dorsal shield of the 1st segment colored normally. The 2nd segment has a short greyish median dorsal line, and two pairs of small greyish markings. The posterior half of each marking of the inner pair tinged black. Besides the markings of the 5th and 8th segments, there is also a pair on the 9th segment. Those on the 5th are nearly oval, pale purplish blue; those on the 8th, and 9th are small greyish yellow dots. The tip of the anal horn and the free edges of the 12th segment and the last abdominal legs are deep brownish yellow. The length of the mature silkworms same as in the 1st variety (Fig. 2. Pl. V.).

Cocoons are either white or light green. The shape is also very irregular. Double cocoons are produced abundantly.

3rd. Body whitish with light bluish and yellowish shades. The head

and the dorsal shield of the 1st segment colored normally. The 2nd segment bears dorsally a broad light greenish band, which gradually broadens behind. At each side of the broader end of this band lies a simple blackish spot. The median dorsal line on the 2nd segment is short and greyish. The dorsal raised wrinkles on the 3rd segment are yellow. The markings on the 5th segment are simple and light greyish purple, while the 8th segment lacks any markings; but the 9th segment possesses dorsally a pair of greyish yellow spots. The tip of the anal horn on the 11th segment as well as the edges of the 12th segment, and of the last abdominal legs are deep brownish yellow. The length of the body same as in the 1st variety (Figs. 3, 3, a. Pl. V.).

Cocoons are deep yellow, and their shape irregular. Double cocoons are very variable in shape.

4th. Body white with light bluish shade. The coloration of the head same as in others; but the dorsal shield of the 1st segment light greyish yellow. The 2nd segment possesses dorsally a pair of large broad blackish patches, whose outer side is occupied by an orange reddish area. The front edge of this area is lined with black, and there is a single tiny blackish dot in the centre. The joints of the remaining segments are embroidered with a dark greyish band, and the joint between the 11th and 12th segments with two bands. Each of these bands except the 1st, 2nd, and 4th, is marked dorsally with two pairs of small blackish dots; but the 5th segment bears dorsally a pair of black hook shaped markings, whose broader end lies within the band running between the 4th and 5th segments. Again each of the anterior three bands are provided on either side with a single light reddish dot, and each of the remaining segments with the pair of the same. From the 4th to the 9th segment, there are one or two dark greyish dots or patches below the spiracle. The free edges of the 12th segment as well as the last abdominal legs are light greyish yellow. The length of the body same as in the 1st variety (Figs. 4, 4, a. Pl. V.).

Cocoons are snowy white, and of variable shape; but normally they are oval and without a constriction at the middle.

5th. Body pale bluish white. Head and the dorsal shield of the 1st segment colored normally. The 2nd segment is marked dorsally with a

dark greyish broad band, which broadens posteriorly. Beside this band, there lie on the posterior half of the same segment, two pairs of blackish markings. A narrow line lying between the two blackish markings on either side of the 2nd segment is light orange yellow. The dorsal raised wrinkles on the 3rd segment are also light orange yellow. The markings on the 5th segment are oval, and enclose a single blackish spot, and their outer edges are lined with black, while their inner halves are of a light orange yellow. Besides the small roundish markings on the 8th segment, there is also a pair of similar markings on the 7th, and both are colored orange yellow. The free edges of the 12th segment and the last abdominal legs have a light greyish yellow color.

The 2nd, 3rd, and the anterior half of the 4th, segments as well as all the remaining segments have a light greyish shade on the pale bluish white ground color. Moreover, the dorsal surface of the 4th to 11th segments is marked with a broad longitudinal dark bluish grey band, which is lined on either side with a pale blue. On the median dorsal line of the 5th to 10th segments, there lies, on each, a λ shaped pale bluish marking. The length of the body same as in the 1st variety (Fig. 5. Pl. V.).

The cocoons of the above mentioned five varieties are equally very variable in shape, and there is no any fixed shape among them; and moreover, double cocoons are more numerous as compared with our white native races. The principal shapes of the cocoons are oval, conical or spindle shaped, but they may be often nearly or perfectly spherical or rarely oval with a slight constriction at the middle (Figs. 6—11. Pl. V.).

The color of the cocoons has some relation to the coloration as well as the markings of the silkworms, thus:—

- the cocoons of the 1st variety are all white
- “ “ “ “ 2nd “ “ white or light green
- “ “ “ “ 3rd “ “ deep yellow
- “ “ “ “ 4th “ “ snowy white
- “ “ “ “ 5th “ “ white or light green.

The cocoons of the 4th variety are much superior in their brilliancy.

The double cocoons, which amount to about 50% of the total, are irregularly roundish or oval, and more or less depressed. Their diameter

varies from 23 to 27 mm., and their height from 12 to 18 mm. These shapes of the double cocoons, which are usually not found in our native races, are very peculiar to the Korean race (Figs. 12—15, Pl. V.).

The following gives some of the qualities of the cocoons of the five varieties selected from the Korean race.

	1st Var.	2nd Var.	3rd Var.	4th Var.	5th Var.
Aver. length of 10 filaments (Bave) of cocoons.	363 Aunes, (432 Metre.)	329 Au. (382 M.)	422 Au. (502 M.)	388 Au. (462 M.)	316 Au. (376 M.)
Aver. titres of ditto at 400 aunes.	1.36 Denier.	1.28 D.	1.21 D.	1.43 D.	1.28 D.
Aver. duvets of ditto.	1.5	2.0	0.6	1.9	2.3
Aver. ruptures of ditto.	0.1	0.2	0.6	0.3	0

As the result of any study on the Korean race, I think, if we spend some time in improving the white cocoons of the 1st and 4th varieties, and the yellow cocoons of the 3rd., it will not be difficult to obtain some excellent varieties of both white and yellow ones from the Korean race studied by me.

Explanation of Plate V.

- Fig. 1. Korean race 1st variety 1 I.
 Fig. 2. Ditto 2nd „ 1 I.
 Fig. 3. Ditto 3rd „ 1 I.
 Fig. 4. Ditto 4th „ 1 I.
 Fig. 5. Ditto 5th „ 1 I.
 Figs. 6—11. Cocoons of Korean race 1/1.
 Figs. 13—15. Double cocoons of ditto 1 I.



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.

Fig. 6.

Fig. 7.

Fig. 3, a, c, e.



Fig. 4, a, d, f.



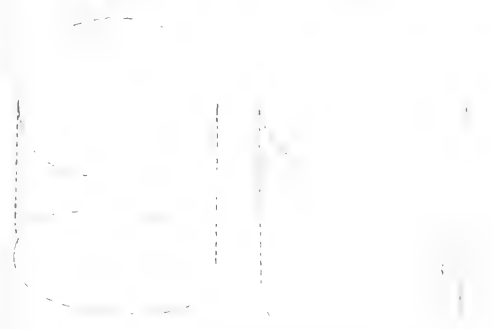
Fig. 8.

Fig. 9.

Fig. 12.

Fig. 13.

Fig. 14.





The Beggar Race (Kojikiko) of Silkworms

BY

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August 1903.

(With Plate VI.)

Two years ago, I had the opportunity of procuring the eggs of the so-called beggar race (Kojikiko) from Shigaken (prefecture near Kyoto). As the name implies, the beggar race eats voraciously not only fresh and clean mulberry-leaves, but also the withered, spoiled or other waste leaves, which are rejected by other races.

Notwithstanding that the meals of the beggar race are of an inferior quality, and its treatment imperfect in every respect, it is still very strong and healthy, and grows well like other silkworms, and moreover, there appear only a few diseased silkworms during its cultivation. However it is very strange that although this race is reared to a limited extent by nearly all cultivators in certain districts as an extra product, it has till now been generally unknown among most of our cultivators.

This race appears twice a year, and its cocoons are yellow, and more or less inferior in quality to the white or green races of our silkworms.

The spring breed reared this year (1903) came forth on May 3rd, and matured on the 7th of June. The worms were fed mostly with withered, or spoiled leaves or those which were destined to be thrown away as a waste. The number of feedings each day as well as the quantity given at a time during cultivation, was intentionally made very irregular. The following shows the ages, dates, the number of days required for cultivation, and the number of meals given each day for the spring breed.

Ages	Dates	Number of days required for cultivation	Number of meals per day
I.	3 V. 1903	1	3
	4 " "	2	4
	5 " "	3	4
	6 " "	4	7
	7 " "	5	5
	8 " "	6	4
	9 " "	7	5
	10 " "	8	6
	11 " "	9	3
	12 " "	10	0
II.	13 " "	11	5
	14 " "	12	4
	15 " "	13	4
	16 " "	14	4
	17 " "	15	6
	18 " "	16	3
III.	19 " "	17	2
	20 " "	18	6
	21 " "	19	0
	22 " "	20	6
	23 " "	21	5
	24 " "	22	5
IV.	25 " "	23	1
	26 " "	24	4
	27 " "	25	5
	28 " "	26	5
	29 " "	27	5
	30 " "	28	5
	31 " "	29	2
V.	1 VI. 1903	30	2
	2 " "	31	4

Ages	Dates	Number of days required for cultivation	Number of meals per days
V.	3 V. 1903	32	4
	4 " "	33	4
	5 " "	34	4
	6 " "	35	4
	7 " "	36	4

On the 7th June 1903, the silkworms became mature and began to spin cocoons. On the 17th June 1903, the moths appeared and laid eggs.

The summer breed came forth on the 4th of July and matured on the 30th. It was similarly treated with the spring breed. The following shows the ages, dates, the number of days required for cultivation, and the number of meals given per day for the summer breed.

Ages	Dates	Number of days required for cultivation	Number of meals per day
I.	4 VII 1903	1	3
	5 " "	2	7
	6 " "	3	7
	7 " "	4	7
	8 " "	5	7
	9 " "	6	0
	10 " "	7	0
II.	11 " "	8	0
	12 " "	9	8
	13 " "	10	8
	14 " "	11	0
III.	15 " "	12	6
	16 " "	13	6
	17 " "	14	6
	18 " "	15	5
	19 " "	16	0

Ages	Dates	Number of days required for cultivation	Number of meals per day
IV.	20 VII 1903	17	6
	21 " "	18	6
	22 " "	19	6
	23 " "	20	7
	24 " "	21	4
V.	25 " "	22	7
	26 " "	23	7
	27 " "	24	6
	28 " "	25	6
	29 " "	26	6

On the 30th July the silkworms matured and commenced to spin cocoons. On the 10th August the moths appeared and laid eggs.

The mature silkworms of the spring breed, which are nearly similar in size and appearance to the summer breed, are about 6.7 cm. in length. There are two varieties of these worms, viz :—1st : Body is white, its anterior and posterior segments of a faintly yellowish hue. Head dull greyish brown, the dorsal shield of the 1st body-segment (thoracic dorsal plate) is light pinkish yellow. The markings on the 2nd and 3rd body segments are entirely absent, those on the 5th is light purple crescent-shaped, and those on the 8th are reduced to merely pale purplish dots. The free end of the last body-segment as well as the last abdominal legs colored light greyish brown. The ventral surface of the body as well as the remaining abdominal legs are also of a yellowish color. (Fig. 1. PL. VI.). 2nd. : The color of the head and body as well as that of the last body-segment, of the last abdominal legs and of the thoracic dorsal plate is similar to that of the 1st variety. At the junction of the 2nd and 3rd body-segments, there lies, on either side, a pair of nearly triangular black markings. The two markings of each pair is separated by a light pinkish streak. The inner marking of each pair is connected together by a blackish streak running transversely on the dorsal surface. The markings on the 5th body-segment are also light purple and crescent shaped and enclose a dark purple dot on their concave side. The

markings on the 8th body-segment are somewhat larger than in the 1st variety and are also purple (Fig. 2. PL. VI.). The cocoons are cylindrical and yellow, having a shallow constriction at the middle (Fig. 3. PL. VI.). The sizes of the cocoons differ in the two breeds, viz: the largest cocoons of spring breed are 2.8 cm. in length and 1.4 cm. in breadth, while those of the summer breed are 3.1 cm. in length, and 1.6 cm. in breadth. Thus, the cocoons of the summer breed are larger than those of the other breed, and the color of the former is deeper than that of the latter (Fig. 4. PL. VI.).

The qualities of the filaments and raw silks reeled from the cocoons of the spring breed of Kojikiko are as follows:—

Quality of filament taken from a single cocoon.

Aver. length of 10 filaments...	2.95 aunes = 351 metre.
Aver. titre of 10 filaments for the length of 400 aunes (476 Metres).	
... ..	1.35 denier
Aver. number of duvets of 10 filaments for the length of 400 aunes.	
... ..	0.9
Aver. ruptures of 10 filaments for the length of 400 aunes	...
... ..	0.3

Quality of raw silk prepared from 6 or 7 cocoons.

Aver. titre of 10 samples of raw silks for the length of 400 aunes.	
... ..	10.2 denier
Aver. tenacity of ditto 40.0 grams
Aver. elasticity of ditto... 10.54 cm.

Although the lengths of the filaments as mentioned above are generally less, and the qualities of the same as well as the raw silk of the race Kojikiko more or less inferior to those of our white races, it will be still profitable to cultivate as an extra product on account of its strong resistance to diseases, as one can procure a sufficient crop without much pain.





Double Cocoon Race of Silkworms.

BY

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August 1903.

(With Plate VI.)

The double cocoon race of the silkworms is aboriginal to the Riu Kiu Islands, and is largely reared there for the purpose of preparing rude coarse or floss silk ; but not for commercial purposes.

As this race is strong and healthy, it can be cultivated by the natives with great ease. It appears once a year, and the duration of its cultivation varies from 28 to 30 days at the temperature of 70° to 75° F. in a breeding chamber, and thus it matures three or four days earlier than the white races of our main island.

There are two varieties of this race ; but both spin yellowish cocoons.

1st variety :—Body is light bluish white ; head and the dorsal shield of the 1st segment are dull greyish brown. The dorsal surface of the 2nd segment is marked with a broad band of light greyish yellow, whose anterior half has a dark greyish streak in the median line. On either side of this broad band, there are two blackish spots of different sizes. The raised wrinkles on the 3rd segment have a pair of light greyish spots lying apart from each other. The markings on the 5th segment are comma shaped. They are greyish yellow, and are bordered with blackish lines. Within each of these markings lies a blackish curved line. The markings on the 8th segment are round and greyish yellow, the peripheries are lined with black, and a small whitish spot lies at the centre of each. The 5th to 11th segments are marked densely with minute greyish dots, and besides these markings, each of the segments bears two pairs of distinct blackish spots. The anal horn and the abdominal legs are tinged yellow. The length of the mature silkworm is 7.6 cm. (Fig. 5. Pl. VI.).

2nd Variety:—Body light yellowish white, head and the dorsal shield of the 1st segment colored same as in the 1st variety. The dorsal band on the 2nd segment, and the raised wrinkles on the 3rd are light greyish yellow. A pair of blackish markings at each side of the band on the 2nd segment is smaller than in the last variety. A pair of large comma-shaped light grey markings on the 5th segment have each a dark greyish line within. The markings of the 8th segment are imperfectly ring-shaped, and are of a light purplish ashy color. The anal horn as well as the abdominal legs are tinged yellow (Fig. 6, Pl. VI.).

The cocoons of this race are almost all double, and the simple cocoons containing a single pupa are much less numerous; while the flossy silk which covers loosely the surface of the cocoons are comparatively abundant than in other races.

The simple cocoons are spindle shaped; but they are often deformed. Their color varies from light to deep yellow. The length of the largest cocoons is about 3.3 cm. and the breadth 1.5 cm. (Figs. 7, 8, Pl. VI.).

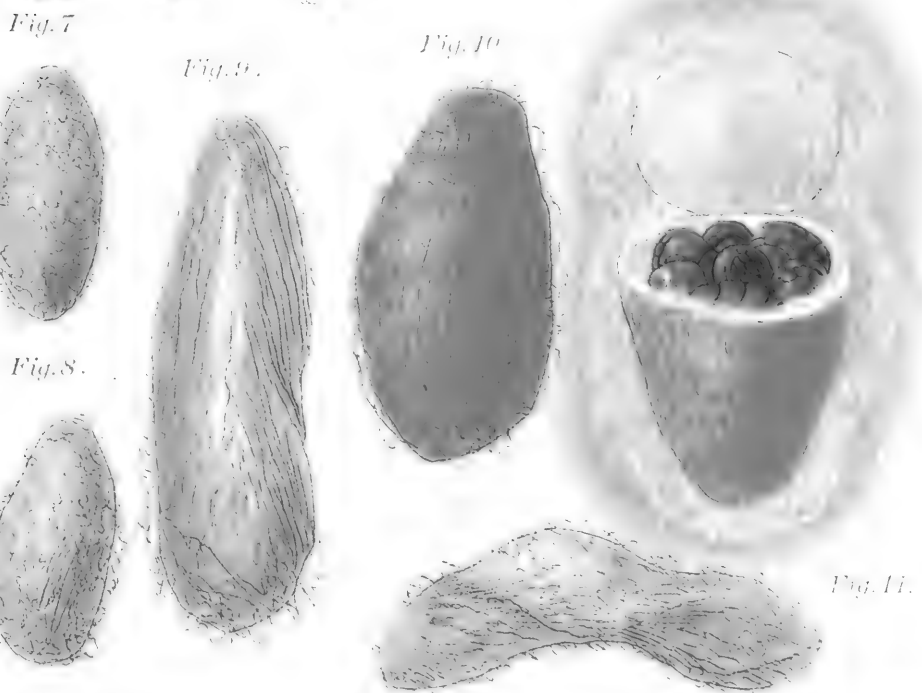
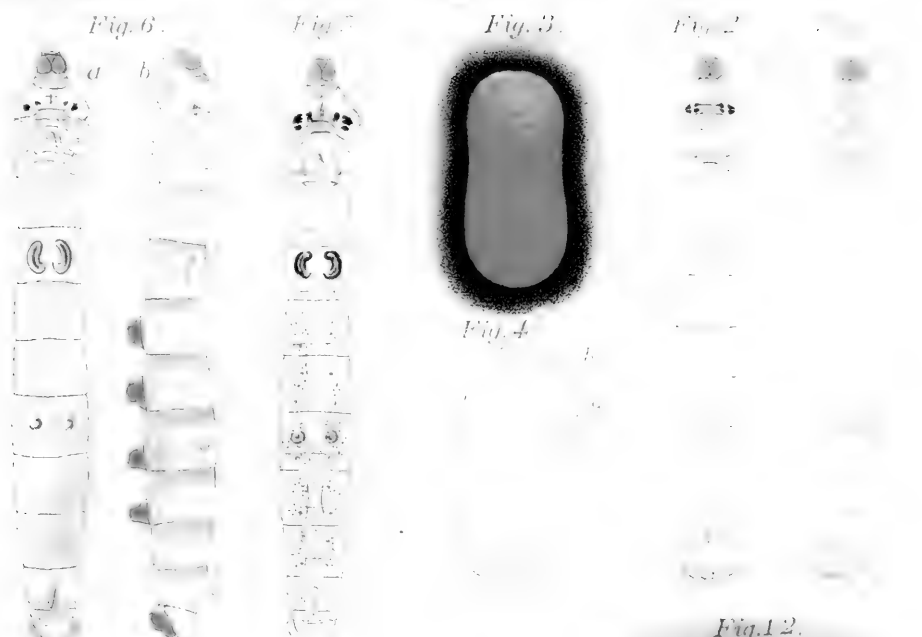
The double cocoons, which are characteristic of this race, are excessively large and very variable in shape; but they are generally hard and compact in texture (Figs. 9, 10, 11, Pl. VI.). They enclose usually more than two chrysalids, and not rarely even seven or eight chrysalids (Fig. 12, Pl. VI.).

The cocoons are mostly ovate-oblong, elongated, triangular and more or less depressed; but still other forms are often met with. The length of the largest cocoons is 7 cm., and the breadth over 3 cm.

Explanation of Plate VI.

- Fig. 1. Mature larva of beggar race without markings 1/1.
Fig. 2. Ditto " " " " with " " 1/1.
Fig. 3. Cocoon showing the coloration.
Fig. 4. Showing the sizes of the cocoons of two breeds.
a, of spring breed. b, of summer breed.
Fig. 5. Mature larva of double cocoon race. 1st variety.
a, dorsal. b, side view.
Fig. 6. Ditto 2nd variety.
Figs. 7, 8. Simple cocoons 1/1.
Figs. 9, 10, 11. Different forms of double cocoons 1/1.
Fig. 12. Double cocoon cut open to show the contained pupae 1/1.
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Sasaki et Yokoyama del.

1-4, Double-Cocoon Race.
5-12, Begger-Race.



On the Feeding of the Silkworms with the Leaves of Wild and Cultivated Mulberry trees.

BY

Prof. C. Sasaki, *Rigakuhakushi*.

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August 1903.

In the year 1900, I made some experiments on the rearing of our native silkworms (Race Aobiki) with two sorts of mulberry trees—wild and cultivated,—in order to examine whether or not these different mulberry trees give any effects on the nature of the filaments, which make up the cocoons. The rearings were carried on by my assistant Mr. Y. Bannai and by a special student, Mr. M. Tokunaga, to whom my acknowledgements are due.

Before entering into details, I think it will not be entirely useless to mention the methods of plantation of our mulberry trees, of which there are two principal ways, viz.—*negari* (cultivated) and *takagi* (uncultivated or wild).

1. The “*negari*” method is extensively practiced in the north-western districts of our main island, where it is esteemed as an improvement. According to this method, the young shoots prepared from cuttings, are planted in a farm in the proportion of 300 to 600 to a single tan.¹

The young shoots thus planted are left for two years without cutting, and in the spring of the third year, the shoots together with the branches, are cut off at the height of about 3 to 5 inches above the ground, and the leaves are used for feeding.

In June and July, many vigorous young branches soon come out from the short stock left after cutting. These branches generally grow to the

¹ Tan is equal to 1/4 acre.

height of several feet or in favourable conditions over ten feet. In the fourth year, the branches left over from the previous year are cut off in May or June exactly in the same manner as mentioned before. The mulberry trees treated by the *negari* method, are usually vigorous, and yield an abundant crop of fine and large leaves; but they have always a tendency to be affected by the so-called dwarf disease widely distributed in our country.

2. The *takagi* method, or that in which the trees are left to their natural growth, is at present largely practiced in the north-eastern as well as the western districts of our main island. The young shoots are usually prepared from seeds, layerings, graftings or cuttings; but those by seeds are mostly employed as stocks for grafting, while the cuttings are only in rare cases selected as shoots or stocks for grafting.

In the spring, some time before the appearance of the leaves, the stems may be cut off at the height of about four feet from their base, and planted in regular files in a farm. In April or May, each stem bears several vigorous branches, which are left over to the next or 2nd year without gathering leaves. In the second year, before the sprouting of the leaves, the branches of the last year, are cut off close to the stem, leaving only three vigorous ones, which may also be cut off at the distance of 2—3 feet from the stem, according to their lengths. From each of these three branches left, may again come out several branchlets, which grow to certain lengths during the same year. The gathering of the leaves from the branches or branchlets for feeding purposes begins in the third year, and thence year after year, the same method of gathering leaves is repeated until the stems die from age. In still other localities, the young shoots prepared from seeds, graftings or cuttings &c., are planted in a farm, and are allowed to grow in a natural condition, no care being taken as to the arrangement or cutting of the branches. It is generally thought that the mulberry trees cultivated after the *takagi* method, yield generally smaller, rigid, and less nutritious leaves than in the *negari* method, whose leaves are larger, more succulent, and look to be richer in nutriment.

It appears to me that, in France and some other European silk-rearing countries as well as in China, the mulberry trees are treated similarly as in our *takagi* method.

In 1898, Mr. F. Lambert,¹ director of the sericultural station at Montpellier, after some experiments on the two sorts of mulberry trees,—wild and cultivated—concluded as follows :—

“ Dix pour cent environ des éducateur font exclusivement usage de la feuille sauvage et paraissent s'en bien trouver. Les bons effets de l'alimentation avec cette feuille plus fine et plus nourrissante que la feuille greffée, poussée dans le même milieu sont manifestes. Les éducateur faites avec cette feuille ont rendu en moyenne plus des 57 Kilogrammes de cocons par once, tandis que les vers nourris avec de la feuille greffe seule or associée à la sauvageonne ont à peine donné 47 Kilogrammes.”

The above experiment tells us that the silkworms reared with the wild mulberry trees yield a larger quantity of cocoons than those reared with the cultivated. It seems, however, the mode of their cultivation differs in details from ours, which makes us quite unable to compare the results of my experiment with that of F. Lambert.

A most renowned weaver, Mr. J. Kawashima of Kyoto, holds the opinion that the raw silk produced in the province of Ōmi, (Shigaken) are far superior in strength and gloss to that of other districts; and for this reason it is used as a material for winding around the hilt of our swords, and also for making the strings of the Japanese guitar (Samisen). The characteristics of this silk depends, without doubt, according to Mr. J. Kawashima, mainly upon the mulberry trees, which are cultivated after the method of *takagi*, and not after the *negari* method. Till now, we have had no opportunity to examine the chemical composition of the leaves of the mulberry trees cultivated by these two different methods.

The main object of this paper is to ascertain whether the silkworms fed with the leaves of these two different sorts of mulberry trees afford silk of different qualities or not.

In order to solve the question, I took 5000 individuals of the race Matakashi (one of the best white race), and reared them in May 1900 at the temperature of 70° to 76 F. Before rearing, they were separated into two equal numbers, and reared with the two different sorts of leaves mentioned before.

¹ Moniteur des Soies No. 1931. 30. IX. 1899.

The number of days in each age or stage of growth, and the average weight and length of the worms in relation to the two different food stuffs, as well as the number of diseased worms during the cultivation, are as follows :—

Race Matamukashi.

The breeding began on the 3rd May and ended on the 6th June, 1900.

Ages.	Number of days in each age.	Aver. weight of 50 full grown silkworms at each age in grms.		Aver. length of 50 full grown silkworms at each age in mm.		Number of diseased worms at end age.	
		Silkworms fed with <i>negari</i> .	Silkworms fed with <i>takagi</i> .	Silkworms fed with <i>negari</i> .	Silkworms fed with <i>takagi</i> .	Silkworms fed with <i>negari</i> .	Silkworms fed with <i>takagi</i> .
I.	7 days 12 hrs.	0.006	0.0062	7	7.5	7	5
II.	5 days 10 hrs.	0.027	0.029	12.5	12.9	110	94
III.	6 days 21 hrs.	0.153	0.173	24.8	25.4	83	68
IV.	7 days 9 hrs.	0.865	0.918	44.55	46.86	13	3
V.	7 days.	3.60	3.70	72.6	73.6	84	116
Number of <i>negari</i> -fed worms which died during spinning cocoons		107		Number of <i>takagi</i> -fed worms which died during spinning cocoons		200	

The average length of ten filaments taken out by unwinding the cocoons of the worms fed with *negari*-leaves was 577.15 metres, while those from *takagi*-leaves was 583.10 metres; the average diameter of the filaments of the former was 0.0264 mm., while that of the latter was 0.0192 mm.

Further, the qualities of the raw silk reeled from 100 momme¹ of the cocoons procured from the worms fed with the two sorts of leaves was as follows :

¹ 1 Momme = equal to 3.75 grms.

	Raw silk reeled from the cocoons of the worms fed with <i>negari</i> -leaves.	Raw silk reeled from the cocoons of the worms fed with <i>takagi</i> -leaves.
Weight of raw silk.....	11,3 momme.	12,7 momme.
Weight of floss silk.....	2,76 „	2,25 „
Tenacity	47,00 grms.	48,00 grms.
Elasticity	11,00 cm.	11,7 cm.
Titre.....	12,9 deniers.	11,1 deniers.

The results of my experiments on feeding the silkworms with two sorts of mulberry leaves, viz. wild (A) and cultivated (B), may be summed up as follows :—

1. Silkworms fed with A and B take the same length of time for their growth.
2. Silkworms fed with A are larger in size at each stage than those fed with B.
3. The weight and length of the silkworms fed with A exceed at each age those fed with B.
4. That the number of diseased silkworms fed with A is larger than those fed with B, depends chiefly upon the presence of parasitic maggots (Larvæ of *Ugimyia sericariæ*, Rond.) which prefer A to B.
5. The length of the filament procured from the silkworms fed with A exceeds that of B.
6. Raw silks reeled from the cocoons of the silkworms fed with A are mostly of superior qualities to those fed with B.





Some Observations on *Antherœa* (*Bombyx*) *Yamamai*, G. M. and the Methods of its Rearing in Japan.

BY

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June 1903.

WITH PLATE VII & VIII.

This well-known moth has been studied by many, both natives and Europeans, and a number of works on this subject have been published both in the Japanese and foreign languages. The principal works are those of C. Personnet¹, Y. Saiki², T. Wardle³, L. Sonthonnax⁴, S. Yaguchi⁵, A. Wallace⁶, &c. With regard to the larva at each stage, the coloration of the moth as well as our methods of cultivation, our knowledge remains still imperfect. This leads me to state some of my observations made on the subject during the past years.

The moth is widely distributed in our country, and we may find it in almost all the mountainous districts. The principal food plants preferred by its larva are *Quercus serrata*, Thunb., *Q. glandulifera*, Bl., *Q. glauca*, Thunb. forma serica, *Q. phyllireoides*, A. Gray and other *Quercus* species.

The larva come forth from the latter part of April, and after the final moult, they attain full size from the end of June to the beginning of July, hence the larval life up to spinning the cocoon lasts for from sixty to seventy days. The larva, after being imprisoned in the cocoon, changes into the pupa at the end of a week or more, and the moth comes forth generally at the end of 40 to 60 days after pupation. The single moth lays from 150 to 300 eggs, and dies at the end of about a week after its appear-

¹ C. Personnet, *Le Ver a soie du Chêne*, 1868.

² T. Wardle, *The Wild Silks of India*, 1881.

³ Y. Saiki, *The Culture of Anthr. Yamamai, G. M.* 1878 (Japanese).

⁴ Laboratoire d'études de la soie Lyon—Essai de classification des Lepiloptères producteurs de soie 1897-1898.

⁵ *Journal of the Silk Industry* Nos. 24, 25, 26, 1895 (Japanese).

⁶ A. Wallace, On the oak feeding silkworm from Japan. *Trans. Ent. Soc.* Vol. V. 1862-64.

ance. The eggs thus laid, hibernate and hatch out in the following spring.

The eggs are nearly roundish, more or less flattened, and are of a dark greyish brown color. The diameter is 3 mm. on the average. They are laid singly or in groups on the stems or branches of the food plants.

Newly hatched larva-Stage I. Length 9 mm. Head dull ochre brown, body light brownish yellow, prothoracic shield light greyish brown bearing a pair of small warts each with 4 yellowish hairs. The five blackish longitudinal streaks (dorsal, subdorsal, and infraspiracular lines) beginning at the 2nd segment of the body, extend as far as the 11th segment, and the dorsal line is much broader than the rest. In the space between the dorsal and subdorsal, the subdorsal and infraspiracular, and the infraspiracular and basal lines, there lies on either side of each of the three thoracic and nine abdominal segments, a single wart provided with more than five blackish or greyish hairs. The subdorsal warts on the 3rd thoracic and the 8th abdominal segments are larger and blackish, and those of the latter lie close to each other. The suranal plate is marked with a blackish triangular, and the outer side of the anal segment (9th abdominal segment) with a blackish marking (Figs. 1, 1, a. Pl. VII.).

End of stage I. Length 13 mm. The head prothoracic shield and warts on the body-segments colored same as in the previous stage; but the body has now changed into bluish green, and the five longitudinal streaks are deep blue (Fig. 2. Pl. VII.).

Stage II. Length 30 mm. Head dull ochre brown as in the previous stage. Body yellowish green. The five longitudinal blackish streaks have disappeared; but there still remains a bluish dorsal line, and there is in addition a pale yellow supraspiracular line. The warts on the body have still the same position and are of the same number, they are now colored orange yellow and are provided each with two sorts of black and yellowish hairs of various length. The warts on the 8th abdominal segment are consolidated into one. The blackish marking on the suranal plate is absent, while that on each side of the anal segment persists (Figs. 3, 3, a. Pl. VII.).

Stage III. Length 42 mm. Head greenish brown. Body, dorsally above the light yellowish supraspiracular line, light yellowish green, and ventrally below the same line, lively green, and covered sparsely with club

shaped light yellowish hair. The supraspiracular line, which begins at the 1st abdominal segment extends to the last segment. The prothoracal shield light greenish yellow. The warts on the subdorsal and supraspiracular lines are reddish brown and are provided with white and blackish hairs of various length; those on the infraspiracular lines are bluish and bear some blackish hairs. Close to the base of the warts on the subdorsal lines of the 3rd thoracic and the 1st and 2nd abdominal segments, as well as on the supraspiracular lines of the 2nd and 3rd abdominal segments, there is a single silvery dot; but it is often absent on the latter lines. The pectoral legs dark brown; the abdominal legs deep green. The suranal plate is bordered at its free edges with a broad brownish marking, which encloses a dark bluish patch. The edges of the anal legs are also colored brown (Fig. 4. Pl. VII.).

Stage IV. Length 58 mm. Head light green, but its color deepens later. The body, dorsally light green, ventrally deep green. A yellowish supraspiracular line which begins on the 1st abdominal segment, and extends to the last abdominal segment, is bordered above with a dark brownish streak, which meets posteriorly with a broad brownish marking on the suranal plate. The warts on the subdorsal, supra. and infraspiracular lines are much reduced in size and are colored blue. They bear brownish or blackish hairs of various lengths. The number of the silvery dots lying on the subdorsal and supraspiracular lines are not definite, and vary in different individuals. The color of the pectoral and abdominal legs are similar as in the previous stage. The following shows the number of the segments which bear silvery spots on the subdorsal and supraspiracular lines.

Nos. of individuals.	Nos. of segments bearing silvery dots on the subdorsal line.	Nos. of segments bearing silvery dot on the supraspiracular line.
1	4	5
2	4, 5, 6	5, 6, 7
3	4, 5, 6	5, 6
4	4, 5, 6	5, 6
5	4, 5, 6	5, 6
6	4, 5	5, 6

Nos. of individuals.	Nos. of segments bearing silvery dots on the subdorsal line.	Nos. of segments bearing silvery dots on the supraspiraculars line.
7	4, 5	5, 6
8	0	0
9	4, 5, 6, 7, 8, 9, 10	5, 6
10	4, 5, 6, 7, 8, 9	5, 6, 7, 8
11	4, 5, 6, 7, 8, 9	5, 6, 7, 8
12	4, 5, 6	5, 6, 7
13	4, 5, 6, 7, 8	5, 6
14	0	5, 6, 7
15	4	0
16	4, 9, 6, 7, 8	5, 6
17	4, 5, 6	5, 6
18	4, 5, 6	5, 6
19	4, 5, 6, 7	5, 6
20	4, 5, 6	5, 6

Silvery dots on the subdorsal lines are usually much larger and more conspicuous on the 5th and 6th segments than on the rest, and those on the supraspiracular lines are generally smaller than the former (Fig. 5. Pl. VII.).

Stage V. Length 95 mm. Head deep emerald green. The prothoracic shield light yellowish green. Body dorsally above the supra-spiracular line, light yellowish green, while ventrally below the same line deep green, and covered all over with short yellowish hairs. The supra-spiracular lines as well as the dark brownish markings on the last abdominal segment and the anal legs are same as in the previous stage. All the warts on the subdorsal, spru. and infraspiracular lines are small, bluish, and provided with greyish or yellowish hairs of different lengths, while those on the subdorsal lines on the 2nd and 3rd segments are yellow. The silvery dots on the subdorsal lines are small roundish and lie close to the warts mostly on the 4th, 5th, 6th, and 7th, segments; while those on the supraspiracular lines lie mostly on the 5th, 6th, 7th and 8th segments, of which those on the 5th, and 6th are much larger and more conspicuous than the rest. The spiracles

dull brown; the pectoral legs light brown, while the abdominal deep green (Fig. 6. Pl. VII.).

Cocoons:—Length 50 mm., breadth 25 mm. on the average. They are oval and compact in texture. The color varies from light greenish yellow to deep green. The surface of the cocoon is rather rough and marked with irregular fine wrinkles. At one end of the cocoon, is a single long silky pedicel with which the cocoon is tightly attached to the branches, from which it hangs down (Fig. 7. Pl. VII.). The cocoon is, in this position, usually protected by leaves nearly on all sides except one. The protected surface of the cocoon is usually light yellowish green, while the exposed surface is deep green, and resembles closely the leaves which protect it.

Pupa.—Large, oval, blackish brown. Length 43 mm. Breadth 18 mm.

Imago ♀.—Head and body bright yellow. Eyes blackish. Antennae plumose with short branches. Prothoracic collar greyish brown. Thorax with a tuft of hairs on each side. Wings are always bright yellow. The costa of the fore wing is greyish brown and forms a continuous band with the prothoracic collar. Fore wing larger than the hind, and with a faint orange line running about the middle from the costa towards the inner margin. At the middle of this line, lies a large transparent eye spot, whose inner side is bordered successively from the inner towards the outer by reddish, brown, white and reddish arcs, and the outer, by yellowish and blackish arcs. A blackish transverse line running between the eye spot and the outer margin of the wing is decorated along its outer side with a whitish line, and then with a reddish brown coloration.

On the area lying between the eye spots and humeral angle, there lies transversely an incomplete zigzag reddish brown line, whose inner side is margined with white.

The eye spot on the hind wing is smaller than that on the fore, and surrounded successively from inner towards the outer by yellow and reddish brown rings. The outer side of the reddish brown ring is decorated by two colored arcs—yellow and black. The upper end of the blackish arc broadens into a large oval dot, while the inner side of the reddish brown ring is also decorated by two colored arcs—whitish and reddish brown. The

colored streaks lying between the eye spot and the outer margin and between the former and the insertion of the wing are almost similar to those of the fore wings. Length 37 mm. Expanse of wings 130 mm. (Fig. 8. Pl. VII.).

Imago ♂.—The coloration is very variable, but there are no specimens, which take the same coloration with the female. The branches of the antenna are very long. The most prevalent colors of the male are of two sorts:—1st. Body greyish brown. Prothoracic collar greyish brown, with the front half white. The fore and hind wings are equally colored greenish brown with light colored inner areas. The number and position of the eye spots and streaks are like those of the female, but their colors change with ground color of the wing. 2nd:—Body and wings dull reddish brown. The prothoracic collar dull brownish white. The fore and hind wings dark reddish brown with lighter colored inner areas. The number and position of the eye spots and streaks same as in the 1st; but their coloration varies more or less according to the ground color of the wing. Length 34 mm. Expanse of wings 127 mm. (Figs. 9, 10, Pl. VIII.).

Methods of culture. The culture of the larva is only practiced by the people in the village Ariakemura in Naganoken on the open grounds, where the food plants are regularly cultivated. The plants selected as the food of the worms are of two species—*Quercus serrata*, Thunb. and *Q. glandulifera*, Bl. They are planted on the ground in the proportion of 2 in every three tsubo.¹ The height of the stems as well as the branches are not allowed to exceed four feet, as otherwise it would be very troublesome and inconvenient in treating the worms.

In spring about a week before the coming forth of the worms, the eggs are pasted on a long and narrow piece of thick and stout paper, and the latter is tied up on the branches of the food plants. When the larva come forth, they crawl on towards the young branches and devour the young tender leaves. Thus the people allow the worms to grow freely in a natural condition. No further care is taken about them, except that the larva are at all times protected from birds, tree frogs, wasps, spiders, &c. If the leaves of a tree are entirely eaten up by the worms, its branches are cut off and transferred to leaf-bearing trees.

¹ Tsubo is equal to 6 feet square.

If the worms attain full growth, they bind together two or more leaves by means of threads, within which they spin a cocoon, so the cocoon is usually protected by the leaves nearly on all sides with only a small uncovered portion. The cocoon is usually colored yellowish green; but its exposed portion is lively deep green. After a week or more, when the exposed surface of the cocoon has the appearance of being covered with a thin white layer (this is the indication of finished pupation), the people collect the cocoons together with the branches on which they are attached, and then hang down the branches on the strings stretched out horizontally under a projecting roof.

When the moths come forth, they are then transferred into a large open worked bamboo basket (diameter about half a metre, height little less than the diameter), within which they are allowed to pair. Each pair is now taken out of the basket, and again transferred to another bell shaped bamboo basket (diameter 20 cm., height 17 cm.) with its wide mouth closed with a large sheet of paper in order to prevent escape. After a while, the female moth will lay the eggs on the inside of the basket successively in two or more days. When egg-laying is finished, the moth is removed, and 6 or 7 empty baskets bearing the eggs on the inside are piled up one upon the other, and may be hung down by means of strings under a projecting roof mentioned before. Later the eggs are scratched off from the basket by the aid of a long piece of bamboo, and then they are spread over on a rectangular wooden frame with a bottom made up of strong grass cloths.

The frame is kept by hanging down in a cool chamber till the following spring, until the eggs can be pasted on the long pieces of paper in order to bind them up around the branches of the food plants as stated before.



Plate VII.

- Fig. 1. Larva of *Antheraea Yamamai*, G.M. 1st stage 5/1.
 Fig. 1, a. Abdominal segment of ditto.
 Fig. 2. Larva of *A. Yamamai*, G.M. at the end of 1st stage 5/1.
 Fig. 3. Ditto 2nd stage 4/1.
 Fig. 3, a. Abdominal segment of ditto.
 Fig. 4. Larva of *A. Yamamai*, G.M. 3rd stage 1/1.
 Fig. 5. Ditto 4th stage 1/1.
 Fig. 6. Ditto 5th stage 1/1.
 Fig. 7. Cocoon of *A. Yamamai*, G.M. 1/1.

Plate VIII.

- Fig. 8. *Antheraea Yamamai*, G.M. Female 1/1.
 Fig. 9. Ditto male 1/2.
 Fig. 10. Ditto male 1/1.



Fig.5.



Fig.4.



Fig.3.



Fig.7.

Fig.1.



Fig.2.

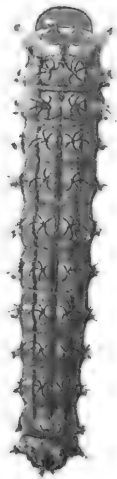


Fig.1.a.



Fig.3,a.



Fig.6.





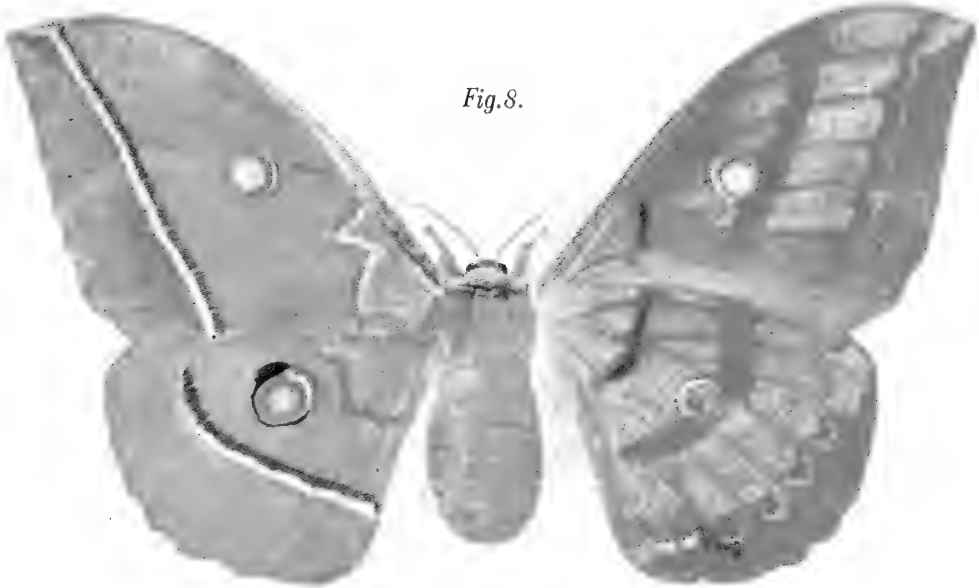


Fig. 8.

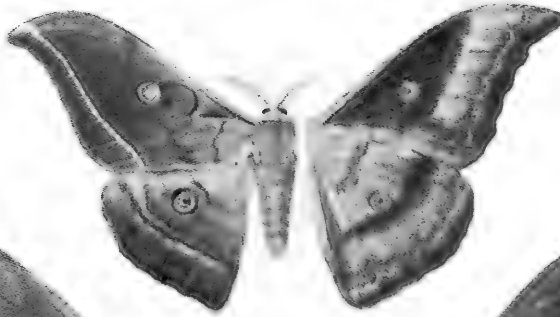


Fig. 9.

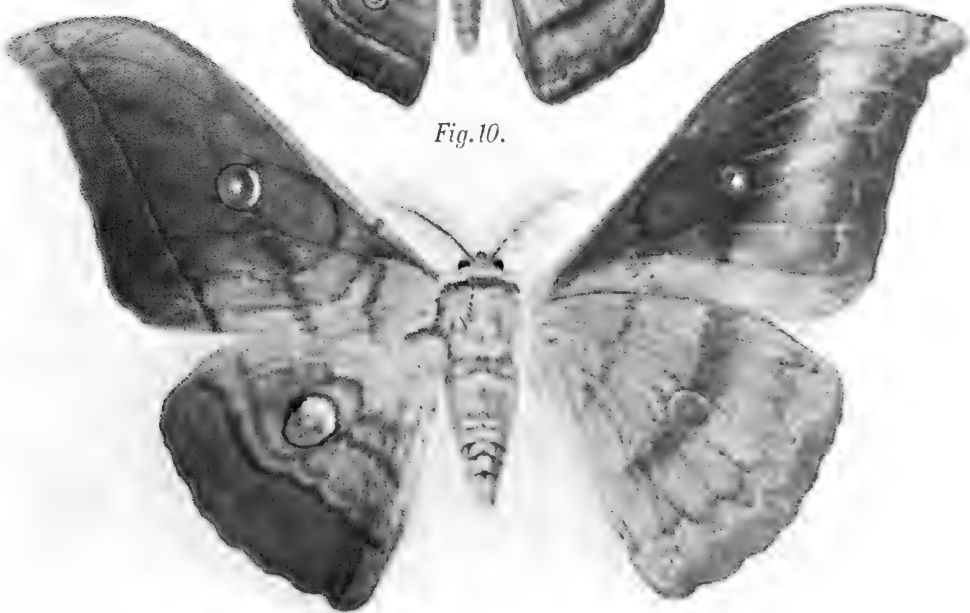


Fig. 10.



A New Field-mouse in Japan.

BY

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May 1903.

(With Plate IX.)

Four years ago, field-mice made their appearance in the prefecture of Ibaraki lying north-east from Tokyo, and the injuries to crops extended not only over the whole prefecture, but also into the neighbouring provinces. In 1900, Dr. S. Onugi¹ and Mr. K. Sanui made some observations on their habits and have carried on their experiments for annihilating them by the inoculation of the so-called mouse typhusbacillus. In January 1901, Dr. S. Onugi² from a study of the characters of the mice and pointing out the differences between the latter and the house-rat, has concluded that the mice was probably of the same species with *Arvicola subterraneus*, Selys. In February of the same year, I also visited the above stated prefecture for the purpose of studying the characters and habits of the mice and made public my results in the Journal of the Agricultural Society of Japan, No. 235.

In 1902, Prof. Y. Kozai³ after two years' experiments for killing the mice by means of Mereskovsky's Bacillus, has obtained a satisfactory result, and at present it is practically employed largely in various provinces.

In the following lines, I will state the results of my studies upon the characters and habits of the hateful mice.

Characters: Body rather small, but plump. Winter pelage: dorsally rusty greyish brown, ventrally greyish white (the dorsal hairs are greyish with rusty yellowish ends, while the ventral are greyish with whitish ends). There is no marked line laterally between the dorsal and ventral colorations. Tail rather short, covered sparsely with hairs, its dorsal hairs dark grey, and the ventral greyish white. Limbs light greyish brown. Snout

¹ Journal of the Agricultural society of Japan, No. 224, 1900.

² Ditto " " " " " " " No. 233, 1901.

³ The Bulletin of the College of Agriculture, Tokyo, Imp. Univ. Vol. IV. No. 5.

rather blunt; eyes dark greyish brown; the upper incisors not projecting beyond the snout. Ears nearly quadrangular (length 13 mm. breadth 8 mm.), about $\frac{1}{2}$ as long as the head, with a few hairs on the inner side, and their outer side as well as the free edges are covered with greyish hairs. They are not completely concealed within the hairs of the head. Incisors yellowish brown on the outer side (Figs. 1; 1, a. Pl. IX.). Hip glands are large and oval. Plantar tubercles 5 (Figs. 3; 3, a. Pl. IX.). Mammeæ 8, inguinal 2—2; pectoral 2—2.

Skull long, smooth and flattened; auditory bulla comparatively large, bulky, and oval in shape (Fig. 2. Pl. IX.). Incisive foramina is very small but distinct. Of the upper jaw, 1st. molar with 4 closed triangles and an anterior loop; 2nd. with three closed triangles, and a posterior triangle with an open base; 3rd. with 3 closed triangles with an anterior and a posterior loop, with 4 inner and 3 outer salient angles; of the lower jaw, 1st. molar with 5 closed triangles, an anterior trefoil and a posterior loop; 2nd. with four triangles and a posterior loop, each triangle on the one side confluent with that on the other; 3rd. with 3 long inner and 3 short outer salient angles (Figs. 4; 4, a. Pl. IX.).

In general aspects and characters, the present species resembles to a certain degree *Arvicola subterraneus*, Selys., which is described by Mrs. H. Leunis¹ and J. R. Bos.²; but it differs in the coloration of the fur, length of the body and tail as well as the size of the ear. This leads us to give it a new name *Arvicola hatanedzumi*.³

Habits: The field mice are mostly found during winter in the farms of wheat, tea, mulberry trees and other plantations. In the day time, they conceal themselves within the subterranean nests, while at night they come out from their hiding places and search for food. If we feed the mice in an enclosure, they remain quiet during the day, but as night approaches they become very active and emit a peculiar cry.

The nest of the mice are usually constructed on the dikes separating rice field or mounds, elevated farms scattered over the same field or along

¹ H. Leunis, *Synopsis der Thierkunde* Band I.

² R. Bos, *Tierische Schädlinge u. Nützlinge*.

³ Hatanedzumi (Jap.) means Farm mouse.

road-sides directly exposed to sun-shine; and moreover even on a plain farm on which various stuffs of halms, stalks or roots of the farms are piled up. The nests are constructed in an oval hollow, excavated usually at the depth of five to seven inches below the ground. Its walls are provided with one or more opening, which communicate with tunnels running in various directions and extending to various distances, where the mice may be able to procure their food. These tunnels open at various distances to the surface of the ground by roundish holes, which serve as a clue to the general direction of the tunnels. The tunnels extend as far as where the food plants are to be detected; and close to the wheat, tea, mulberry and other farms which are preferred by the mice, there open usually one or more holes. In the case of wheat, the mice come out to the surface from the holes opening close by, cut off the stalks or leaves at a height of less than an inch above the ground, and eat them on the spot, or else they carry them to their nests; thus the holes and newly cut stalks or leaves of the wheat indicate, without doubt, the presence of the mice. But in the case of tea and mulberry trees, the mice do injury only to the root by gnawing the cortical layer leaving series of traces of their teeth on the surface of the woody layer (Fig. 5, Pl. IX.).

The nests (Fig. 6, Pl. IX.) are generally oval (length about 22 cm., breadth 14 cm.), or nearly roundish, more or less flattened, consist of a single chamber. The materials employed for the construction of the nests are generally fine strips of straw or fibrous roots of various plants. The inner layer of the nest consists of a much finer and softer stuff than the outer. The nest is provided with the same number of openings as the hollow within which it lies, thus giving the mice free passage towards the tunnels. Close to the hollow in which the nest lies, is excavated another small chamber or hollow mainly used for preserving food. The principal food stuffs, so far as I could find in the store chamber, are strips of the roots of tea or mulberry trees, roots of *Lappa major*, Gærtn., *Daucus carota*, L., the stalks or leaves of the tobacco plant, ears of the rice plant and others. The roots are cut off nearly to an equal length and piled up horizontally in a regular manner in the store chamber; and especially the ears of the rice plant are equally cut off to the length of four to five inches, and then they are piled up

horizontally by arranging regularly the grain bearing end of each ear on the same side so as nearly to fill the chamber.

Generally a single nest is constructed at one spot, but sometimes more are found. The interior of the nest is always very clean and free from dusts or excrements.

On the surface of the ground below which a nest lies, usually open one or more holes, and in the case of the nests formed beneath the inclined surface of dykes, boundaries &c., the holes are not far removed from the nest, and open always more or less below the level of the nest so as to avoid the entrance of rain water. If the nests are constructed below the inclined surface as stated above, the particles of soils will flow out from the holes on the same surface. When the particles of soils look fresh, the mice are almost always present in the nest, while if not fresh they are usually absent, thus we can easily judge of the presence or absence of the mice by the appearance of the particles.

In winter, there may be found several individuals in a single nest; but during the breeding season, it is most probable that a single pair inhabits a single nest.

In capturing the mice, if we dig out the nest slowly, it is always very difficult to find them, for by their acute sense of hearing, they will soon notice the approach of men, and escape through the tunnels running out from the nest. But after having located the nest, let several persons dig out the ground around the nest at the same time at a distance of a few feet from the latter so as not to allow their escape through the tunnels, then the mice can be easily captured.

The pairing season of the mice is not yet accurately known, but they seem to breed several times during the warmer months of the year. They are herbivorous in habit, but when starved they do not hesitate to devour their weaker and inactive mates.

Explanation of Plate IX.

(Figs. 1; 1, a, 6. drawn by K. Yokoyama)

- Fig. 1. *Arvicola hatanadzumi*, Sasaki 1/1.
Fig. 1, a. Ditto.
Fig. 2. Skull of ditto ♀ 1/1.
Fig. 3. Right fore foot 1/1.
Fig. 3, a. Right hind foot 1/1.
Fig. 4. Molar series of right upper jaw 4/1.
Fig. 4, a. Ditto of left lower jaw 4/1.
Fig. 5. Root of mulberry tree with the trace of teeth;
a blackish line shows the level of ground.
Fig. 6. Nest with two openings 1/3.





Fig. 1.



Fig. 1. a

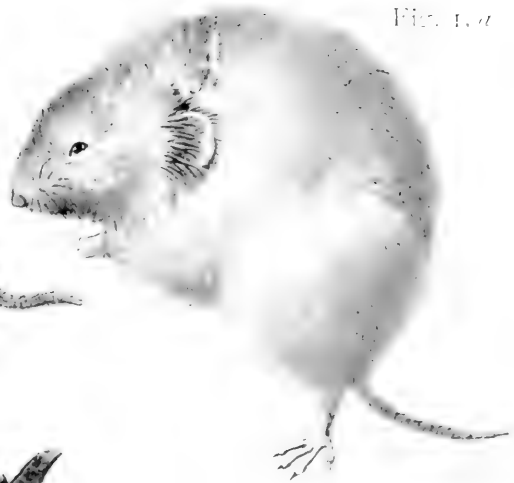


Fig. 2.



Fig. 5.



Fig. 4.

Fig. 4. a



Fig. 6.

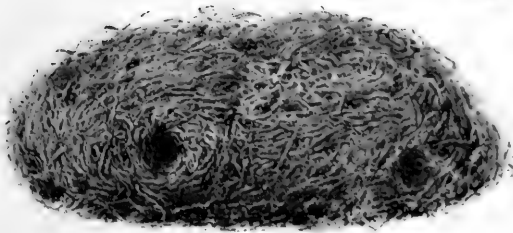


Fig. 3.

Fig. 3. a





Studies on the Lability of Enzymes.

BY

K. Aso.

The cause of the chemical powers of enzymes has frequently been the object of speculation. According to the theory of O. Loew,¹ this activity is intimately connected with the lability of the enzymes, in as much there exist in them certain labil groupings which exert chemical energy—a kind of atomic motion—which can cause chemical changes in certain other compounds. A condition for the action of enzymes is that the compound to be acted upon, shows a certain configuration as was shown by *E. Fischer*.

Various compounds can destroy the activity of enzymes what can be explained by their causing the migration of atoms from the labil to the stable position within the enzyme molecule. But, in most of such cases no conclusion can be drawn as to the nature of the labil groups. Thus, for instance, carbonate of soda in 1 per mille solution will soon destroy the action of pepsin and takadiastase. This is a special case of the phenomenon that alkalies and acids can change various labil compounds to stable ones. In order to be able however to draw certain conclusions as to the *chemical nature of the labil groupings* we must select such compounds that have quite specific actions, even in high dilution and in perfect neutral solution. *Loew* suspected formerly that the lability of enzymes is caused by the simultaneous presence of amido—and aldehyde groups, but his own tests with alkaline silver solution failed.² The presence of aldehydegroups would provide a plausible view, as *Vernon*³

¹ Pflüg. Arch. 27., 212; Die chemische Energie der lebenden Zellen, p. 149; Journ. f. prakt. Chem. 1888, p. 194.

² Pflügers Archiv, für die ges. Physiologie, vol. 27, p. 212.

³ Journ. of Physiology, vol. 29, p. 331 [1903].

has pointed out: "it may, for instance by alternate hydration into CII (OH)₂ groups and subsequent dehydration be able to effect the hydrolysis of proteids, whilst di-amido-or other aldehyde groups by the reverse process may be able to effect the dehydration of caseinogen into casein." As to the action of zymogens, *Vernon* expresses himself as follows: "Let us also provisionally accept *Loew's* hypothesis that ferments differ from inactive proteids in virtue of their containing aldehyde groups. Then we may assume that the formation of ferments in the cells of digestive glands consists in the activation of ordinary proteid molecules by the reduction of some or all of their COOH or acid groupings into CHO or aldehyde groupings."

It is also possible that according to a later view of *O. Loew*, the zymogens contain ketonegroups, and that the activation process consists in the opening of lactamgroups in the zymogen molecule, labil amidogroups thereby being generated.¹ Amidoketones also are very labil bodies as seen from the behavior of diamidoacetone which spontaneously changes to an indifferent substance (*Rügheimer* and *Mieschel*). In regard to the amidogroups *O. Loew* infers their presence from his observation that dilute formaldehyde easily destroys the action of enzymes at the ordinary temperature.² It is well known that formaldehyde easily attacks amidogroups of a certain lability, e.g.:



Thus if labil amidogroups in enzymes would be changed in an analogous manner, the activity of this grouping would of course be lost, since the amidogroup as such has disappeared. The following table shows the more or less intense action of formaldehyde on enzymes.

¹ *Centrallbl. f. Bakteriologie* 12, p. 445.

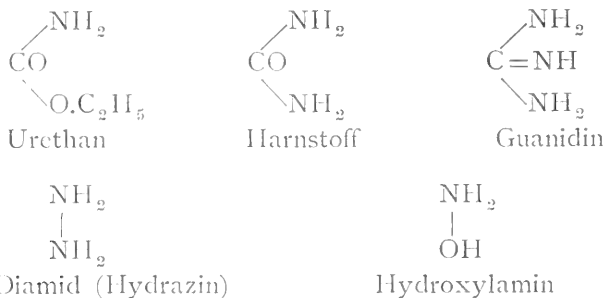
² *Journal für prakt. Chemie* 1888, vol. 37, p. 104. Such observations were later on made also by various authors.

Enzym.	Formaldehyde. ¹	Time in which the enzym is killed.	Author.
Diastase	1%	in 24 hours	Bokorny.
Myrosin	5%	soon	„
Rennet	0.5%	„	„
Zymase	0.05%	„	Wroblewsky
Sucrase	5%	one hour at 54° C.	Pottevin
Catalase	4%	1 hour	Loew
Pepsin	5%	24 hours	„
Pepsin	4%	24 hours	Sawamura
Papain	0.4%	Soon at 40°	Vines

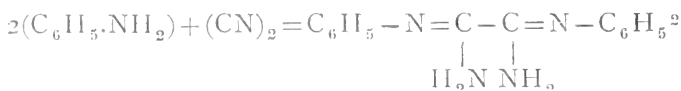
It is true that formaldehyde will act also on hydroxylgroups and produce methylene-compounds as, e.g., with the polyvalent alcohols, yielding the so-called "formals," but in order to accomplish this, application of heat in presence of hydrochloric acid is required, hence the conditions are far different from those just mentioned. The inference that amidogroups of a certain lability are concerned in the activity of enzymes would receive further support, if it could be shown, that the enzymes take

¹ The commercial formalin or formal contains about .40% formaldehyde.

up free cyanogen and would lose thereby their activity.¹ As to the different lability of amidogroups *Loew* expresses himself as follows: "Die Amidogruppe kann unter gewissen Umständen stabil, reactionsunfähig, unter anderen aber wieder äusserst labil und reaktionsfähig sein. Die Amidogruppe ist Z. B. im Urethan sehr stabil, im Harnstoff labiler, noch mehr im Guanidin. Im Hydroxylamin und Diamid aber ist sie so energisch geworden, dass diese Stoffe selbst bei grosser Verdünnung noch in alles lebende Protoplasma ohne Ausnahme eingreifen können, d. h. Gifte allgemeinen Charakters sind. Folgende Formeln lassen die Einflüsse benachbarter Gruppen auf die Energie der Amidogruppe erkennen:"



When dicyanogen acts on amines, it forms as chief compounds addition products with two molecules of amine. Thus



When, however, it acts on amidocompounds several products may result. Thus the chief product with amidobenzoic acid



¹ Also hydroxylgroups can take up cyanogen at the ordinary temperature as *Loew* has shown with pyrogallol. But this is the only case known thus far. (Journ. f. prakt. Chem. Vol. XV. 326.) Very labil methylene groupings as in the acetylacetic ether also can react with cyanogen but only in presence of sodium ethylate $\text{C}_2\text{H}_5\text{ONa}$ (W. Traube). Thus far, however, only a few such cases have been described.

² This formula has been shown by *Villmann* to correspond better to the behavior of the cyananiline than the former imidoformula.

It is noticeable, however, that not every amido compound and amide can combine with dicyanogen, and imidogroupings are not acted upon at all. Neither hydrazobenzol nor asparagine nor urea are acted upon. But *Andreasch* has shown that methylthiourea enters into reaction. The peculiar behavior of free cyanogen towards highly labile amidogroupings have induced *Loew* and *Tsukamoto*¹ to test the behavior of a highly diluted aqueous solution of dicyanogen towards living organisms of the most different kind. These tests have revealed highly poisonous properties of dicyanogen rendering the presence of labile amido groupings in the proteins of living matter highly probable. It was now of interest to test whether it could also kill the enzymes. Since most enzymes belong in all probability to the protein group it could hardly be doubted that dicyanogen would act on enzymes when applied in excess to a concentrated solution, since *Loew* has shown that cyanogen combines with ordinary albumin.² He applied solutions containing 10–25% albumin. In my experiments with enzymes, the dilution was a much higher one, since it was to be expected that very labile amido groupings would take up dicyanogen in high dilution, hence a reaction under this condition would admit a safer conclusion as to the degree of lability.

Experiment with Pepsin.

2 grams of commercial pepsin³ were dissolved in 200 c.c. water and divided into halves.⁴ Cyanogengas developed from 5 grams mercuric cyanid was passed through one of these bottles which, well closed, was left for 12 hours, Thereupon 10 c.c. of 2% hydrochloric acid were added and some fibrin, previously swollen in dilute hydrochloric acid, and kept for 24 hours at 28° C. The fibrin was dissolved rapidly in both cases

¹ Forschungsberichte über Lebensmittel, Vol. I, No. 7;—Journ. College of Science, Tokyo, 1896.
Cf. also These Bulletins Vol. II, No. 7.

² Journ. f. prakt. Chem. 1877.

³ The solution of this sample was acid.

⁴ Some ether was added to the control flask to prevent bacterial growth.

and neither the precipitation with nitric acid nor the saturation with ammonium sulfate did show any difference; nor the colorimetric comparison of the biuret reaction. In a second experiment, 2g of pepsin were dissolved in 200 c.c. of water and 20 c.c. of a 2% hydrochloric acid added. One half served as control, while the other half was treated with the same quantity of cyanogengas as before, but in this case, the solution was kept at 35-40°C during the treatment. Moreover this liquid was placed in the incubator at 28°C for 20 hours before testing its proteolytic action. Equal quantities of fibrin and thin square slices of boiled egg white were now added to both liquids which were kept at 28°C for one hour. The fibrin was dissolved also here and the egg-albumin was almost wholly digested after 3 hours in both cases. In the third experiment the conditions were again changed. Pepsin, 1g, was dissolved in 200 c.c. water and 1g of sodium carbonate (anhydrous) added. 100 c.c. of this solution were treated with the same quantity of cyanogen as before and left for 12 hours. After neutralizing 10 c.c. of 2% hydrochloric acid were added and fibrin. The result showed that the pepsin had been killed by the sodium carbonate. This result is not surprising considering the great sensitivness of pepsin toward alkaline liquids. *Green* reports that pepsin is injured by 0.002% soda solution after 1-2 hours at bodily temperature.¹ Nevertheless, a further experiment was made with the modification that the pepsin solution was rendered but very slightly alkaline with sodium carbonate. The passing of cyanogen from 5 grms of mercuric cyanide on heating took about one hour. Afterwards 10 c.c. of 2% hydrochloric acid were added to 100 c.c. of pepsin solution, the treated one, as well as the control. The same quantity of fibrin was added in both cases, but no digestion took place in either case. In the final experiment the acidity was hardly perceptible to litmuspaper; the further treatment was the same as just mentioned. In both cases, the fibrin was quite dissolved after one hour while the slices of boiled egg disappeared after 12 hours.

¹ *Langley* and *Eves* found a distinctly inhibitory action to be manifested by the presence of as little as 0.0015% of sodium carbonate.

Experiment with Trypsin.

1 gram of commercial trypsin was dissolved in 200 c.c. of water containing 0.4g. sodium carbonate and divided into halves, one serving as control and the other being treated with cyanogen gas developed from 5 grms of mercuric cyanid. After 12 hours an equal quantity of fibrin and two thin square slices of boiled egg were put in each solution. After two hours at 28°C the fibrin was almost completely digested in both solutions, also the egg slices were very much attacked, but did not disappear completely after 20 hours. In the next experiment, the quantity of mercuric cyanid was doubled, but the result was the same as before.

Experiment with Emulsin.

A solution of 0.1% emulsin with 0.1% Na_2CO_3 was treated with cyanogen gas developed from 5 grms mercuric cyanid. After standing for 48 hours, 0.1g. of amygdalin was added to 10 c.c. of the solution. After one hour at 28°C, the peculiar odor of benzaldehyde was plainly perceptible like in the control case. The decomposition of amygdalin was also shown by the reaction with ammoniacal silver solution and with fuchsin solution decolorized by sulphurous acid, proving the formation of the decomposition products of amygdalin, viz. of glucose as well as of benzaldehyde.

Experiment with Takadiastase.

0.2g. of commercial takadiastase were dissolved in 200 c.c. of water. This solution had a faint acid reaction and was rendered faintly alkaline by sodium carbonate. One half was treated with cyanogen developed from 5 grms mercuric cyanid while the other half served as control. After 24 hours standing the amylolytic action was compared. 10 c.c. of these solutions were mixed with 10 c.c. of 0.1% starch paste suspension

and kept at 30°C for 2 hours. On addition of iodine solution, no blue reaction set in, showing that the starch was transformed equally well in both cases.¹ On boiling with Fehling's solution, a strong sugar reaction was obtained and upon warming with ammoniacal silver solution, a silver mirror appeared in both cases. The enzyme had therefore not lost its activity by the treatment with cyanogen.

Experiment with Oxidases.

45g. of a fresh radish root were triturated with addition of 100 c.c. water. Through 50 c.c. of this extract, cyanogen gas developed from 5 grms mercuric cyanid was passed while 50 c.c. served as control. After standing 15 hours, the cyanogen gas was replaced by air and the liquid tested for oxidizing enzymes in the usual manner, but the treated solution gave the color tests much weaker than the control. The experiment was repeated with the result, that when after 24 hours standing the color tests were made, they failed almost completely. Since dicyanogen in aqueous solution soon forms some prussic acid it was possible that some prussic acid had paralyzed the action of the oxidizing enzymes. Hence in the next trial the treated liquid was left to evaporate at 40-50°C to remove the prussic acid, whereupon the guaiac and the guaiacol test for peroxidase were readily obtained, only the guaiac test for oxidase was somewhat weaker.

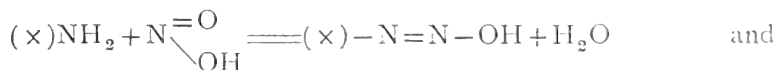
In all the cases here described *dicyanogen has failed to destroy the activity of the enzymes*, what reveals a great chemical difference between the lability of enzymes and the lability of the active proteins in the living protoplasm.

Loew and *Tsukamoto* (l. c.) have observed that a fresh solution of dicyanogen in water in a dilution of 1 : 5000 kills bacteria and of 1 : 10000

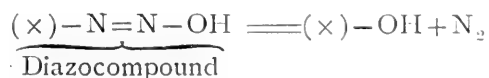
¹ Although this solution had a weak alkaline reaction, the diastase was not perceptively injured. *Chittenden* and also *Grützner* have observed an injurious action of small quantities of alkalies, but *Epstein* and *Schulze* found that hereby this enzyme is not destroyed, but merely "paralysed," because by neutralization the activity is again restored, at least partially.

phaenogams, algae and lower aquatic animals. Here exists then another striking difference in the chemical behavior of living protoplasm and enzymes.

The apparent indifference of enzymes to dicyanogen shows either that the amido groupings in the enzymes are not of sufficient lability or that they are protected by other neighboring atomic groupings, forming a steric obstacle in the molecule. The inference that there are no amido groups present at all would be improbable considering the behavior of enzymes towards formaldehyde. Further tests therefore seemed necessary to demonstrate the participation of the amidogroups in the activity of the enzymes. Here the behavior towards nitrous acid promised to furnish some clue. In my experiment with enzymes I added to highly diluted solution of sodium nitrate and sodium nitrite the theoretically necessary quantity of sulphuric acid.¹ With those enzymes which are injured very easily by any kind of acids special care was necessary to apply nitric and nitrous acid in a very high dilution. The cause of the injurious action of nitric acid would of course be very different from that of nitrous acid. The former in high dilution would act like dilute sulphuric acid causing atomic migration of labil atoms while nitrous acid would act on labil amido groups in the following manner:



in the case of aliphatic compounds development of nitrogen would immediately follow the formation of a diazocompound:



Experiment with Pepsin.

1). 0.3g of pepsin were dissolved in 300 c.c. water, and divided into three equal parts. To one potassium *nitrite* and sulphuric acid in

¹ In this connection it is also an interesting fact that a very high diluted nitrous acid (1 : 100000) is much more poisonous for lower organisms than nitric acid, as *Loew* and *Bokorny* have shown.

high dilution were added in the calculated proportions to produce free nitrous acid of 0.1% in the liquid. To another potassium *nitrate* and sulphuric acid were added in such proportion as to produce 0.1% nitric acid, while the third portion served as control. The nitrous acid soon caused a yellowing of the pepsin solution, while nitric acid did not. After standing 24 hours,¹ an equal quantity of fibrin previously swollen in dilute hydrochloric acid and washed, further two square slices of boiled egg white and 10 c.c. of a 2% hydrochloric acid were added.

	After 1/2 hour at 40°C.	After 1 hour at 40°C.
Control.	Fibrin dissolved; egg white not yet.	Egg white wholly dissolved.
Nitrous acid.	Fibrin dissolved; egg white almost unchanged.	Some egg white still remained.
Nitrous acid.	Some pieces of fibrin dissolved; egg white remained intact.	Egg white not attacked.

An equal quantity of fibrin was again added;

	After 1/2 hour.	After 1 hour.	After 17 hours.	After 3 days.
Control.	Fibrin and egg white dissolved.	—	—	—
Treated with nitric acid.	Fibrin as well as egg white attacked.	Both almost dissolved.	—	All dissolved.
Treated with nitrous acid.	Fibrin and egg white not dissolved.	Both unchanged.	Fibrin dissolved, but not egg white.	Egg white a little attacked.

2). In a second experiment 0.2% nitric acid and nitrous acid were applied in the same way as before. In the control solution however, 0.2% sulphuric acid was added to show the effect of the mere acidity. The solutions were kept at the ordinary temperature for 24 hours.

¹ The solution treated with nitrous acid developed a peculiar odor of certain nitro-compounds.

Hereupon 10 c.c. of a 1% hydrochloric acid were added to each solution further an equal quantity of fibrin and two square slices of boiled egg white. These solutions were kept at 40°C.

	After ¼ hour.	After ½ hour.	After 1 hour.	After 2 days. (at ordinary temp.)
0.2% sulphuric acid.	Fibrin dissolved very little.	Fibrin almost dissolved; Egg white unchanged.	Fibrin dissolved. Egg white unchanged.	Egg white dissolved.
0.2% nitric acid.	Fibrin little dissolved.	Fibrin almost dissolved. Egg white unchanged.	Fibrin dissolved. Egg white unchanged.	Egg white dissolved.
0.2% nitrous acid.	Not dissolved.	Some fibrin dissolved. Egg white not.	—	Some fibrin and egg white still unchanged.

A further quantity of fibrin and egg white was added and kept at 40°C :

	After ½ hour.	After 1 hour.	After 20 hours.
0.2% sulphuric acid.	Fibrin almost dissolved. Egg white unchanged.	All fibrin dissolved. Egg white hardly unchanged.	Egg white dissolved very much.
0.2% nitric acid.	Fibrin almost dissolved. Egg white unchanged.	All fibrin dissolved. Egg white hardly unchanged.	ditto.
0.2% nitrous acid.	Much fibrin unattacked. Egg white unchanged.	Much fibrin and all Egg white unchanged.	Egg white unchanged; Some fibrin still present.

3). In the third test, 0.1g. of nitrous, nitric and sulphuric acid were added respectively, each bottle holding 100 c.c. of 0.1% pepsin solution, and immediately heated at 35°C for one hour. 10 c.c. of 1% hydrochloric acid, further eggwhite and fibrin were then added.

	After 2 hours at 40°	After 24 hours at 40°
0.1% sulphuric acid.	Fibrin dissolved.	Egg white almost dissolved.
0.1% nitric acid.	"	"
0.1% nitrous acid.	Some fibrin unattacked.	Egg white unchanged.

0.1 gm. of nitrous, nitric and sulphuric acid was again added to these solutions respectively, and kept at 40° for one hour. An equal quantity of fibrin was then added.

	After 1 hour at 40°
0.2% sulphuric acid.	All fibrin and all egg white dissolved.
0.2% nitric acid.	" " "
0.2% nitrous acid.	All fibrin and egg white undissolved.

Experiment with Trypsin.

Into three flasks holding 100 c.c. of a 0.5% trypsin solution, 0.05 grams of nitrous, nitric and sulphuric acid were added and kept at 40° for one hour. These solutions were now neutralized and 10 c.c. of a 2% sodium carbonate solution added and some fibrin.

	After 2 hours at 40°	After 17 hours at 40°
0.05% sulphuric acid.	Some fibrin dissolved.	All fibrin dissolved.
0.05% nitric acid.	" " "	" " "
0.05% nitrous acid.	Fibrin undissolved.	Fibrin undissolved.

Experiment with Emulsin.

This test was made in the same way as in the last mentioned case. After 20 hours, 10 c.c. taken from each flask received 0.1g. of amygdalin. After 15 minutes at 40°C, the following was observed.

Control.	Odor of benzaldehyde and prussic acid. Reduction with Fehling's solution and with ammonical silver solution.
0.1% nitric acid.	No odor developed. No reduction took place with the above-named reagents.
0.1% nitrous acid.	” ” ”

The result did not differ when the amygdalin was added after neutralisation of the liquids.

2). In the next experiment, the quantity of nitric and nitrous acids was reduced to 0.05%, while to the control solution sulphuric acid was added in the same concentration. After keeping for 16 hours at 18° these solutions were neutralized and 0.5g. amygdalin added. On keeping these mixtures now at 40° for three hours, the following was observed:

0.05% sulphuric acid.	The characteristic odor of benzaldehyd appeared very plainly.
0.05% nitric acid.	” ” ”
0.05% nitrous acid.	No odor developed.

These tests with pepsin, trypsin and emulsin show therefore, that nitrous acid destroys the activity of these enzymes more easily than do nitric and sulphuric acids in the same high dilution.

In order to test for *ketone groups*, experiments were made with hydrazine, methylhydrazine and hydroxylamine. The solutions of the salts of these bases having an acid reaction were neutralised with sodium carbonate. From the amount of salt weighed out, the amount of the free bases was calculated.

1) *Hydrazine*.

Pepsin: 100 cc. 0.1% pepsin solution + 1% free hydrazine.

After two hours at 40° 0.2% hydrochloric acid and three flocculi of fibrin were added and kept again at 40°C.

	After $\frac{1}{2}$ hour.	After 2 hours.	After 4 hours.
1% free hydrazine.	Fibrin not attacked at all.	Not dissolved.	Not attacked at all.
Control.	Fibrin wholly dissolved.	—	—

Trypsin: 1% trypsin solution + 1% free hydrazine.

After two hours at 40°C, 0.2% sodium carbonate and three flocculi of fibrin were added, keeping the mixtures in the incubator.

	After 2 hours.	After several days.
1% free hydrazine.	Fibrin unattacked.	Not attacked.
Control.	Almost dissolved.	All dissolved.

Diastase: Solution of 0.1% diastase + 1% free hydrazine.

After keeping at 40°C for 2 hours some 0.1% starch paste was added and kept at 40°C, for 2 hours. After evaporating and removing the hydrazine with alcohol the residue was treated with water and tested with iodine dissolved in potassium iodid.

1% free hydrazine.	Blue starch reaction.
Control.	No starch reaction.

Emulsin: 0.1% emulsin solution + 1% free hydrazine.

After keeping at 40°C for 4 hours, 0.1 gm. amygdalin was added to 10 c.c.

1% free hydrazine.	The odor of benzaldehyd and prussic acid developed very weak.*
Control.	The odor was very strong.

These solutions were kept at 40°C and tested in the same way again:

	After 6 hours.	After 8 hours.
1% free hydrazine.	A very weak odor appeared.	Trace of odor.
Control.	Very strong odor.	Very strong odor.

I have observed further that also zymase is easily killed by a 1% solution of hydrazine.

2) *Methylhydrazine.*

I. *Experiment.*

Emulsin } 0.2% solution + 0.032% free methylhydrazine.
Pepsin }

Trypsin 0.5% „ + „

After keeping at 40°C for 1 hour was added:

To emulsin: 0.1g. of amygdalin

„ Pepsin: 0.2% HCl

„ Trypsin: 0.2% Na₂CO₃

Emulsin: After keeping at 40°C for 15 min. the enzyme was still active but weakened somewhat by methylhydrazine.

* It might be objected, that the diminution of the odor might have been due to the formation of benzyliden hydrazine but the control tests with the fresh mixture and that which had been tested after 8 hours digestion at 40° revealed a great difference in the intensity of the odor. The formation of benzyliden hydrazine from benzaldehyde and hydrazine in such high dilution does not take place instantaneously.

	Pepsin	Trypsin.
After 2 hours.	Fibrin dissolved.	Still some fibrin.
" 4 "	"	All dissolved.

II. *Experiment.*

Emulsin 0.1% + free methylhydrazine 0.64%
 Pepsin 0.1% + " " "

After 2 hours at 40°C was added :

To Emulsin 0.2g. amygdalin,

To Pepsin 0.2% HCl and 3 flocculi of fibrin.

After 15 Min. at 40°, the tests showed that emulsin was still active in the 0.64% solution of free methylhydrazine, but it was weaker than in the control case.

After one hour at 40°C,

Pepsin	{	0.64% free methylhydrazine—Fibrin not dissolved at all, even not after 2 days.
		Control: All fibrin dissolved.

III. *Experiment.*

Emulsin 0.1% + free methylhydrazine 0.64%.

Kept at 32°C for 20 hours, and 0.1 grm. of amygdalin added and warmed.

Immediately tested :

Control:—Odor of benzaldehyde.

Treated:—No odor at all.

IV. *Experiment.*

- Takadiastase 0.1% + free methylhydrazine 0.32%.

After 20 hours at 24° 10 c.c. of a 2% starch paste were added, the mixture kept at 40° for 2 hours, then evaporated and extracted with alcohol to remove the methylhydrazine. The residue was treated with some water.

Control:—Already after $\frac{1}{2}$ hour, no longer any iodine reaction for starch.

Treated:—Blue starch reaction.

These tests leave no doubt that hydrazine and methylhydrazine injure enzymes very much or kill them.

3) *Hydroxylamine.*

Pepsin 0.1% + free hydroxylamine 1%

Trypsin 0.5% + { free hydroxylamine 0.5%
 „ „ „ „

Diastase 0.1% + hydroxylamine 1%

Emulsin 0.1% + „ „

After keeping at 40° for 2 hours was added:

to pepsin: 0.2% HCl and three flocculi of fibrin.

to trypsin: 0.2% Na₂CO₃ and „ „ „

to diastase: 0.1 gram. of starch in the form of starch paste.

to Emulsin: 0.1 gram. amygdalin.

and the mixtures kept at 40°C for 2 hours:

Pepsin: Control:—Fibrin dissolved completely.¹

1% hydroxylamine:—Fibrin undissolved.

Trypsin: Control:—Almost all fibrin dissolved.

0.5% hydroxylamine:—Fibrin unattacked.

1% „ „ :— „ „

¹ The fibrin had dissolved already after 30 Min.

Even after 24 hours standing at the ordinary temperature, the fibrin was not dissolved where hydroxylamine had been added.

Diastase: Control:—No starch reaction with iodine.

1% hydroxylamine:—Strong starch reaction.¹

Emulsin: immediately tested after adding amygdaline.

Control:—Strong odor developed.

1% hydroxylamine:—Slight odor of prussic acid and benzaldehyde.

This test was repeated by adding amygdalin after heating the enzym solution with hydroxylamine to 40° for 4 hours.

Control:—Strong odor.

1% hydroxylamine:—No odor.

These observation on the injurious action of the hydroxylamine on enzymes are in accord with a former observation of *O. Loew* on diastase.²

The following table shows the results obtained:

	Pepsin.	Trypsin.	Emulsin.	Diastase.
Free NO ₂ H	Kills at 0.2% in one hour at 40°C.	Kills at 0.05% in one hour at 40°C.	Kills at 0.05% in 16 hours at 18°.	—
Free N ₂ H ₄	Kills at 1% in two hours at 40°C.	Kills at 1% in two hours at 40°C.	Nearly kills at 0.1% in 8 hours at 40°C.	Kills at 1% in two hours at 40°C.
Free N ₂ H ₂ .CH ₃	Kills at 0.64% in two hours at 40°C.	Injures at 0.032% in one hour at 40°C.	Kills at 0.64% in 20 hours at 32°C.	Kills at 0.32% in 20 hours at 24°C.
Free NH ₂ .OH	Kills at 1% in 2 hours at 40°C.	Kills at 1% in 2 hours at 40°C.	Kills at 1% in 4 hours at 40°C.	Kills at 1% in two hours at 40°.

Conclusion.

1. Enzymes in high dilution are not killed by small quantities of dicyanogen. Hereby another essential difference between the chemical behavior of the living protoplasm and that of enzymes is established.

¹ Small doses of iodine are changed to HI by hydroxylamine, hence an excess must be added here to obtain the starch reaction.

² Journ f. prakt. Chem. 1888. p. 104.

2. Nitrous acid in very high dilution is more injurious for enzymes than equally diluted nitric acid.

3. Hydrazine, methylhydrazine and hydroxylamine in dilute neutral solutions destroy the activity of enzymes. This would be best explained if the active grouping in the enzymes are either aldehyde or ketone groups. According to *Loew's* present view, ketone groups alone can come here into consideration.





Ueber fungicide Wirkungen von Pilzculturen.

VON

Y. Kozai und O. Loew.

Es ist seit lange bekannt, dass die Culturen mancher Bacterienarten Stoffe enthalten, welche das Wachstum anderer Bacterienarten hemmen oder verhindern. Bei den Culturen des *Bac. pyocyaneus* ist es ein Enzym, welches geradezu manche andere Bacterien auflöst.¹ Auch die Entwicklung von Schimmelpilzen ist öfters auf Culturflüssigkeiten nicht möglich, in denen sich gewisse Bacterienarten entwickelt haben, obwohl es sich hier nicht um Aufloesung der Mycelfäden handelt. In neuerer Zeit haben ferner *E. Bourquelot* und *Hérissey*² beobachtet, dass ein Extract von *Aspergillus niger* die Gärtätigkeit der Hefe beeinträchtigt, die Hefe selbst aber nicht tötete. Diese Wirkung wurde selbst nach dem Aukochen der Loesung nicht aufgehoben.

Die Beobachtung nun, dass der in Japan unter dem Namen Miso bekannte vegetabilische Käse selbst in der heissesten Zeit des Sommers nicht schimmelt, trotzdem er in hoch feuchtem Zustande dem Staube der Luft angesetzt in offenen Läden feil gehalten wird, bewog uns, auch die Culturflüssigkeit des *Aspergillus oryzae* auf fungicide Eigenschaften zu prüfen. Jener Miso wird nämlich mit Hülfe dieses Pilzes, resp. dessen Enzymen aus gekochten Soyabohnen dargestellt.

Er enthält 50-60% Wasser, 5-11% Kochsalz, 6-12% Rohprotein, 5-6.5% Fett und 13-24% Kohlehydrate und Extractstoffe. Die Reaction ist meist ganz schwach sauer. Der Kochsalzgehalt ist zu gering, als

¹ Siehe R. Emmerich und O. Loew, Z. Hyg. 1898.

² Jahresber. f. Thierchemie 1895, S. 623.

dass derselbe das Schimmeln verhindern könnte. Wir liessen ihn in einem offenen Becherglase während des Monats August bei einer alltäglich auf 33–35°C steigenden Temperatur stehen und beobachteten dabei eine allmählich sich entwickelnde Hefeschichte, welche dann von *Sarcina* überwuchert wurde. Schliesslich wurde auch diese durch *Bac. prodigiosus* verdrängt. Es wurde keine Spur von *Penicillium* oder *Aspergillus* entwickelt. Die Reaction war schliesslich alkalisch geworden. Um nun zu prüfen ob *Aspergillus oryzae* Stoffe produciren kann, welche auf ihn selbst sowohl als auf nahestehende andere Fadenpilze schädlich wirken, wurde jener Pilz auf folgender Loesung cultivirt:

Pepton	—	1%
Zucker	—	0.5%
$K_1H_2PO_4$	—	0.2%
$MgSO_4$	—	0.02%

Es wurden 4 Kolben, je 500 cc. dieser Loesung enthaltend, aufgestellt und nach dem Sterilisiren inficirt, am 18 September. Um die Sporenbildung zu verhindern und die Mycelbildung zu begünstigen, wurden die Kolben täglich umgeschüttelt. Einer der Kolben wurde am 6 October geöffnet, der Inhalt (der noch etwas unzersetztes Pepton enthielt) durch sterilisirte Filter in sterile Kolben filtrirt und ein Teil direct, der andere nach dem Aufkochen mit *Penicillium*sporen inficirt. Nach einigen Tagen zeigte sich in beiden Flaschen eine sehr langsam sich entwickelnde Schimmelvegetation. Es wurden desshalb die anderen Kolben noch länger stehen gelassen, bis das Mycel den Nährboden erschöpft hatte und dem Absterben unterlag. Das letztre schien uns aus der allmählich eintretenden Schwarzfärbung der Flüssigkeit (Folge von austretenden oxydirenden Enzymen?) zu folgen.

Am 20 November wurden nun zu einem Kolben 2 g sterilisirtes Pepton gesetzt und dann wie oben verfahren. Es ergab sich, dass diesmal selbst nach 11 Tagen bei 10–16°C. keine Spur einer Schimmelvegetation eintrat. Auffällig war, dass auch in der einen Moment aufgekochten Portion sich keine Entwicklung nach der Impfung zeigte.

Es muss also die Production eines gewissen fungiciden Stoffes durch

den Pilz *Aspergillus oryzae* gefolgert werden. Diese Substanz ist aber für verschiedene Pilze nicht in gleichem Maasse schädlich.

Im Anhang hiezu wurde noch ein zweiter Versuch mit *Penicillium* sporen gemacht, welche diesmal auf eine nur schwach alkalisch reagierende Culturflüssigkeit des *Bac. pyocyaneus* ausgesät wurden. Es fand auch nach Wochen keine Entwicklung von *Penicillium* statt, während im Controlversuch dieselbe sehr lebhaft war.





Zur Frage der Existenz des Pyocyanolysins.

VON

O. Loew und Y. Kozai.

Bulloch und *Hunter* hatten vor mehreren Jahren beobachtet, dass die Culturen des *Bac. pyocyaneus* einen Körper enthalten, welcher Blutkörperchen auflöst. Sie fanden ferner, dass dieser Körper vorzugsweise in den Zellen bleibt, so dass Filtrate der Culturen weit schwächer wirken, als sterilisirte unfiltrirte Culturen. Darauf hin haben wir gesucht, Bedingungen zu finden unter welchen dieser Körper, den *Bulloch* und *Hunter* für ein Enzym hielten und Pyocyanolysin nannten, in besonderem Maasse entsteht.² Wir beobachteten dabei, dass eine Peptoncultur bei reichlichem Luftzutritt die Eigenschaft Blutkörperchen zu lösen besonders stark zeigte, aber für Mäuse ganz harmlos war, während die Bouilloncultur bei nur geringem Luftzutritt toxische Eigenschaften hatte und in weit geringerem Maasse Blut löste. Es war für uns allerdings etwas überraschend, dass ein Blut lösendes Enzym bei subcutaner Injection harmlos für Mäuse sein sollte. Indessen die Beobachtung von *Bulloch* und *Hunter* waren auch von *Weingcroff*,³ ferner von *Nencki* und *Sieber* gemacht worden,⁴ Da ferner *B. pyocyaneus* besonders reichlich enzymbildend ist⁵ war auch die Bildung eines blutlösenden Enzyms nicht

¹ Centralbl. f. Bakt. Band 28, S. 866.

² Diese Bulletins, Bd. 4, No. 5.

³ Centralbl. f. Bakt. Bd. 29, S. 777.

⁴ Briefliche Mitteilung von M. Nencki an den einen von uns.

⁵ In neuerer Zeit constatirte *Eijkmann* (C. Bakt. Bd. 29, S. 848) die Bildung von Lipase, ferner die eines elastische Fasern lösenden Enzyms (Ibid. Bd. 35, S. 2), durch den *Bac. pyocyaneus*. Nach *Eijkmann* erkennt man am einfachsten die blutlösenden Eigenschaften mit Blut-Agar. C. Bakt. 29, S. 847.

unwahrscheinlich, da ferner unsere Culturen nur schwach alkalisch reagierten, suchten wir den Grund der Blutloesung auch nicht im Alkaligehalt, um so weinger als nach *Myers*¹ hinreichend schwaches Ammoniak keine Haemolyse verursacht, wenn die Blutkörperchen in physiologischer Kochsalzloesung suspendirt sind.² Immerhin war es uns auffallend, dass die haemolytische Wirkung durch Kochen der Loesung nicht aufgehoben wurde und sich keine giftige Wirkung bei Mäusen constatiren liess.

In neuester Zeit hat nun *Jordan*³ ebenfalls die haemolytische Wirkung von *Pyocyanus*kulturen beobachtet, aber dieselbe lediglich als Folge des Alkaligehalts der Culturen erklärt. Werden die Culturen genau neutralisirt, so bleibt die Haemolyse aus. Wir haben diesen Versuch wiederholt und können diese Beobachtung im Wesentlichen bestätigen.

Als 2 cc. einer 5% Aufschwemmung von Blutkörperchen mit 2 cc. der 15 Minuten auf 60° erwärmten Cultur 24 Stunden im Brutschrank blieben, war Lösung der Blutkörperchen eingetreten; als aber die Cultur vorher genau neutralisirt wurde, war nach 24 Stunden keine Haemolyse eingetreten. Erst nach einen weiteren Tag trat allmählich Loesung ein, doch kann dieses kaum auf eine Enzymwirkung gedeutet werden. Weitere Versuche haben ergeben, dass schon auffallend geringe Mengen von Natriumcarbonat Haemolyse herbeiführen können. Als 2 cc. einer Blutkörperchenaufschwemmung mit 2 cc. einer 0.001% Sodaloesung 2½ Stunden bei 32° gehalten wurden, war schon etwa die Hälfte, nach 20 Stunden alles gelöst. Es ist dieses um so auffallender, als das Blut im lebenden Thier doch auch eine alkalische Reaction besitzt.

¹ C. Bakt., 28, S. 237.

² Erst wenn eine gewisse Menge secundäres Natriumphosphat zugefügt wird, erfolgt Haemolyse.

³ C. Bakt., Bd. 35, S. 274.



On the Microbes of the *Nukamiso*.

BY

S. Sawamura.

The name of *Nukamiso* is given in Japan to a preparation, resulting from the spontaneous fermentation of a mixture of rice bran, common salt and water. It is used for softening and rendering palatable certain fruits and roots. According to *Inouye*¹ it has following composition.

Water.	75.6%
Lactic acid.	2.6
Sugar.....	3.4
Sodium chlorid.....	8.1
Proteids, amidocompounds, fats, } mineral matters, starch. }	10.4

The most striking chemical change caused by that fermentation is the increased production of sugar and acids. The writer estimated the quantity of acides and sugar in *Nukamiso* fermented by keeping the fresh mixture in a warm (20—25°C) place for 20 hours.² The quantity of normal soda solution necessary for neutralizing 100 cc of the filtrate was 5.2 cc, while the acidity of the original rice bran mixture was only 0.4 cc. The quantity of sugar calculated as dextrose was as follows:—

Original mixture	0.041%
<i>Nukamiso</i>	0.534 ..

By preparing a plate-culture of chalk-glucose medium with freshly prepared *Nukamiso* four kinds of bacilli which produced acids were isolated. The microbes have the following properties.

¹ This bulletin Vol. II. No. 4.

² The mixture consisted of 100 gr of rice bran, 50 gr of common salt and 1 litre of water.

No. 1.

Form. The cell cultured in bouillon for 24 hours at 37°C is 0,5 μ wide and 1—2 μ long. Two are generally united; they are motile by peritric flagella. Spore formation is not observed.

Staining. Gram's method negative.

Oxygen. Aërobic.

Bouillon. A feeble scum and a little deposit are formed.

Gelatin plate-culture. A round, light, yellow, moist, bright, sharply defined colony. An elevated point is observed in the centre. It grows to a moderate size. Deep colony appears as a white point.

Gelatin streak-culture. A light yellow, moist, homogeneous colony. Gelatin is not liquefied.

Gelatin stab-culture. Thread-like growth to the bottom.

Agar streak-culture. A white, homogeneous colony, condensed water clear.

Potato culture. An elevated, moist, at first gray but afterwards yellow colony.

Milk culture. It is coagulated with an acid reaction.

Gas. It is evolved in glucose-bouillon. The gas consists of CO₂ and H₂.

Indol reaction. Positive.

Chemical activity. It does not saccharify starch. Acid produced in a mixture of 10 gr of rice bran and 100 cc of water for 3 days at 36°C was 0.780% calculated as lactic acid from the quantity of normal soda solution necessary for neutralisation.

No. 2.

Form. The cell is 0.6 μ wide and 2—4 μ long, and has rounded ends.

It is motile and flagella seem to grow in one end of the rod.

Gram's method. Positive.

Oxygen. Aërobic.

Bouillon. A feeble scum and a moderate deposit are produced, but in glucose-pepton water a thick scum which finally breaks.

Gelatin plate-culture. A round, sharply defined, somewhat transparent,

homogeneous, moist colony, which never grows larger than 2 mm in diameter.

Gelatin streak-culture. A light yellow, moist, homogeneous colony.

Gelatin is not liquefied.

Gelatin stab-culture. Thread-like growth to the bottom.

Agar plate-culture. A round, elevated, somewhat transparent, moist colony which does not grow larger. By weak magnification it is the same. Deep colony is a white point.

Agar streak-culture. A yellowish white, moist, bright, homogeneous colony.

Potato culture. A dark yellow, moist, bright, homogeneous colony.

Milk culture. It is coagulated with an acid reaction.

Gas. It is not evolved in glucose-bouillon.

Indol reaction. Negative.

Chemical activity. Starch is not saccharified, and sugar is not formed also in *Nukamiso* by this bacillus. Acid produced in a mixture of 10 gr of bran and 100 cc of water for 3 days at 36°C was 1.327% calculated as lactic acid, and that produced in glucose-bouillon containing some Ca CO₃ in 3 days at 36°C was 1.007% of lactic acid calculated from CaO dissolved. The acid produced was found to be lactic acid by examining the properties of the zinc salt.¹

Some alcohol is formed from glucose, which was confirmed by the formation of iodoform.

Since the already known lactic ferments such as *Bacillus acidi lactici Hueppe*, *Bacterium acidi lactici Grotenfeldt* and *Kozai's bacilli* are all not motile this microbe seems to be a new species.

No. 3.

Form. The cell is 0.4 μ wide and 1 μ long, and it is motile by peritric flagella.

¹ The nutritive solution of glucose with CaCO₃, in which this bacillus was cultured, was filtered. The filtrate, after having been acidified with P₂O₅, was evaporated to dryness. The residue was treated with ether and filtered. The filtrate was evaporated but no crystal was formed, the residue being a syrupy mass. It was neutralised with Zn CO₃, and by examining the crystal-form of the zinc salt and its behavior towards alcoholic ammonia, it was proved to be zinc lactate.

- Two are usually united, and spore-formation is not observed.
- Gram's method. Negative.
- Oxygen. Aërobic.
- Bouillon. It becomes turbid, but no scum is formed.
- Gelatin plate-culture. A round, elevated, sharply defined, white, moist, bright, homogeneous colony. By weak magnification it is the same. Deep colony is a white point.
- Gelatin streak-culture. A white, moist, bright, homogeneous colony. Gelatin is not liquefied.
- Gelatin stab-culture. Thread-like growth to the bottom.
- Agar streak-culture. A white, moist, bright, flat colony.
- Milk culture. It is coagulated with an acid reaction.
- Potato culture. A gray moist, elevated colony. Gas bubbles are formed on the colony.
- Gas. Gas consisting of CO₂ and H₂ is vigorously produced in glucose bouillon.
- Indol reaction. Negative.
- Chemical activity. It does not saccharify starch. Acid produced in *Nukamiso* above described for 3 days at 36°C was 0.654% calculated as lactic acid and that produced in glucose bouillon containing CaCO₃ in 3 days at 36°C was 0.453% of lactic acid calculated from dissolved CaO. This microbe can produce the characteristic smell of *Nukamiso*. This is probably *Bacillus chologenes Kruse*, which resembles very much the coli-bacillus.

No. 4.

- Form. The cell is 0.5—0.7 μ wide and 2—4 μ long and is motile by peritric flagella. Spore-formation is not observed.
- Gram's method, positive.
- Oxygen. Aërobic.
- Bouillon. A stick scum is formed, the medium remaining quite clear.
- Gelatin plate-culture. A round, flat, gray, roughly defined colony which grows very large.
- Gelatin streak-culture. It liquefies quickly gelatin.

Gelatin stab-culture. Thread-like growth to the bottom, the surface being quickly liquefied.

Agar streak-culture. A light brown, folded, characteristic colony.

Milk culture. It is coagulated with an acid reaction.

Potato culture. Characteristic folded colony which is at first faintly red, and changes to gray afterwards.

Gas. It is not evolved.

Indol reaction. Positive.

Chemical activity. It saccharifies starch, and produces 0.296% of glucose in a mixture of 10 gr of bran and 100 cc of water for 24 hours at 36°C, and acid produced in the same mixture for 3 days at 36°C was 0.770% calculated as lactic acid. A feeble red tint is produced in *Nukamiso*.

By these properties this microbe is regarded as *Bacillus mesentericus ruber* *Globig*.

From an old *Nakamiso* the writer isolated a bacillus which was identified to be *Bacillus mesentericus vulgatus* *Flügge* and a kind of Kahlm-yeast, which produces a weak alcoholic fermentation on a nutritive solution containing glucose.

In order to see which microbe produces most acid they were inoculated into a sterilized *Nukamiso* and kept for 24 hours at 36°C. The quantity of normal soda necessary to neutralize 100 cc of the filtrate from the above culture was as follows:—

No. 1.	12cc
No. 2.	20,,
No. 3.	10,,
No. 4.	12,,
No. 2.+No. 4.	22.5,,
No. 1.+No. 2.+No. 3.+No. 4.						25.0,,

From these figures it is clear that the chief acid producer is *Bacillus* No. 2, but the symbiosis with the other microbes increases the production of acid. The smell characteristic for *Nukamiso* is probably produced by a *Mesentericus* species, and the organism No. 3. The production of sugar in *Nukamiso* is solely due to the activity of the *Mesentericus* species.

The species of saccharomyces present does not participate in the fermentation, since its fermentative power is nearly nil. The writer observed in the case of saccharification of mannan by *Bac. mes. vulgatus*, the accelerating action of a certain wild yeast on this bacillus.¹ In order to see whether the function of this wild yeast be analogous to it the mixture of *Bac. mes. vulgatus* with the saccharomyces was cultured for 3 days at 20°C in glucose-bouillon containing CaCO₃ and the quantity of CaO dissolved by the acid formed was determined.

Control.	0.618%
<i>Bac. mes. vulgatus</i> + the Saccharomyces.	0.910,,

In the second experiment a mixed culture of *Bac. mes. vulgatus* and that yeast were left to act upon starch, 2% of which were suspended in bouillon. The sugar formed in one week at the ordinary temperature was as follows :—

Control.	0.79%
<i>Bac. mes. vulgatus</i> + the wild yeast.	0.82,,

From these figures it follows that the saccharomyces has indeed some accelerating effect upon the action of the bacteria.

We may conclude as follows :—

- I. By the fermentation of *Nukamiso* sugar and acids are formed in a moderate quantity.
- II. In fermented *Nukamiso* there are present various microbes, of which the writer isolated four kinds of bacilli and a Saccharomyces.
- III Sugar is produced exclusively by a Mesentericus species.
- IV. Several microbes that can produce acid are present in *Nukamiso*, but the chief acid-producer is a bacillus, which seems to be a new species.
- V. The aroma characteristic for *Nukamiso* seems chiefly to be produced by a Mesentericus species.
- VI. The Saccharomyces present in *Nukamiso* seems to have no other effect than to accelerate somewhat the bacterial actions.

¹ This bulletin Vol. V No 2.

Ueber den Kalkgehalt verschiedener tierischer Organe.

VON

M. Toyonaga.

Das Muskelfleisch hat nach mehreren Autoren einen höheren Gehalt an Magnesia als an Kalk, was wahrscheinlich mit der relativ geringen Zellkernmasse zusammenhängt.¹ Es zeigt sich jedoch ein Unterschied zwischen dem Kalkgehalt der Muskeln von Batrachiern und Fischen einerseits, und demjenigen der Muskeln der Warmblüter andererseits; so ergibt sich im Durchschnitt für 1000 Teile frischen Muskels von Warmblütern 0,0954 Teile Calcium, bei Kaltblütern aber 0,2913 Teile Calcium.

Ferner ist das Verhältniss $\frac{\text{Ca}}{\text{Mg}}$ der Muskelgewebe beider Tiergruppen sehr verschieden.

Muskel der Kaltblüter.	Muskel der Warmblüter. ²
$\frac{\text{Ca}}{\text{Mg}}$ 1.26	0.34

Von einigem Interesse schien es nun, die glatten mit den quergestreiften Muskeln in dieser Hinsicht zu vergleichen, da die glatten Muskeln eine niedrigere Entwicklungsstufe des Muskelgewebes darstellen, und die relative Grösse der Zellkerne verschieden ist. Vergleichende chemische Untersuchungen beider Muskelarten sind nur spärlich vorhanden.

¹ Vergleiche meine früheren Abhandlungen in diesen Bulletins, Band 5.

² Durchschnitt aus mehreren Bestimmungen von Muskeln verschiedener Tiere nach Kütz.

Von Interesse ist die Beobachtung Vincents,¹ dass die glatten Muskeln 6-8 mal so viel Nucleoproteid enthalten als die quergestreiften, und dass der Herzmuskel einen Uebergang zwischen beiden bildet. Beide Muskelarten geben ein Salzplasma, welches entweder von selbst gerinnt oder durch Verdünnung. Ob das Mehr von Nucleoproteid in den glatten Muskeln gänzlich dem relativ grösserem Zellkern der glatten Muskelfasern zuzuschreiben ist, wäre noch zu untersuchen. Immerhin schien es von Interesse, das Verhältniss $\frac{\text{Ca}}{\text{Mg}}$ in beiden Muskelarten von demselben Tiere zu bestimmen. Ich wählte die Schenkelmuskeln des Pferdes und verglich sie mit den Bauchmuskeln. Leider stellen die letzteren Muskeln keineswegs nur ein Gewebe aus glatten Muskelfasern dar, sondern enthalten noch quergestreifte Muskelfasern und Bindegewebe. Es können daher die erhaltenen Zahlen nur annähernde Werte für die glatten Muskelfasern sein. Ich verfuhr im Wesentlichen nach der in meinen früheren Arbeiten (l. c.) erwähnten Methode und erhielt die folgenden Resultate.

In 1000 Teilen frischer Substanz sind enthalten :

	CaO	MgO	$\frac{\text{Ca}}{\text{Mg}}$
Quergestreifter Muskel des Pferdes,	0,064	0,322	$\frac{0,24}{1}$
Glatter Muskel des Pferdes,	0,07	0,292	$\frac{0,29}{1}$

Man erkennt hieraus, dass in der Tat die glatten Muskeln, obgleich noch gemischt mit quergestreiften, einen etwas höheren Kalkgehalt ergeben als die quergestreiften.

¹ Zeitschrift f. physiolog. Chemie, Band 34, S. 417, (1902).

Hodensubstanz.

Obgleich die Hoden drüsige Organe sind, so erscheint doch die Zellkernmasse derselben geringer als die der Leber oder Pancreasdrüse. Kalk- und Magnesiagehalt der Drüsen scheint manchmal, vielleicht unter pathologischen Einflüssen, abnormen Schwankungen zu unterliegen, jedoch nicht in dem Sinne, dass der Magnesia-gehalt über den Kalkgehalt steigt, sondern dass der Kalkgehalt enorm zunimmt und der Magnesiagehalt abnimmt. So fand, *Lüning*¹ in der Asche der Pancreasdrüsen von zwei krebsskranken Frauen das eine Mal (a) Ca=2,55% und Mg=1,48%, das andere Mal (b) Ca=16,94% und Mg=0,37%.² Die Aschenprocente der frischen Drüsen waren nahezu gleich, nämlich 1,04 und 1,02 resp.

Auf 1000 Teile frischer Substanz berechnet würde sich ergeben :

(a)	(b)
Ca—0.2663	1.7356
Mg—0.1541	0.0383

Dieses abnorme Sinken des Magnesiumgehalts im Falle (b) beim Pancreas, erinnert an einen ganz ähnlichen Fall, beobachtet an der Leber, von *Oidtmann*.

Dieser³ fand (1858) in der Leber 1.1% Asche und in dieser Asche = 3.62% CaO und nur 0.19% MgO, oder 0.289 Teile Ca und 0.017 Teile Mg auf 1000 Teile frischer Substanz ; es ist also in diesen abnormen Fällen :

	Pancreas (b).	Leber (nach Oidtmann).
$\frac{Ca}{Mg} =$	45.3	17.0

¹ Die anorganischen Bestandteile des Pancreas, Würzburg 1899.

² Die eine Frau (a) litt an Magenkrebs, die andere (b) an Eierstockkrebs.

³ In der Asche der Milz fand dieser Autor auf 7,18% Kalk nur 0,49% Magnesia ; es ist also hier

das Verb. $\frac{Ca}{Mg} = 18.1$

Sonst bewegt bei Drüsen sich dieses Verhältniss $\frac{\text{Ca}}{\text{Mg}}$ zwischen 1 und 6.

Aus Hoden von Fischen wurden bekanntlich interessante Substanzen gewonnen, die Protamine, aber es existirt doch noch keine vollständige quantitative Analyse des Hodens von Säugetieren,¹ woraus wir die Menge Albumin, Globulin, Nucleoprotein, Mucin, Lecithin, etc. entnehmen könnten. Sogar die Trockensubstanz ist nicht genau bestimmt worden. *Miescher* gibt zwar an, dass der Wassergehalt 75 procent und die Trockensubstanz 25 procent betrage; wahrscheinlich hatte er aber den Hoden gemischt mit Bindegewebe der Bestimmung unterworfen. Bei meiner Bestimmung entfernte ich das Bindegewebe so sorgfältig wie möglich und fand dann den Wassergehalt weit bedeutender, nämlich 85.39%. Ich analysirte die Hodensubstanz des Pferdes und des Stieres² mit folgendem Resultat:

	In 1000 Teilen.			$\frac{\text{Ca}}{\text{Mg}}$
	Total Asche.	CaO	MgO	
Pferdehoden	9.550	0.096	0.256	$\frac{0.45}{1}$
Stierhoden a)	9.943	0.102	0.214	$\frac{0.51}{1}$
b)	10.109	0.091	0.237	

¹ Vor Kurzem hat *Levene* aus Rinderhoden eine Nucleinsäure dargestellt, welche bei Spaltung unter andern Guanin, Thymin und Cytosin lieferte.

² Den Chlorgehalt der Stierhodenasche fand ich zu 0.418%. Bei der Pancreasasche beträgt derselbe 2.5-2.6%.

Beim Vergleich der Trockensubstanzen ergibt sich :

	Quergestreifter Muskel des Pferdes.	Milchdrüse des Kindes.	Hoden des Pferdes.
CaO	0.0323%	0.2517%	0.668%
MgO	0.1619%	0.0639%	0.1773%
$\frac{Ca}{Mg}$	0.24	4.67	0.45 ¹

Wir finden daher, dass der Kalkgehalt des Hodens geringer ist als der von Pancreas, Milz und Leber, dass aber andererseits das Verhältniss $\frac{Ca}{Mg}$ ein weiteres ist als bei den Muskeln der Warmblüter. Das Secret des Hodens ist aber sehr kalkreich, in Uebereinstimmung mit dem anschnlichen Gehalt an Zellkernen (Spermatozoen). Es enthält nach *Z. Slovzoff*² frisches Menschengesperma = 0,90% Asche, ferner 9.68% Trockensubstanz und 0.199% Nuclein (2% des trocknen Spermas). Der Kalkgehalt in der Asche von zwei Alkoholfraktionen des Spermas betrug 22,40% und 15,08%.

Analytische Belege.

	Frische Substanz.	Wasser.	Asche.	CaCO ₃ (in der Hälfte).	Mg ₂ P ₂ O ₇ (in der Hälfte).
Quergestreifter Muskel.	233.6916 g.	187.243 g.	2.032 g.	0.0134 g.	0.1045 g.
Glatter Muskel.	231.0862 „	187.1316 „	2.1236 „	0.0147 „	0.0938 „
Hoden des Pferdes.	120.6913 „	103.3148 „	1.1521 „	0.0103 „	0.0427 „
Hoden des Stiers (a)	201.5540 „	175.0597 „	2.004 „	0.0184 „	0.0609 „
(b)	186.8451 „	161.9256 „	1.9196 „	0.0152 „	0.0612 „

¹ Das Verhältniss $\frac{Ca}{Mg}$ in der Lunge nähert sich mehr dem in den Hoden als dem in der Leber und Milchdrüse. Schmitt fand in 1000 Teilen Lunge (Mensch) 1,9 CaO und 1,9 MgO, also

$$\frac{Ca}{Mg} = \frac{1.18}{1}$$

² Zeitschrift f. physiolog. Chemie, 35, p. 358.

Behufs einer Übersicht über die bisher in Bezug auf den Kalkgehalt tierischer Organe erhaltenen Resultate lasse ich folgende Zusammenstellung folgen.

In 1000 Teilen frischer Substanz sind enthalten Calcium :

Muskel des Warmblüter	0.057 (Bunge, Mittel).
„ „ „	0.095 (Katz [1896], Mittel).
Quergestreifter Muskel (Pferd)	0.046 (Toyonaga [1902]).
Glatter Muskel (Pferd)	0.050 „
Weisse Hirnsubstanz (Kalb)	0.041 „
„ „ (Pferd)	0.037 „
Graue Hirnsubstanz (Kalb)	0.263 „
„ „ (Pferd)	0.778 „
Milchdrüse (Kuh)	0.600 „
Hoden (Pferd)	0.069 „
„ (Stier)	0.069 „
Leber (Mensch)	0.284 (Oidtmann).
Periphere Nerven (Pferd)	0.568 (Toyonaga).

Es zeigt sich ferner, dass mit Zunahme des Kalks in drüsigen Organen die Zunahme der Magnesia keineswegs gleichen Schritt hält, sondern in manchen Fällen sogar sehr gering bleibt. Vergleichen wir das Verhältniss Ca/Mg beim Muskel und der weissen Hirnsubstanz mit dem in drüsigen Organen und der grauen Hirnsubstanz, so ergibt sich :

	Ca/Mg.
Muskel der Warmblüter	0.34 (Katz, Mittel).
Quergestreifter Muskel (Pferd)	0.24 (Toyonaga).
Glatter Muskel (Pferd)	0.29 „
Weisse Hirnsubstanz (Pferd)	0.30 „
„ „ (Kalb)	1.14 „
Graue Hirnsubstanz (Pferd)	2.80 „
„ „ (Kalb)	1.72 „
Milchdrüse (Rind)	4.69 „
Niere	1.84 (Aloy).
„ (Rind)	2.98 (Gossmann).

	Ca/Mg.
Niere (Mensch)	4.25 (Gossmann).
Milz (Rind)	2.52 (Ribaut, Mittel).
„ „	2.79 (Aloy).
Milzpulpa (Rind)	2.70 (Ribaut).
Bindegewebe der Milz (Rind)	3.45 „
Pankreas (Mensch)	1.73 (Lüning 1900).
„ „	4.75 (Gossmann).
Lunge	1.20 (Schmidt).
„ (Pferd)	1.36 (Toyonaga).
Hoden (Stier)	0.51 „
„ (Pferd)	0.45 „
Periphere Nerven (Pferd)	1.56 „





On the Influence of Different Ratios of Lime to Magnesia on the Growth of Rice.

BY

K. Aso.

A number of experiments to find the most favorable ratio of lime to magnesia for plant-growth have been made at this Institute, under Prof. Loew. Furuta studied in this regard the behavior of buckwheat, oats and cabbage in soil culture and the writer¹ that of barley, soy-bean and onion in water culture, and of the mulberry-tree in water as well as in soil culture. Recently, Katayama² has also carried out several sand and soil cultures with onion, oats and buckwheat on this line. In all those cases, it became evident that the maximum yield depended, other things being equal, upon a distinct ratio of lime to magnesia and that the best ratio is not the same with every kind of crops.

Since rice-culture is a most important factor³ in agriculture of Japan, it occurred to me that this principle should be also studied with the rice crop. The soils differ widely in chemical composition and the farmer never knows whether liming would be in order or not. Generally, however, the farmers of Japan apply too much lime and the injuries thus produced have induced the local government of Kiushiu to issue a law prohibiting the use of lime. Besides the depression of the harvest also a greater brittleness of straw and grains and a relative decrease of protein result from excessive liming.⁴ The content of lime and magnesia in the straw and grains of rice plants are the following in an average :

¹ Bull. College of Agric. Tokyo, Vol. IV, No. 5 and Vol. No. 4.

² This Bulletin, p. 102.

³ The annual production of rice in Japan (without Formosa) amounts in average to 41 millions Koku (= 7.4 Mill. Liter.) while the importation amounts to a value of at least 5 million yen annually.

⁴ Bull. College of Agric. Tokyō, Vol. I, No. 9.

In 100 parts of air dry matter :

	CaO	MgO	CaO : MgO	
Paddy rice	straw	0.26	0.19	1.4 : 1
	not whitened grains	0.03	0.09	1. : 3
Upland rice	straw	0.31	0.24	1.3 : 1
	not whitened grains	0.02	0.07	1. : 3.5

It will be seen from these figures, that the ratio of lime to magnesia in rice is smaller than that in many other plants.

My experiment was carried out as follows :—

Seven *Wagner's* porcelain pots were filled with 7 kilo of airdry sifted soil taken from a paddy field which had not been cultivated for several years. The quantity of available lime and magnesia in this soil was determined by extracting the soil with cold 10 % hydrochloric acid for 48 hours with the following result :

In 100 parts of dry soil ;

CaO	0.70
MgO	0.60

The ratio of lime magnesia was now changed in six pots by mixing the soil with calcium carbonate or pure magnesite¹ (finely powdered) to reach the following ratios :—

Pots.	Quantity of Calcium carbonate added. <i>gr.</i>	Quantity of Magnesite added. <i>gr.</i>	CaO : MgO
a	166.24	0	5 : 1
b	122.86	0	4 : 1
c	79.46	0	3 : 1
d	36.07	0	2 : 1
e (original)	0	0	1 : 1 (nearly)
f	0	68.3	1 : 2
g	0	127.4	1 : 3

¹ This mineral was imported from Germany and contained only minute quantities of lime.

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As general manure for each pot served :

Ammonium sulphate	15 gm.
Sodium phosphate	15 gm.
Potassium carbonate ¹	10 gm.

On July 13, the young rice plants² (about 36 cm. long) were transplanted from the seed bed. Each pot received three bundles. One bundle was made up of three individuals of equal size. Although this experiment was carried out in a glass house, the treatment was the same as in the field. Towards the end of August the difference in plant growth became very marked, the plants in e showed the best growth of all. These plants also flowered first (Sept. 9). On September 18, all plants were in flower and on that day a photograph was taken which is reproduced on plate X and which exhibits the difference in development very well. It might be surmised that some ammonia of the sulfate was transformed into carbonate by the potassium or calcium carbonate added and volatilized; and this loss of some nitrogen might have something to do with the difference in growth. But it must be remembered that not only was the dose of nitrogen a very heavy one (ratio: 600 kilo N per ha), and that much more nitrogen was present than could be possibly utilized, but also that the soil was so rich in humus (11%) that a considerable absorption of ammonia was undisputable. Nevertheless further experiments are contemplated with such a modification that even a small loss of nitrogen will be practically excluded,³ viz. P_2O_5 will be applied as superphosphate and K_2O as sulfate.

On November 6, the crop was harvested and left to become airdry. The weight was as follows :

¹ This was applied separately, later.

² The variety name was Satsuma.

³ *P. Wagner* has calculated from many trials with oats, that in average 1 gram of ammonia nitrogen can yield 56.97g. straw + 41.33g. of grains, while 1 gram of nitrate nitrogen, 57.03g. straw + 42.53g. of grains. From these data it may be seen, that there was a considerable excess of nitrogen in my manured soil compared with the harvest of rice, which in all probability would show not a very different behavior from the related oats.

Pots.	$\frac{\text{CaO}}{\text{MgO}}$	Full grains.	Empty grains.	Straw.	Total.
a	$\frac{5}{1}$	<i>gr.</i> 20.5	<i>gr.</i> 2.0	<i>gr.</i> 53.5	<i>gr.</i> 76.9
b	$\frac{4}{1}$	30.5	1.5	59.5	91.5
c	$\frac{3}{1}$	44.0	2.0	65.5	111.5
d	$\frac{2}{1}$	58.5	3.5	96.0	158.0
e	$\frac{1}{1}$	98.5	6.5	125.0	230.0
f	$\frac{1}{2}$	84.0	3.0	95.5	182.5
g	$\frac{1}{3}$	79.0	4.0	106.0	189.0

It will be observed from these figures, 1) the lime factor¹ for rice agrees nearly with that of other Gramineae which is between 1 and 2; 2) the rice plant seems to possess a relatively considerable resistance power against an excess of magnesium carbonate² since this does not depress the yield so much as the same excess of lime; 3) Rice-culture demands special attention to the proper ratio of lime to magnesia, since the maximal yield depends to a great degree upon the ratio 1 : 1.

These facts induced me to examine the ratio of lime and magnesia in various soils of Japan. The analyses of soils were kindly furnished by Dr. *Tsunetō* and his colleagues of the Geological Survey of the Japanese Empire, who have published a compendious volume on the composition of soils in Japan. These analyses were made in the usual style and do therefore not exactly distinguish between the readily assimilable amounts and the total amounts of lime and magnesia soluble in hot concentrated hydrochloric acid. In examining the long list of analyses I have observed numerous cases in, which the ratio $\frac{\text{CaO}}{\text{MgO}}$ would

¹ Bull. College of Agric. Tokyo, Vol. IV, No. 5.

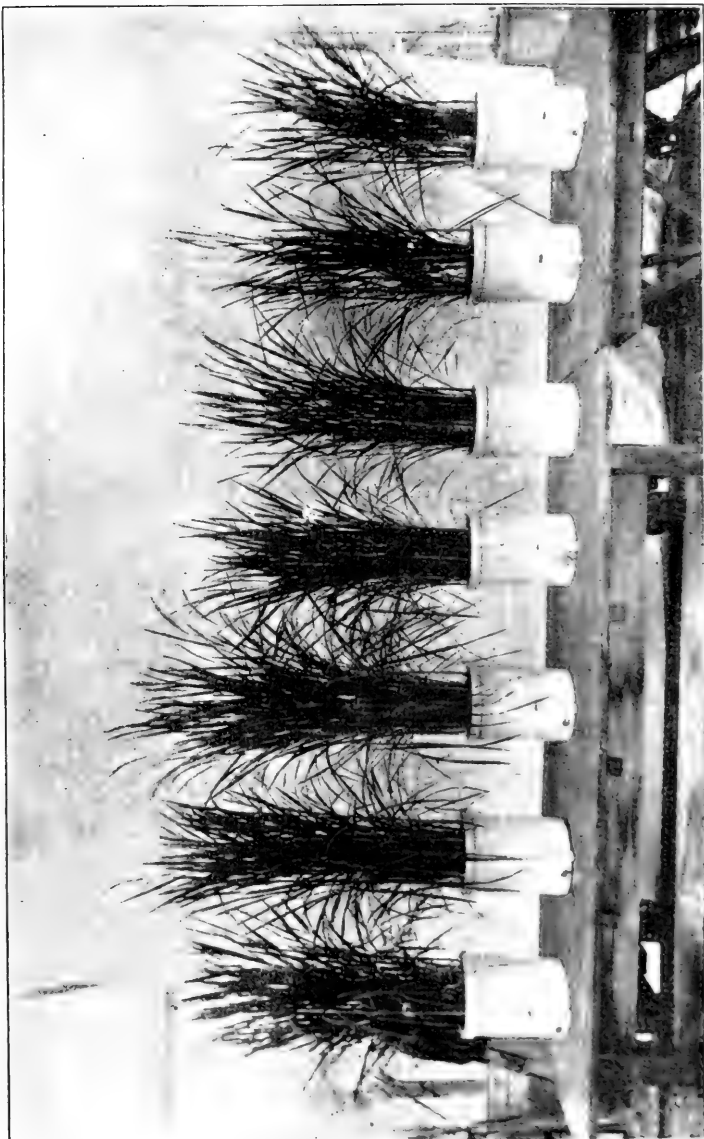
² At least in presence of manure of alkaline reaction; an acid reaction of the manure would probably change this behavior.

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not be favorable for a maximal yield of rice. Many of these unfavorable cases are mentioned in the following table:

Locality.	Mother Rock.	In 100 parts of fine dry soil.		Locality.	Mother Rock.	In 100 parts of fine dry soil.	
		CaO	MgO			CaO	MgO
Kurosakamura, Hinogōri, Hoki.	Granite.	0.376	0.986	Hasamamura, Ōitagōri, Buzen.	Volcanic rock.	1.445	0.312
Kitayatomura, Ikumagōri, Yamato.	Granite.	0.308	0.888	Fujinamura, Fujigōri, Suruga.	Lava and Lapilli.	0.389	1.784
Mikagemachi, Mukogōri, Settsu.	Granite.	0.058	0.488	Kanaokamura, Suntōgun, Suruga.	Volcanic Rock.	0.450	1.735
Nishinemura, Igugōri, Iwaki.	Granite.	0.412	1.913	Tatsukawamura, Iwatagun, Tōtōmi.	Crystalline Schist.	1.440	5.890
Nakanomura, Iishigōri, Idzumo.	Granite.	trace.	0.475	Onishimachi, Tanogōri, Kōtsuke.	Crystalline Schist.	0.778	2.189
Nagayasumura, Nakagōri, Iwami.	Porphyritic.	0.946	0.158	Kitamura, Suchigōri, Tōtōmi.	Chichibu-paleozoic.	0.612	3.236
Nishinamura, Kamogōri, Idsu.	Dia base.	1.618	6.307	Komamura, Irumagōri, Musashi.	Chichibu-paleozoic.	1.610	0.480
Dōshimamura, Kawarumagōri, Iwasyiro.	Andesitic tuff.	0.130	0.004	Meijimura, Minamiumibegori, Bungo.	Chichibu-paleozoic.	0.575	0.112
Daikonjima, Yatsukagōri, Idsumo.	Basalt.	1.259	0.368	Kamisannomiya-mura, Yamagōri, Iwashiro.	Tertiary tuff.	0.391	0.010
Tsudamura, Yatsukagōri, Idsumo.	Basalt.	0.295	0.923	Ogimura, Shusugōri, Noto.	Tertiary tuffaceous sandstone.	1.100	0.407
Inomura, Nakagōri, Iwami.	Basalt.	0.180	0.812	Ōtamura, Chichibugōri, Musashi.	Tertiary Shale.	0.330	0.940

Locality.	Mother Rock.	In 100 parts of fine dry soil.		Locality.	Mother Rock.	In 100 parts of fine dry soil.	
		CaO	MgO			CaO	MgO
Takidamura, Awagun, Awa.	Tertiary tuff.	0.567	0.033	Gyotokumachi, Higashikatsu- shikagori, Shimōsa.	Alluvial.	1.545	0.021
Nissakamura, Ogasagōri, Tōtōmi.	Tertiary Sandstone.	0.531	2.172	Nikaidōmura, Yamabegōri, Yamato.	Alluvial.	0.739	0.200
Tokachihara, Hokkaido.	Diluvial.	2.129	0.400	Hiraidsumimura, Nishiwaigōri, Rikuchū.	Alluvial.	0.904	0.130
Yoshiminehara, Iwasegōri, Iwashiro.	Diluvial.	1.516	0.096	Ōsumura, Shidagun, Suruga.	Alluvial.	0.120	1.670
Kikyōgahara, Higashichiku- magun, Shinano.	Diluvial.	0.448	1.423	Yoshiwaramachi, Fujigun, Suruga.	Alluvial.	0.146	1.602
Kugamura, Katorigun, Shimōsa.	Diluvial.	0.706	0.004	Sekimura, Nagōgōri, Kadsusa.	Alluvial.	0.867	0.029
Ōmagarimura, Senhokugun, Ugo.	Alluvial.	0.351	1.616	Miomura, Abegōri, Suruga.	Alluvial.	0.272	1.428
Nakazatomura, Takatagun, Idsu.	Alluvial.	2.075	0.461				



CaO $\frac{1}{3}$ $\frac{1}{2}$ $\frac{1}{1}$ $\frac{2}{1}$ $\frac{3}{1}$ $\frac{4}{1}$ $\frac{5}{1}$

MgO $\frac{1}{3}$ $\frac{1}{2}$ $\frac{1}{1}$ $\frac{2}{1}$ $\frac{3}{1}$ $\frac{4}{1}$ $\frac{5}{1}$

This plate shows the influence of different ratios of lime and magnesia upon rice.

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On the Determination of the Available Amounts of Lime and Magnesia in the Soil.

BY

T. Katayama.

It has been shown by experiments of O. Loew and W. May in Washington¹ and of K. Aso and T. Furuta² in Tokyo that the best development of a plant depends, other things being equal, upon a certain ratio of the amounts of lime to the amount of magnesia available to the roots.

In order to reach a maximum harvest, it is necessary to determine the *available* amounts of lime and magnesia in the soil, and then to provide for the proper ratio between these two on the basis of the result obtained, by the addition of the calculated amounts of lime and magnesia compounds.

The extraction of the soil with hot concentrated hydrochloric acid yields very probably more lime and magnesia than can be dissolved by the roots, while the proposed extraction with ammonium chlorid will yield in most cases certainly too small numbers.³

Also, the size of the particles to be separated from the soil, previously to the treatment with hydrochloric acid, forms a very important question. Some authors separate all particles smaller than 0.5mm. diameter and treat that fraction with hydrochloric acid of 10% or also of 30% at the ordinary temperature, while others apply boiling heat. Furuta separated all particles smaller than 0.25mm. and treated this fine sand + silt + clay'

¹ Bul. No I, Bureau of Plant Industry Washington 1901.

² Bul. of the College of Agriculture, University of Tokyo, vol IV, No. 5.

³ *Immendorf* proposed recently boiling with $\frac{1}{2}$ normal sulphuric acid for 30 minutes and to determine in this solution the easily available lime and magnesia.

with hot conc. hydrochloric acid. I have tried the following modification :

I. The fine earth, $<0.25\text{m.m}$ was treated with hydrochloric acid of 5% at the ordinary temperature, in the proportion of 25g : 75c.c, for 24 hours.

II. The fine earth, $<0.25\text{m.m}$ was treated with hydrochloric acid of 10% at boiling temperature for 50 minutes, in the proportion of 25g : 50c.c.¹

The determination by the former method yielded, however, too small a number for magnesia, leading to a ratio $\frac{\text{CaO}}{\text{MgO}}$ which would differ too much from the observations of *Aso* and *Furuta* made a year previous with water and soil cultures.¹

Hence only the second method was adhered to. The percentages of lime and magnesia thus obtained served as a basis for the calculations. In order to observe whether different soils would give equally reliable results with the same method, I have compared the sandy soil from an orchard near Kawasaki, about 15 miles south-west from Tokyo, with a loamy soil, from Komaba, a suburb of Tokyo, the same soil which had served *T. Furuta* for his trials. Both soils were examined in the air dry state.

The analysis gave the following data :

	Lime		Magnesia	
Fine earth of the soil of Kawasaki = 68.8%	{ 0.615% } { 0.650, ,, }	average 0.63%	{ 0.823% } { 0.786, ,, }	average 0.80%
Fine earth of the soil of Komaba = 76.33%	{ 0.597% } { 0.587, ,, }	average 0.60%	{ 0.486% } { 0.516, ,, }	average 0.49%

The pots for the cultures with the Kawasaki soil held 5 kilo, which amount of soil contained therefore 21.7g Ca O and 27.5g Mg O. In order to procure the desired ratios the following additions were necessary :

¹ After this treatment with HCl, 200c.c of water was added and heated again to boiling for 10 minutes, then left standing for 15 hours before the filtration.

² *Söderbaum* observed recently that a hydrochloric acid of 2% extracted in 48 hours at the ordinary temperature only a part of the available plant nutrients.

I.	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{0.75}{1}$	No addition.
II.	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{2}{1}$	33.3g Ca O = 59.4g Ca CO ₃
III.	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{3}{1}$	60.8g Ca O = 108.6g Ca CO ₃
IV.	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{4}{1}$	88.3g Ca O = 157.7g Ca CO ₃

The pots for the cultures with the soil from Komaba¹ held 4 Kilo, which amount of soil contained therefore 18.3g Ca O and 14.9g Mg O. Hence the following additions were here required :

I.	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{1.2}{1}$	No addition.
II.	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{2}{1}$	11.5g Ca O = 21.8g Ca CO ₃
III.	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{3}{1}$	26.2g Ca O = 46.9g Ca CO ₃
IV.	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{4}{1}$	41.3g Ca O = 73.6g Ca CO ₃

Now if my determination of lime and magnesia in the soils corresponds really to the available amounts, the results obtained with those soils limed in certain degrees, must agree with those obtained with cultures in quartz-sand or water, in which the lime and magnesia were present in the same ratios and in form of soluble salts. The soils were manured in the ratio of 10g of monopotassium phosphate, 6.3g potassium sulfate, 10g sodium nitrate for 5 Kilo.

Sand culture.

About 15 Kilo of pure quartzsand were left with conc. hydrochloric acid for 3 days, and then well washed with water, until every trace of acid reaction had disappeared.

Five flower pots received each 700 grms of this sand, and 14g of

¹ This soil from a depth of one foot was analysed some years before by T. Furuta, while for my analysis and cultures served the surface soil only, hence some small discrepancies are easily accounted for.

precipitated air dry aluminium silicate (2% of the sand) to increase the water holding capacity.

The solutions applied had the following composition :

$$\text{General Manure : } \left\{ \begin{array}{l} 0.1\% \text{ KCl} \\ 0.3\% \text{ K NO}_3 \\ 0.2\% \text{ K}_1\text{H}_2\text{PO}_4 \\ \text{trace Fe SO}_4 \end{array} \right.$$

(1)	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{1}{1}$	0.3% Ca O = 0.879% Ca (NO ₃) ₂ 0.3 ,, Mg O = 0.895 ,, Mg SO ₄
(2)	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{2}{1}$	0.4% Ca O = 1.117% Ca (NO ₃) ₂ 0.2 ,, Mg O = 0.596 ,, Mg SO ₄
(3)	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{3}{1}$	0.45% Ca O = 1.318% Ca (NO ₃) ₂ 0.15 ,, Mg O = 0.447 ,, Mg SO ₄
(4)	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{4}{1}$	0.48% Ca O = 1.406 ,, Ca (NO ₃) ₂ 0.12 ,, Mg O = 0.358 ,, Mg SO ₄
(5)	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{5}{1}$	0.5% Ca O = 1.465 ,, Ca (NO ₃) ₂ 0.1 ,, Mg O = 0.298 ,, Mg SO ₄

The pots in each of the four series received 330c.c of the above mineral solution respectively.

Since the evaporation of water from sand is rapid, a special arrangement was provided to secure a constant supply of water consisting in a band of loose cotton freed from the adhering fat, and immersed with one end in water. These bands encircled the pots; thus moisture due to capillary attraction was present always in the pots.

For my experiments the growth of onion plant was observed in these soil and sand mixtures.¹

¹ Experiments with oats, and bean and buckwheat also had been commenced but unfortunately insect pests and parasitic fungi caused so much damage that I was compelled to abandon them.

Experiment with the onionplant in sand culture.

10 seeds were sown, on March 13, and the number of shoots reduced to five of nearly equal size on April 1.

The height of the young plants was measured, April 29 with the following results:

	Ratio of Ca O : Mg O.				
	1 : 1	2 : 1	3 : 1	4 : 1	5 : 1
	8.0 cm	10.5	7.7	8.2	8.0
	7.8 „	10.2	7.0	8.2	7.3
	7.4 „	9.8	6.8	7.4	7.0
	6.0 „	8.6	6.1	7.2	6.9
	5.6 „	7.9	6.0	6.9	6.8
average	6.84 „	9.30	6.72	7.52	7.20

A photograph was taken, on May 1, see Plate XI

This result shows the ratio $\frac{\text{Ca O}}{\text{Mg O}} = 2$ the best for onion.

Experiments with Onionplants in Soil from Kawasaki.

Fifteen seeds of onion sown on May 2.

The rate of germination was as follows:

Date	$\frac{\text{Ca O}}{\text{Mg O}} = \frac{0.75}{1}$	$\left(\frac{\text{Ca O}}{\text{Mg O}} = \frac{2}{1}\right)$	$\left(\frac{\text{Ca O}}{\text{Mg O}} = \frac{3}{1}\right)$	$\left(\frac{\text{Ca O}}{\text{Mg O}} = \frac{4}{1}\right)$
May	3	—	5	5
„ 10	6	6	10	14
„ 12	8	10	13	14
„ 14	12	14	14	14

The number of the young shoots was reduced to 5 on May 15. The number of branches and the height of the young plants were measured on June 30, with the following results :

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches		Length, cm.	
$\frac{0.75}{1}$	a	4		22.5
	b	5		29.0
	c	4		22.5
	d	4		25.5
	e	4		27.0
	sum	21	average	25.3
$\frac{2}{1}$	a	5		35.0
	b	6		36.0
	c	5		34.5
	d	4		23.0
	e	5		34.0
	sum	25	average	33.3
$\frac{3}{1}$	a	4		31.5
	b	4		31.0
	c	5		25.0
	d	5		24.5
	e	5		29.0
	sum	23	average	28.2
$\frac{4}{1}$	a	4		27.4
	b	5		28.0
	c	4		26.7
	d	5		32.0
	e	3		18.0
	sum	21	average	26.4

Lime and Magnesia in the Soil.

These onion plants were harvested on July 12, and yielded the following results :

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Length of shoots, cm	Number of shoots	Total weight, g.
$\frac{0.75}{1}$	a 29.40	5	2.65
	b 35.70	5	3.40
	c 27.30	4	1.95
	d 30.30	4	2.30
	e <u>33.75</u>	<u>5</u>	<u>4.10</u>
	average 31.29	sum 23	sum 14.40
$\frac{2}{1}$	a 42.90	5	4.80
	b 44.10	6	7.15
	c 42.60	5	4.51
	d 39.00	5	4.60
	e <u>41.10</u>	<u>5</u>	<u>4.45</u>
	average 41.54	sum 26	sum 25.51
$\frac{3}{1}$	a 39.00	5	4.05
	b 36.60	5	4.00
	c 30.60	5	2.60
	d 29.70	5	3.15
	e <u>36.60</u>	<u>5</u>	<u>4.50</u>
	average 36.50	sum 25	sum 18.30
$\frac{4}{1}$	a 36.00	5	3.50
	b 36.60	6	5.15
	c 33.20	5	4.15
	d 37.80	5	4.25
	e <u>21.90</u>	<u>3</u>	<u>1.50</u>
	average 32.50	sum 24	sum 18.55

Experiments with Onionplant in Soil from Komaba.

Fifteen seeds of onion were sown on May 2.

The rate of germination was as follows:

Date	$\left(\frac{\text{Ca O}}{\text{Mg O}} = \frac{1}{1}\right)$	$\left(\frac{\text{Ca O}}{\text{Mg O}} = \frac{2}{1}\right)$	$\left(\frac{\text{Ca O}}{\text{Mg O}} = \frac{3}{1}\right)$	$\left(\frac{\text{Ca O}}{\text{Mg O}} = \frac{4}{1}\right)$
May 8	—	2	2	2
„ 10	1	3	2	5
„ 12	3	5	8	8
„ 14	5	8	8	8

The number of young shoots were reduced to 5 on May 15. The number of branches and the height of the young plants were measured on June 30, with the following results ;

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches	Length, cm
$\frac{1.2}{1}$	a 4	28.5
	b 4	34.0
	c 4	24.0
	d 3	19.0
	e 4	20.5
	sum 19	average 25.2
$\frac{2}{1}$	a 5	33.5
	b 4	34.5
	c 5	34.8
	d 5	29.0
	e 5	36.0
	sum 24	average 33.5

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length, cm.	
$\frac{3}{1}$	a	4		36.8
	b	5		33.5
	c	3		28.5
	d	4		30.0
	e	5		36.5
	sum	21	average	33.0
$\frac{4}{1}$	a	4		22.0
	b	4		31.5
	c	5		35.0
	d	3		28.0
	e	5		28.5
	sum	21	average	31.0

On harvesting (July 12) the following result was obtained ;

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Length of shoots, cm.		Number of shoots.		Total weight, g.	
$\frac{1.2}{1}$	a	37.80		4		2.30
	b	38.40		5		2.30
	c	30.90		4		1.50
	d	21.60		4		0.45
	e	28.50		4		2.00
	average	31.44	sum	21	sum	8.55
$\frac{2}{1}$	a	40.50		5		5.60
	b	36.60		4		4.50
	c	42.00		5		4.70
	d	36.00		5		3.20
	e	43.80		5		0.25
	average	39.78	sum	24	sum	24.25

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Length of shoots, cm.	Number of shoots.	Total weight, g.
$\frac{3}{1}$	a 41.40	5	5.20
	b 39.90	5	4.80
	c 41.40	4	3.75
	d 37.80	5	3.45
	e 36.00	5	2.75
	average 39.34	sum 24	sum 19.95
$\frac{4}{1}$	a 36.6	5	3.30
	b 36.6	5	4.10
	c 42.0	5	3.95
	d 37.8	4	3.10
	e 37.8	5	3.80
	average 38.01	sum 24	sum 18.25

Hence we find in all these cases that the best ratio of $\frac{\text{Ca O}}{\text{Mg O}}$, or the lime factor for the onionplant is ≈ 2 .

The chief results with two different soils agree therefore very well with the results in sandculture, where the total content of lime and magnesia was certainly present in an easily available form. It may be safely concluded that the modification I proposed to determine the available amounts of lime and magnesia in soils is in close agreement with the actual results and furnishes therefore a reliable basis for the calculations regarding the liming of soils.

It might be objected that the conditions of the absorptive powers in certain soils might alter the availability of lime and magnesia for the roots. But in my investigation the two soils applied differed very much in character and nevertheless yielded results which agree with each other and with those of the sand culture.

Only soils with a unusually high percentage of clay or humus might yield results differing somewhat from my expectations, but such soils are from the outset not favorable for agriculture.

The experiments of the year 1902 just mentioned were repeated in

1903. The same pots served for this second series, the soil was not renewed, but it was manured again uniformly with 20g KH_2PO_4 and 15g $(\text{NH}_4)_2\text{SO}_4$ for each 5 kilo. The pots were kept in the green house.

Second experiment with onion plants in the soil from Kawasaki.

15 seeds were sown per pot, on January 7, and the number of the young shoots reduced, on March 2, to six per pot, all being of equal size. The height of the young plants and the number of branches was measured, on May 1, with the following result:

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.	Length cm.
$\frac{0.75}{1}$	a 4	32.0
	b 4	33.0
	c 4	29.5
	d 3	30.5
	e 5	27.0
	f 4	34.0
	sum 24	average 31.0
$\frac{2}{1}$	a 4	33.0
	b 4	32.5
	c 5	46.0
	d 5	32.5
	e 4	31.5
	f 4	34.0
	sum 26	average 34.9
$\frac{3}{1}$	a 4	34.0
	b 4	32.5
	c 4	30.5
	d 4	38.0
	e 5	33.0
	f 4	29.0
	sum 25	average 32.7

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length cm.
$\frac{4}{1}$	a	4	30.0
	b	4	30.5
	c	3	25.5
	d	4	32.5
	e	4	29.0
	f	4	35.5
	sum	23	average 30.5

Up to this time, almost every day 200CC water for irrigation was applied, but the quantity was gradually increased to 300CC.

On harvesting, on June 7, the following result was obtained :

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length cm.	Total fresh weight, gm.
$\frac{0.75}{1}$	a	6	58.0	22.5
	b	6	61.0	17.5
	c	5	57.0	17.0
	d	6	57.0	30.5
	e	7	52.0	14.5
	f	6	62.0	22.0
	sum	36	average 57.8	sum 124.0
$\frac{2}{1}$	a	6	59.0	24.5
	b	8	58.0	31.0
	c	7	74.0	49.5
	d	8	57.0	19.5
	e	7	60.0	28.0
	f	7	61.0	25.5
		43	average 61.5	sum 178.0
$\frac{3}{1}$	a	7	60.0	29.0
	b	6	62.0	24.5
	c	7	63.0	25.0
	d	7	59.0	31.0
	e	6	63.0	25.0
	f	6	45.0	17.5
		39	average 58.6	sum 152.0

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length, cm.	Total fresh weight, gm.
$\frac{4}{1}$	a	5	55.0	25.5
	b	6	57.0	25.0
	c	7	60.0	22.0
	d	6	55.0	22.0
	e	5	52.0	16.0
	f	7	60.0	28.0
	sum	36	average 56.5	sum 133.5

These figures, as well as the photograph taken shortly before harvesting and reproduced on Plate XI show plainly again the superiority of the ratio $\frac{\text{Ca O}}{\text{Mg O}} = \frac{2}{1}$ for the onion plants.

Second experiment with onion plants in soil from Komaba.

15 seeds were sown into each pot, on Febr. 10, and the number of the young shoots reduced, on March 20, to six of equal size.

The height of the young plants and the number of branches were measured, on May 12, with the following result :

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length, cm.
$\frac{1}{1}$	a	5	36.0
	b	5	41.0
	c	3	37.0
	d	4	36.0
	e	4	40.0
	f	4	37.0
	sum	25	average 37.8
$\frac{2}{1}$	a	6	47.5
	b	5	40.0
	c	5	45.0
	d	5	45.5
	e	6	43.0
	f	3	38.0
		30	43.1

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length, cm.
$\frac{3}{1}$	a	5	45
	b	3	40
	c	5	38
	d	5	40
	e	4	42
	f	<u>3</u>	<u>38</u>
	sum	25	average 40.6
$\frac{4}{1}$	a	5	40.0
	b	5	41.0
	c	5	39.5
	d	4	37.5
	e	3	<u>42.0</u>
	f	<u>3</u>	40.0
		25	

A photograph was taken shortly before harvesting, on June 15; it is reproduced on Plate XI.

On harvesting, the following result was observed :

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length, cm.	Total weight, grm.
$\frac{1}{1}$	a	5	45	18.5
	b	5	48	18.5
	c	4	47	12.5
	d	5	45	10.5
	e	5	49	15.0
	f	<u>5</u>	<u>47</u>	<u>8.5</u>
	sum	29	average 45.5	sum 83.5
$\frac{2}{1}$	a	7	63	28.5
	b	6	53	19.0
	c	6	58	22.5
	d	6	58	25.5
	e	6	55	22.0
	f	<u>5</u>	<u>56</u>	<u>17.2</u>
	sum	36	average 57.2	sum 134.7

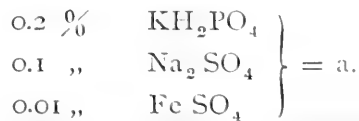
Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches,	Length cm.	Total weight gram.
$\frac{3}{1}$	a 6	59	19.0
	b 5	53	15.5
	c 6	50	19.0
	d 5	50	18.0
	e 5	50	21.0
	f 5	53	18.0
	sum 32	average 52.5	sum 110.5
$\frac{4}{1}$	a 6	50	19.7
	b 5	52	21.0
	c 6	54	23.0
	d 5	53	17.0
	e 5	55	16.0
	f 5	45	16.0
	sum 32	average 51.5	sum 112.7

Also this result shows that the lime factor for the onion plant is = 2.

Second series of Sandculture.

A large amounts of sea sand (particles smaller than 1 mm. diameter) was soaked in conc. HCl for about a month, and well washed with distilled water until every trace of chlorine reaction had disappeared, then left to dry.

Five pots received each 1.5 litres of this air dry sand, and Soc.c of nutritive solutions which had the following composition :



(1) $\frac{\text{Ca O}}{\text{Mg O}} = \frac{1}{1}$ $0.3 \% \text{ Ca O} = 0.879 \% \text{ Ca (NO}_3)_2$
 $0.3 \text{ ,, Mg O} = 1.098 \text{ ,, Mg (NO}_3)_2 + a$

(2) $\frac{\text{Ca O}}{\text{Mg O}} = \frac{2}{1}$ $0.4 \% \text{ Ca O} = 1.117 \% \text{ Ca (NO}_3)_2$
 $0.2 \text{ ,, Mg O} = 0.732 \text{ ,, Mg (NO}_3)_2 + a$

$$(3) \quad \frac{\text{Ca O}}{\text{Mg O}} = \frac{3}{1} \quad \begin{array}{l} 0.45\% \text{ Ca O} = 1.318\% \text{ Ca (NO}_3)_2 \\ 0.15 \text{ ,, Mg O} = 0.549 \text{ ,, Mg (NO}_3)_2 + a \end{array}$$

$$(4) \quad \frac{\text{Ca O}}{\text{Mg O}} = \frac{4}{1} \quad \begin{array}{l} 0.48\% \text{ Ca O} = 1.406\% \text{ Ca (NO}_3)_2 \\ 0.12 \text{ ,, Mg O} = 0.439 \text{ ,, Mg (NO}_3)_2 + a \end{array}$$

$$(5) \quad \frac{\text{Ca O}}{\text{Mg O}} = \frac{5}{1} \quad \begin{array}{l} 0.5 \text{ \% Ca O} = 1.465\% \text{ Ca (NO}_3)_2 \\ 0.1 \text{ ,, Mg O} = 0.366 \text{ ,, Mg (NO}_3)_2 + a \end{array}$$

At the beginning of this experiment, the total concentration of the mineral nutrients for each pot was 2.14—2.38 per mille. The pots were kept in the green house.

20 seeds of onion were sown, per pot, on March 6, and the number of young shoots reduced, on April 7, to 8 of equal size.

The height of the young plants and the number of branches was measured May 1 with the following result :

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.	Length, cm.	
$\frac{1}{1}$	a	4	21.5
	b	5	23.0
	c	5	25.5
	d	5	23.5
	e	4	25.3
	f	3	18.5
	g	4	22.7
	h	4	21.2
	sum	34	average 22.6
$\frac{2}{1}$	a	5	30.1
	b	4	24.0
	c	5	27.8
	d	5	26.2
	e	4	24.9
	f	5	25.0
	g	4	28.9
	h	5	26.2
	sum	37	average 26.6

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length, cm.
$\frac{3}{1}$	a	4	21.2
	b	4	27.5
	c	4	20.5
	d	5	25.5
	e	5	18.0
	f	5	26.5
	g	4	24.0
	h	5	23.0
	sum	36	average 23.4
	$\frac{4}{1}$	a	5
b		4	20.2
c		4	21.5
d		5	25.5
e		4	28.3
f		5	28.5
g		4	22.3
h		5	18.6
sum		36	average 23.9
$\frac{5}{1}$		a	4
	b	5	21.7
	c	5	26.5
	d	4	20.1
	e	4	22.0
	f	4	17.0
	g	4	18.3
	h	3	18.7
	sum	33	average 21.8

The mineral solutions above mentioned were added in the following proportions :

Date.	March 9	April 20	May 15	June 7
Quantity.	25 c.c	30	40 c.c	40 c.c

In the beginning of this culture, every day 25c.c distilled water for irrigation was applied, and the quantity was gradually increased to 70c.c, but sometimes to 100c.c in very warm weather.

The plants were harvested June 27, and the following results observed:

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.	Length, cm.	Total weight, gram.
$\frac{1}{1}$	a 8	30.1	10.8
	b 6	31.4	8.2
	c 7	25.5	10.7
	d 4	34.0	6.3
	e 6	37.2	10.3
	f 3	22.3	1.9
	g 5	26.1	5.3
	h 5	26.9	11.1
	sum 44	average 29.2	sum 64.6
$\frac{2}{1}$	a 7	30.2	9.2
	b 5	30.0	6.8
	c 7	40.3	9.5
	d 7	33.1	10.0
	e 7	35.2	11.3
	f 5	35.3	9.0
	g 6	37.4	6.5
	h 7	38.0	11.6
	sum 51	average 34.9	sum 73.9
$\frac{3}{1}$	a 7	22.1	6.3
	b 6	30.2	10.5
	c 8	24.7	8.7
	d 7	35.0	10.7
	e 6	30.5	8.8
	f 5	38.1	8.9
	g 4	31.3	6.5
	h 6	28.9	6.4
	sum 49	average 29.7	sum 66.8

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Number of branches.		Length, cm.	Total weight, gm.
$\frac{4}{1}$	a	4	30.3	4.7
	b	5	23.5	5.7
	c	7	27.1	7.8
	d	7	30.4	9.0
	e	8	31.0	8.4
	f	8	29.2	7.5
	g	5	29.0	10.2
	h	7	28.0	11.2
	sum	51	average 28.6	sum 64.5
	$\frac{5}{1}$	a	7	29.1
b		8	29.6	12.2
c		6	31.3	8.7
d		4	27.0	5.3
e		6	23.2	7.2
f		4	29.5	5.7
g		6	26.1	7.1
h		5	30.2	8.0
sum		46	average 28.2	sum 63.9

Additional Experiments.

Experiment with pea in the soil from Kawasaki.

All principal conditions were the same as in the first onion experiments above described. 15 seeds of pea were sown, on Febr 9 and the number of young plants reduced to five of equal size on March 18. The formation of flowers commenced on May 3 and ended on the 24th of the same month. Up to the flowering period, almost every day 400c.c. of water for irrigation were applied but later on the quantity was increased to 600c.c. When the fruits had reached the ripening stage, on June 7, watering was stopped and

the plants left to dry. The harvest on June 15 yielded the following result:

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	average length.	number of branches.	weight of fresh fruits.	weight of air dry seeds.	weight of air dry straw.	air dry total weight.
$\frac{0.75}{1}$	98 cm	7	18.3 gm	15.7 gm	11.8 gm	29.0 gm
$\frac{2}{1}$	115 "	9	33.0 "	27.5 "	17.0 "	49.3 "
$\frac{3}{1}$	129 "	10	38.3	31.3	21.5	58.0
$\frac{4}{1}$	125 "	9	38.5	31.0	20.4	57.3

Experiment with pea in soil from Komaba.

The number of plants, time of sowing and harvesting was the same as in the case just described.

On harvesting, on June 15, the following results was obtained:

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	average length.	number of branches.	weight of fresh fruits.	weight of air dry seeds.	weight of air dry straw.	air dry total weight.
$\frac{1}{1}$	120 cm	8	35.5 gm	29.7 gm	18.5 gm	52.8 gm
$\frac{2}{1}$	125 "	11	38.0	31.8	23.0	59.4
$\frac{3}{1}$	137 "	11	41.5	33.9	27.4	66.7
$\frac{4}{1}$	123 "	11	36.5	31.2	22.6	58.7

Both results of experiments with different soils show that the ratio $\frac{3}{1}$ is the best for the pea.

Experiment with oats in soil from Kawasaki.

15 seeds were sown December 15, and the number of young shoots reduced to six all of equal size, on March 6; all conditions were essentially the same as in the former cases.

On harvesting (June 29) the following result was obtained;

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Average height.	Number of stems.	Weight of seeds.	Total weight.
$\frac{1}{2}$ ¹	94	26	41.4	118
$\frac{1}{1}$	105	28	46.1	143
$\frac{2}{1}$	103	28	45.7	141
$\frac{3}{1}$	101	26	42.9	115

Experiment with oats in the soil from Komaba.

These experiments were made at the same time as those with onion. The result was as follows ;

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$	Average height.	Number of stems.	Weight of seeds.	Total weight.
$\frac{1}{2}$ ²	112	21	40.1	99.5
$\frac{1}{1}$	117	24	43.3	113
$\frac{2}{1}$	114	25	43.8	117
$\frac{3}{1}$	107	18	37.6	103

Both results of experiments with oats in different soils show that the ratios $\frac{1}{1}$ and $\frac{2}{1}$ are the best.

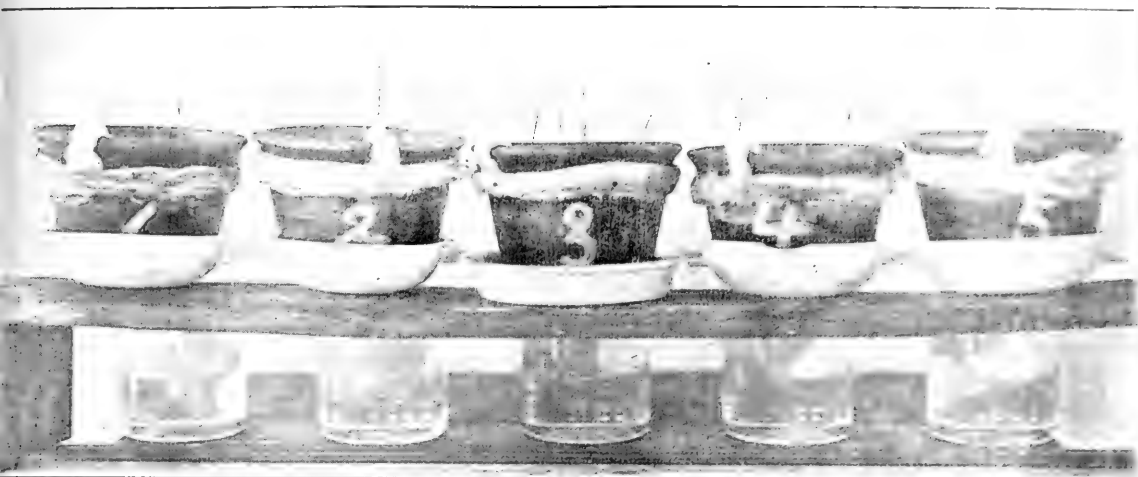
¹ In order to procure the ratio $\frac{1}{2}$, this pot received 15.9 gr Mg O = 33.1 gr Mg CO₃.

² In order to procure the ratio $\frac{1}{2}$, this pot received 21.7 gr Mg O = 45.2 gr Mg CO₃.

Conclusion.

It will be noticed from the second series of experiments with onions, that the results fully confirm the results of the first series that is: Sand-culture as well as the cultures in two soils differing widely in character from each other yielded the best results when the available amounts of lime and magnesia were present in the ratio 2 : 1, in other words the onion has the limefactor 2. Lime and magnesia in the sand culture were added in form of solutions, hence the total amount of these salts were easily available even if precipitated as finely divided phosphates. As to the soil culture the "available amounts" of lime and magnesia were determined according to my modification of the usual method and their ratios changed by adding carbonate of lime in such quantities as to reach the fixed ratios of the sandculture. Since in all my experiments of 1902 and of 1903 the ratio $\frac{\text{Ca O}}{\text{Mg O}} = \frac{2}{1}$ proved the most favorable for the onion plant, the determination of the available amounts must have been made by a reliable method. Hence my modification of the usual determination of the available amounts of lime and magnesia may be stated again: I propose to separate all particles <0.25 m.m, to determine the percentage of this fraction, and to extract this fraction for 50 minutes with boiling hydrochloric acid of 10% in the ratio of 25g : 50c.c.





CaO	$\frac{1}{1}$	$\frac{2}{1}$	$\frac{3}{1}$	$\frac{4}{1}$	$\frac{5}{1}$
MgO					



CaO	$\frac{1}{1}$	$\frac{2}{1}$	$\frac{3}{1}$	$\frac{4}{1}$
MgO				

Sand culture. To page 100.

Soil of Kawasaki. To page 100.

Soil of Komaba. To page 111.

Experiments of 1901.



Ueber den Einfluss des Mangans auf Waldbäume.

VON

Oscar Loew und Seiroku Honda.

Da Manganverbindungen einen günstigen Einfluss auf landwirtschaftliche Gewächse äussern,¹ war es wünschenswerth, auch über den Grad des Einflusses auf Waldbäume einige Anhaltspunkte zu gewinnen. Manganoxyd ist schon häufig in der Asche verschiedener Hölzer, in Blättern und Früchten verschiedener Bäume gefunden worden, die Mengen desselben variirten aber je nach dem Standorte ebenso wie die Mengen des Eisenoxyds beträchtlich,² wie die folgenden Daten, welche mit Ausnahme der letzten Zahlen den Aschentabellen *Wolff's* entnommen sind, erkennen lassen:

Object.	Procente in der Asche.		Analytiker.
	Mn ₃ O ₄	Fe ₂ O ₃	
Weidenholz	0.15	0.53	Reichard
Birkenholz I	3.94	3.00	Wittstein
Birkenholz II	4.13	0.59	Berthier
Lindenholz	0.88	0.15	„

¹ Siehe diese Bulletins, Bd. 5.

² Nach *Wolff* (Aschenanalysen, 2 Teil, S. 159) nimmt der Mangangehalt der Bäume zu, wenn es an Kalk mangelt, was von *Cowder* bestätigt wurde (Zeitschr. f. Forst- und Jagdwesen, Bd. 14, S. 113 [1882] und Bd. 35, S. 391 [1903]. Allerdings beobachtete letzterer auch, dass in den kalkreichsten Organen (Nadeln) der Waldbäume auch der Mangangehalt grösser war als in den anderen Theilen der Bäume.

Object.	Procente in der Asche.		Analytiker.
	Mn ₃ O ₄	Fe ₂ O ₃	
Fichtenrinde	0.65	2.67	Wittstein
Kirschbaumrinde	1.02	0.09	Palm
Ulmenrinde	0.68	0.90	Zeyer
Buchenblätter I	11.25	1.07	Fresenius
Buchenblätter II	1.86	12.00	Wittstein
Weidenblätter	0.29	0.96	Reichard
Birkenblätter	6.73	1.14	Wittstein
Kastanienfrucht	5.48	1.03	Richardson
Buchensamen	3.10	2.66	Suchay
Fichtenpollen	1.12	1.95	Ramann

Man ersieht hieraus, dass der Mangangehalt nicht selten den Eisengehalt weit übertrifft, Dass dieser Mangangehalt irgend einen Einfluss auf die Waldbäume äussern könne, war unbekannt; man hielt ihn für unwesentlich und für zufällig aus dem Boden aufgenommen.

Wir wählten zu unsren Versuchen die für Japan so wichtige *Cryptomeria japonica*. Am 29 April 1902 pflanzten wir auf Beeten von zwei Quadratmeter Grösse die jungen nahezu gleichgrossen Bäume ein. Zu dem Versuch dienten sechs solche Beete, welche von einander durch eine etwa 1 Meter breite mit niederen Brettern abgegränzte Fläche getrennt waren. Der Boden war ein humoser Lehmhoden von nur mässiger natürlicher Fruchtbarkeit. Jedes Beet erhielt anfangs neun Pflanzen, die aber später auf acht reducirt wurden, da einige eingiengen. Auf Beet No. 6 waren nach einiger Zeit nur sieben Pflanzen erhalten. Die Beete wurden genau wie alle jungen Pflanzungen der Förstereien behandelt und im Winter gegen Frost geschützt. Die Loesungen wurden nicht gleichmässig über die Beete verbreitet, sondern in eine kleine Vertiefung um jeden Stamm die berechnete Menge gegossen. Die Loesungen wurden zehnpocentig vorrätig gehalten und die jedesmal nötige Menge auf das hundertfache verdünnt.

Beet No. 1 erhielt von 1 Mai bis 1 November 1902 allmonatlich 0.5g Mangansulfat, nur bei der ersten Begiessung die doppelte Menge; im Jahre 1903 aber vom 1 Mai bis 1 Nov. (inclusive) allmonatlich 1g. Es erhielt also jede der 8 Manganpflanzen im Ganzen 1.5 gramm jenes Salzes.

Beet No. 2 erhielt Eisensulfat (Eisenvitriol) in gleicher Weise und Menge wie Beet No. 1 das Mangansulfat. Beide Sulfate waren die krystallisirten, nicht chemisch reinen, Producte des Handels.

Beet No. 3 erhielt lediglich die jenen Loesungen entsprechende Menge Wasser, und diente wie Beet No. 5 und No. 6 als Controlbeet.

Beet No. 4 erhielt Kochsalz, No. 5 Natriumnitrat, No. 6 Calciumnitrat in denselben Mengen wie Beet No. 1 das Mangansulfat. Diese Nitrate wurden angewandt, um zu beobachten, ob eine teilweise Düngung eine ähnliche Wirkung äussern könnte, wie Mangansulfat. Das Chlornatrium auf Beet No. 4 sollte Aufschluss geben, ob die Holzbildung befördert wird; denn in der Landwirtschaft ist bei einem gewissen Chlornatriumgehalt des Bodens ein schnellerer Verbrauch von Stärkemehl und Zucker zu Gunsten der Ausbildung der Holzfasern beobachtet worden, oder es wird wenigstens ein derartiger Einfluss des Kochsalzes für wahrscheinlich gehalten.

Vier Monate nach der Behandlung war noch kein deutlicher Unterschied im Höhenwachstum wahrzunehmen, erst im fünften Monat war ein Voraneilen der Manganpflanzen deutlich zu erkennen. Dieses Voraneilen nahm aber im folgenden Frühjahr ein rascheres Tempo an, bis im Herbst des zweiten Jahres ein höchst auffallender Höhenunterschied erreicht wurde, wie aus folgender Tabelle ersichtlich wird. Die Zahlen geben die Höhen in Centimetern an.

No. der Pflanze	Manganesulfat Mn SO ₄ + 4 aq.			Ferrosulfat Fe SO ₄ + 7 aq.			Control			Chlornatrium			Natriumnitrat			Calciumnitrat		
	Mai 1./02	Nov. 10./02	Nov. 10./03	Mai 1./02	Nov. 10./02	Nov. 10./03	Mai 1./02	Nov. 10./02	Nov. 10./03	Mai 1./02	Nov. 10./02	Nov. 10./03	Mai 1./02	Nov. 10./02	Nov. 10./03	Mai 1./02	Nov. 10./02	Nov. 10./03
1	18.0	49.3	135	19.7	29.5	115	18.7	33.5	130	19.5	31.0	90	18.7	31.0	115	22.7	36.7	110
2	19.5	47.5	168	22.0	34.0	85	18.3	35.0	89	20.7	36.0	97	19.9	32.0	106	20.5	24.4	54
3	19.7	34.0	133	19.0	27.3	105	10.2	19.0	45	19.6	29.0	95	10.2	19.0	62	18.7	35.0	65
4	17.0	44.0	145	21.3	31.2	95	19.0	27.0	110	17.8	23.0	60	19.0	41.5	143	23.0	32.7	111
5	19.5	31.0	100	17.5	31.5	104	16.5	34.8	105	18.7	24.5	81	16.5	34.8	96	20.6	33.0	110
6	21.5	32.8	114	20.2	31.5	98	20.3	30.0	99	18.5	27.0	95	20.3	30.0	121	20.0	32.7	103
7	19.5	35.5	137	18.0	27.3	108	17.7	25.5	90	16.3	23.0	70	17.7	25.5	97	16.1	20.0	71
8	20.3	24.5	89	18.5	37.5	138	16.4	21.0	93	13.7	18.0	58	16.4	21.0	77	—	—	—

Hieraus berechnet sich für die Zeit vom 1 Mai 1902 bis 10 Nov. 1903 das durchschnittliche Zuwachsprocent bei Behandlung mit:

Mangansulfat zu...	558.7
Ferrosulfat ...	445.5
Chlornatrium ...	345.5
Natriumnitrat ...	426.7
Calciumnitrat ...	340.6
und für die Controlpflanzen ...	448.2

Man erkennt hieraus, dass *Mangansulfat* das Höhenwachstum stark förderte, dass das *Ferrosulfat* diese fördernde Wirkung nicht hatte und *Chlornatrium* sowohl als *Calciumnitrat* hemmend gewirkt haben.¹ Bemerkenswert ist noch, dass die Düngung mit *Chilesalpeter* das Höhenwachstum nicht förderte. Indessen der *Eisenvitriol* sowohl als der *Chilesalpeter* haben das Wachstum der Zweige begünstigt wie aus dem Vergleich der Totalgewichte erhellt.

Am 17 November wurden die Bäume am Grunde abgesägt und frisch gewogen mit folgendem Resultat bei:

Manganosulfat • ...	5871 gramm.	} 8 Pflanzen.
Ferrosulfat ...	3395 "	
Chlornatrium ...	1390 "	
Natriumnitrat ...	3355 "	
Calciumnitrat ...	2497 "	
Control ...	2535 "	(8 Pflanzen)

Es ergibt sich daher als Durchschnittsgewicht für eine Pflanze:

bei Manganosulfat...	733.8 g
„ Ferrosulfat ...	424.5 "
„ Chlornatrium ...	173.8
„ Natriumnitrat ...	419.4
„ Calciumnitrat ...	356.7
„ Control ...	316.9

¹ Auch *Loughridge* (Calif. Stat. Bul. 133) und *Kossowitsch* (Journ. f. Exper. Landw. 1903, p. 44) beobachteten einen sehr schädlichen Einfluss von Kochsalz auf Bäume. Natriumsulfat ist nach diesen Autoren bedeutend weniger schädlich.

Die Durchschnittshöhe bei der Pflanzung der Bäumchen war auf dem Manganbeet = 19.4, auf dem Controlbeet aber = 17.1 cm.

Berechnet man nun das Erntegewicht bei den Manganpflanzen auf gleiche Anfangshöhe wie bei den Controlpflanzen um, so hat man für eine Manganpflanze = 646.7g. für die Controlpflanzen gleicher Anfangshöhe = 316.9g.

Die Manganpflanzen hatten also für dieselbe Anfangshöhe nach $1\frac{1}{2}$ Jahren die Controlpflanzen um das doppelte (2.03 fach) an Massenzunahme übertroffen, wie auch wohl aus der in Tafel XII reproducirten Photographie abgeschätzt werden könnte.¹

¹ Von einigem Interesse ist noch der Unterschied in der Wirkung von Natriumnitrat und Calciumnitrat, weil solche Unterschiede zu Gunsten des Natriumnitrats auch in der Landwirtschaft beobachtet sind. Es wirkt eben auch das Natron in der Form des Nitrats stimulierend, während Chlornatrium in Folge des Chlorgehaltes wieder hemmend wirkt, wenigstens in grösseren Dosen. In relativ geringerer Menge kann aber auch dieses eine mässig stimulierende Wirkung auf Feldgewächse ausüben. Fichten reagiren nicht so energisch auf Mangan als Cryptomerien. Versuche in grösserem Massstabe wird der eine von uns (Honda) weiter führen.

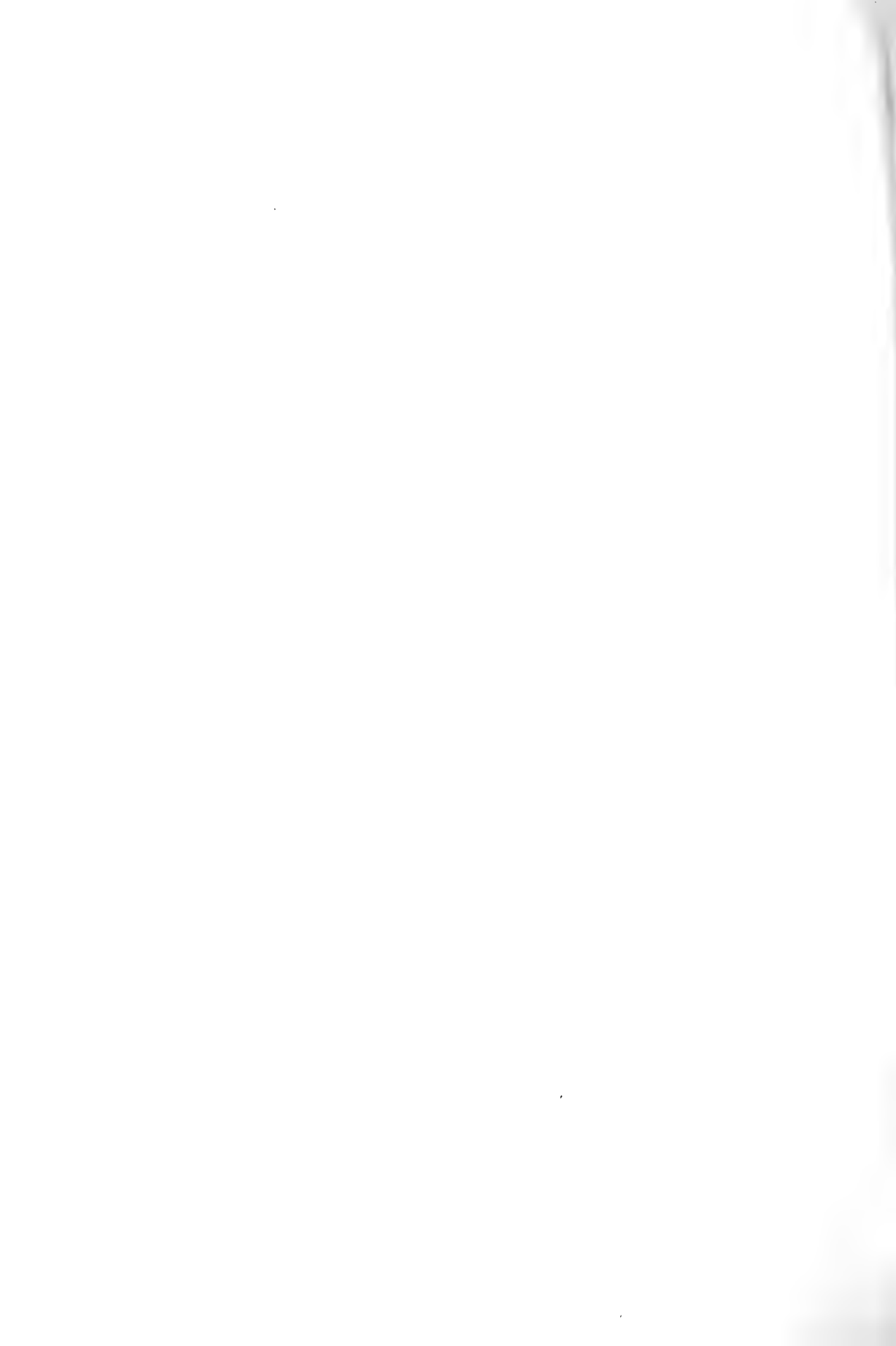




Control plants.

Manganese plants.

Plate showing the stimulating effect of manganous sulphate upon the growth of *Cryptomeria japonica*. To page 130.



On the Practical Application of Manganous Chlorid in Rice-culture.

BY

K. Asō.

In the last volume of this Bulletin, several communications regarding the stimulating action of manganese upon plant-growth were published. Loew and Sawa¹ observed this action with plants in water and soil culture and also the author² with various plants in waterculture. Nagaoka³ further carried out a field experiment with rice in wooden frames and obtained an increase of one third of the harvest in grains by the application of manganous sulphate at the rate of 35 kilo $Mn_3 O_4$ per hectar. In all these experiments, manganous sulphate was used. But, since manganous chlorid is a cheap by-product in the bleaching powder factories, it seemed to me of some practical importance to make also an experiment with this salt.

My experiment was carried out in the paddy field with rice in quite the same manner as the practical farmer does. Two square-shaped plots, each of 30 sq. metre, were selected in a field which had not been manured for several years. Each plot received 27 kilo barnyard manure, 15.5 kilo rotten human excrement, 230 grams double superphosphate and afterwards 570 grams wood ash. Besides, one plot received 200 grams crystallized manganous chlorid⁴ (corresponding to 25 kilo $Mn_3 O_4$ per ha.), while the other served as control.

On July 3, the young rice plants⁵ from the seed-bed were transplanted,

¹ Bul. College. Agric. Tokyō. Vol. V. No. 2.

² *ibid.* No. 2.

³ *ibid.* No. 4.

⁴ This was applied separately after manuring.

⁵ The variety was the Satsuma.

each plot receiving 306 bundles of twelve equally developed individuals. The irrigation and the drainage were made in each plot separately in the same manner as practically carried on and care was taken to avoid the passing of drainage water from one plot to the other.

Towards the end of July a difference in regard to the development was quite marked and became gradually more noticeable. On September 3, all plants in the manganese plot flowered and four days later in the control plot. The weather conditions were very favorable for rice culture throughout the whole summer, and all possible attention was paid to avoid damages by insect pests. On November 6, the plants were harvested.

The harvest was weighed in the air dry state :

	Manganous chlorid.	Control.
Total harvest	23.74 k.	16.73 k.
Straw	12.10 k.	8.19 k.
Total grains	11.49 k.	8.46 k.
Full grains.....	11.23 k.	8.23 k.
Empty grains	0.26 k.	0.23 k.
Husked full grains	8.66 k.	6.65 k.
Weight of 1 Litre of unhusked full grains.	616 g.	619 g.

Now, if the yield of the control plot is taken as unit, the following figures are obtained :

Manganous chlorid plot.

Total yield	1.42
Straw	1.48
Full grains (unhusked)...	1.36
Full grains (husked).....	1.30

These figures show an increase of one third of the grains by the application of 25 kilo. $Mn_2 O_3$ per hectar in the form of manganous chlorid,¹ which is in full coincidence with the result of Prof. Nagaoka who had applied the same amount of $Mn_2 O_3$ in the form of the sulphate. Since the area of one plot corresponded to $\frac{1}{330}$ hectar and the quantity of manganous chlorid applied was 200 grams, 66 kilo of this salt would be required per hectar. The cost would be only 4.4 yen,² while the value of the increased harvest is 137.33 yen.³

These experiments will be continued for a series of years on the same plots.

¹ As to pot cultures manganous sulphate would be preferable to the chlorid, since chlorids often exert an injurious influence on the yield. In field culture—especially with paddy field—this depressing factor is removed by rains and irrigation water. The manganous chlorid of course changes in the soil into other manganese compounds.

² The price of 100 pounds of the crystallized salt $Mn Cl_2 \cdot 4 aq.$ is three yen (about six Mark).

³ Compare Bul. College. Agric. Tokyō. Vol. V. No. 4. p. 472.



On the Stimulating Action of Manganese upon Rice, II.

BY

M. Nagaoka.

In a former Bulletin (vol. V, No. 4) I had shown that manganese compounds can increase the yield of paddy rice. The experiment was repeated under essentially the same conditions in the same frames as before, the only difference being that no fresh doses of manganese sulphate were applied since this time it was the chief object to *observe any after effects of the first doses* given the previous year. The crop was harvested on Nov. 11 and weighed two months later after being well air dry. The results are seen from the following table :

No. of Frames.	Mn ₂ O ₃ per ha kg.	Full grains gr.	Empty grains gr.	Straw gr.	Average.			Total
					Full grains.	Empty grains.	Straw.	
1	No manure	136.5	2.1	217.9	186.7	3.4	272.0	462.1
13	and no	196.2	4.0	288.1				
25	Mn ₂ O ₃	227.5	4.0	309.9				
2	No Mn ₂ O ₃	182.0	3.1	271.9	207.6	3.5	295.7	509.8
14		204.7	3.3	295.3				
26		236.0	4.2	319.9				
3		212.5	3.7	293.1	203.7	3.9	286.8	497.4
15	10	179.9	3.7	277.4				
27		217.0	4.4	298.9				
4		221.7	3.6	305.4	224.0	4.0	304.0	533.5
16	15	213.0	4.0	289.9				
28		240.0	4.3	318.4				

No. of Frames.	Mn ₂ O ₃ per ha kg.	Full grains gr.	Empty grains gr.	Straw gr.	Average.			Total.
					Fell grains.	Empty grains.	Straw.	
5		238.0	4.1	323.4				
17	20	248.0	4.8	219.4	230.3	4.4	316.4	551.1
27		205.0	4.3	306.4				
6		244.0	5.1	324.8				
18	25	238.0	4.8	315.6	235.5	4.9	308.8	549.2
30		224.5	4.7	285.9				
7		247.4	5.2	329.9				
19	30	251.7	4.9	324.2	242.7	5.0	322.2	569.7
31		229.0	4.9	312.4				
8		196.5	4.6	295.9				
20	35	270.0	4.1	339.7	231.2	4.6	313.5	549.3
32		227.0	5.2	354.9				
9		202.9	5.1	298.7				
21	40	227.0	4.0	314.4	215.0	4.6	306.6	526.2

The harvest in full grains was therefore greatest in the frame that had received manganous sulphate at the rate of 30 kilo Mn₂O₃ per ha; very near to this comes the frame with the ratio of 25 kilo Mn₂O₃ per ha, which in the first year yielded the greatest weight of full grains. The increase over the manured frame without manganese was 16.9% while the maximum increase in the first year was 37%.

On the Influence of Manganese Salts on Flax.

BY

Y. Fukutome.

In former experiments, described in the Bulletins of the College of Agriculture, was shown that manganese salts can exert a stimulant action on various plants serving as food. It seemed interesting to make further observations also on plants that are cultivated for their fibres.

I selected for this purpose flax and compared here the action of manganous chlorid with that of ferrous sulphate and Cobalt nitrate. The soil came from the experiment grounds of our College of Agriculture. Each pot containing eight kilo soil was manured with 16 g. superphosphate, 10 g. potassium sulphate, 8 g. each of ammonium sulphate, and sodium nitrate.

40 seeds were sown in each pot on September 21 and the shoots singled out in October to 15 of equal height (= 5 cm.).

Pot I. had received 0.4g crystallized manganous chloride ($\text{Mn Cl}_2 \cdot 4\text{H}_2\text{O}$)

Pot II. „ 0.4g ferrous sulphate ($\text{Fe SO}_4 \cdot 7\text{H}_2\text{O}$)

Pot III. „ 0.4g cobalt nitrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$)

Pot IV. „ 0.02g „ „ „

Pot V. „ 0.4g ($\text{Mn Cl}_2 \cdot 4\text{H}_2\text{O}$) + 0.4g ($\text{Fe SO}_4 \cdot 7\text{H}_2\text{O}$)

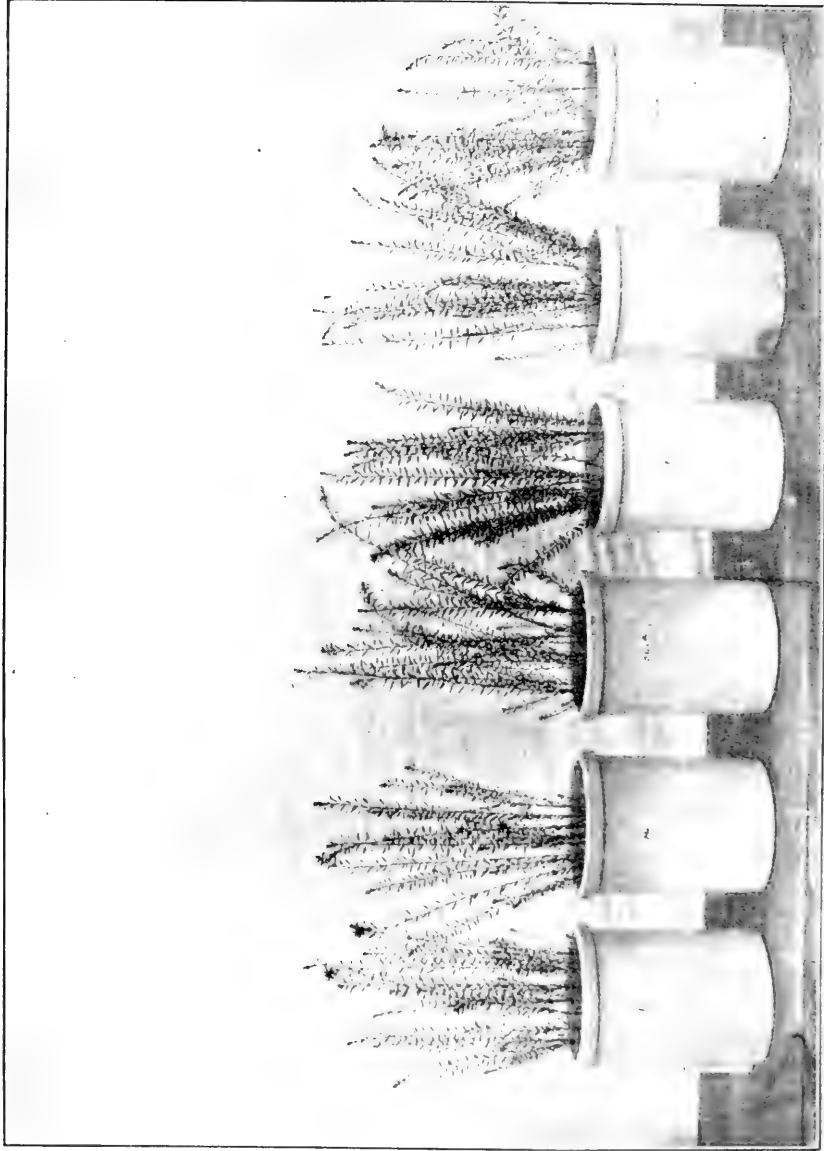
Pot VI served as Control.

On Nov. 30 the plants were measured and a photograph taken, reproduced on Plate XIII. It shows that a stimulating action had taken place. On Dec. 21 the stem and branches were again measured, whereupon the plants were cut and left to become air dry. At that time only two flowers opened, one had in pot IV and one in pot V.

The results were as follows :

	Nov. 30	Dec. 21				
	Height, cm. average	Height, cm. average	Number of Buds	Number of branches	Average length of branches	Weight, g. (airdry)
I. Mn.....	46.9	65.7	2	19	26.7	10.7
II. Fe	47.4	67.8	5	17	24.5	11.6
III. Co (0.4 g.)...	48.6	68.5	3	19	27.4	11.0
IV. Co (0.02 g.)..	48.9	69.6	5	19	33.8	12.8
V. Mn+Fe ...	51.7	71.9	4	19	32.9	12.9
VI. Control	43.4	60.1	0	19	25.	10.5

This result shows that the joint application of iron and manganese had a marked effect on the growth, while each alone but little in the dose of 0.4 g per 8 kilo soil. Also cobalt nitrate in the small dose of 0.02 g per pot exerted a stimulating effect.



Flax under the influence of ferrous sulphate, manganous chlorid and cobalt nitrate.
To page 138



Can Potassium Bromid Exert any Stimulating Action on Plants ?

BY

K. Asō.

Potassium bromid is only a very weak poison for plants, certainly very much weaker than potassium iodid.¹ The question whether very small quantities of that salt can exert any stimulating action was thus far only tested by *Völcker*, who found that barley soaked for a short time in a potassium bromid solution of 1%, yielded afterwards an increased harvest.² It was desirable to collect some further information in this regard.

Four pots containing 2.5 k. air dry soil were manured as follows :

Sodium nitrate.....	3 g.
Ammonium sulfate.....	1 g.
Monopotassium phosphate	3 g.
Potassium carbonate	3 g.

One pot received 10 mg. potassium bromid, another 100 mg. and the third 500 mg. potassium bromid for each kilo soil, while one pot served as control. On April 23, ten seeds of *Phaseolus* (Dwarf variety) were sown and on May 25, the plants were reduced to two of equal height in each pot.

The plants measured on July 2 :

Control plants	Potassium bromid plants		
	10 mg per kilo.	100 mg per kilo.	500 mg per kilo.
cm.	cm.	cm.	cm.
22	22	22	22
21	24	24	19

¹ Cf. O. Loew, Ein natürliches System der Giftwirkungen, p. 108.

² Journ. Roy. Agr. Soc. Engl. (III) 11, p. 566.

On July 24, the plants were harvested.

	Control	Potassium bromid		
		10 mg per kilo.	100 mg per kilo.	500 mg per kilo.
Number of fruits (ripe).....	4	7	6	6
„ (unripe).....	2	0	1	2
Weight of pods (air dried)	5.9 g.	11.0 g.	8.8 g.	7.4 g.
Number of ripened seeds.....	15	26	21	21
Weight of ripened seeds (air dried)	4.4 g.	8.5 g.	6.5 g.	5.7 g.

These figures leave no doubt that 10 milligram potassium bromid per kilo soil had exerted a stimulating action and further that this beneficial action decreased with the increase of that salt. But even 500 mg. potassium bromid per kilo soil had still a slight stimulating action.¹

Another experiment was made with upland rice. The manuring and the quantity of potassium bromid were quite the same as in the case with *Phaseolus*. On April 23, ten seeds of upland rice were sown into each pot and the young plants reduced to seven of equal size on June 4.

Later on two more were removed from each pot on account of damage by fungi. The height on July 24 was :

Control	Potassium bromid		
	10 mg per kilo.	100 mg per kilo.	500 mg per kilo.
cm.	cm.	cm.	cm.
60	80	69	68
68	82	74	68
80	74	69	72
67	71	60	64
67	78	70	58
—	—	—	—
Average length. 66	77	68	68

¹ The seeds of these plants yielded again normal plants.

On October 5, the plants were harvested.

	Control	Potassium bromid		
		10 mg per kilo.	100 mg per kilo.	500 mg per kilo.
Average length	81.4 cm.	87.6 cm.	83.6 cm.	75.0 cm.
Weight of grains (air dry).	10.5 g.	11.2 g.	8.7 g.	8.0 g.
Weight of straw (air dry).	18.5 g.	22.2 g.	18.2 g.	14.5 g.

This result shows, like in the former case, that 10 milligram potassium bromid per kilo soil exerts a stimulating action but this action diminishes with the increase of the amount; 100 milligram potassium bromid per kilo soil depressed the weight of grains and 500 milligram potassium bromid injured the normal growth of the plants.

A further experiment was made with fungi. The culture solutions contained:

- 0.5 % pepton
- 1.0 % glycerol
- 0.2 % monopotassium phosphate
- 0.02% magnesium sulphate.

Potassium bromid was added at the rate of 0.1%, 0.01% and 0.001%. These solutions were infected with a trace of spores of *Aspergillus Oryzae* and kept in diffused day-light at the ordinary temperature.

For control the solutions received equal amounts of potassium chlorid. After four days, the fungus mass was collected on weighed filters and dried. The result was as follows:

		Weight of the fungus mass.	
0.1% KCl	{ a	0.013 g.	
	{ b	0.015 ..	
0.01% ..	{ a	0.012 ..	
	{ b	0.015 ..	

		Weight of the fungus mass.
0.001% KCl	{	a 0.013 g.
		b 0.013 „
0.1% K Br	{	a 0.010 g.
		b 0.013 „
0.01% „	{	a 0.022 „
		b ———
0.001% „	{	a 0.012 „
		b 0.010 „
Control (no KCl and no K Br).....		0.018 „

These figures show quite undecisive differences.

Summary.

Potassium bromid at the rate of 10 milligrams per kilo soil exerts a stimulating action on bean and rice. This effect diminishes with the increase of the amount of that salt. With fungi (*Aspergillus Oryzae*) no stimulating effect of potassium bromid was observed at doses of 0.001—0.1%.



Can Thorium and Cerium Salts Exert any Stimulating Action on Phaenogamous Plants ?

BY

K. Asō.

Thorium became recently one of the most interesting elements on account of its compounds showing radioactivity.¹ It seemed to me therefore of some interest to observe its action on living plants. In a 1% solution of thorium nitrate ($\text{Th}(\text{NO}_3)_4$) young barley plants 12—15 cm. high were killed after about one day. This can not surprise us, however, since thorium nitrate is of strong acid reaction which in itself would be sufficient to kill the plants within that time. In a 1 per mille solution of that salt no injurious action whatever was observed on such plants even after eight days. It can therefore be inferred that thorium nitrate has no poisonous properties, which confirms the observation of Bokorny made about ten years ago.

Observations on the action of thorium compounds on plants in full nourishing solutions are hardly possible, since the phosphates would precipitate the thorium as phosphate almost completely, which phosphate settling as a fine powder, remains out of reach by the roots. Hence some experiments with plants in soil culture were instituted.

Two pots containing eight kilo soil received each the following manure :

KCl	5 g.
K_2CO_3	7 g.
Na NO_3	6 g.
$(\text{NH}_4)_2 \text{SO}_4$	6 g.
Common superphosphate	18 g.

¹ *Rutherford and Soddy* (Chem. News, Vol. 86. No. 2236) assert that radio-activity is a manifestation of subatomic chemical change.

One pot received thorium nitrate 10 mg. and the other 100 mg. for each kilo of soil. The third pot served as control. Twenty seeds of buckwheat were sown March 12. On March 26, the young shoots were thinned out to five of equal size in each pot. On April 30, the plants were measured with the following result:

	Thorium nitrate 10 mg per kilo.	Thorium nitrate 100 mg per kilo.	Control.
	cm.	cm.	cm.
	52	45	50
	55	49	52
	55	52	57
	69	57	61
	70	69	62
	-----	-----	-----
Average.....	56.2	54.4	56.4

This shows that there was no stimulating action exerted in regard to the development in height. On July 9, the plants were cut and the straw and grains weighed in the air dry condition with the following result:

	Thorium nitrate 10 mg per kilo.	Thorium nitrate 100 mg per kilo.	Control
	g.	g.	g.
Full grains	17.8	16.0	28.3
Unripe grains ...	4.7	4.5	4.0
Straw	18.7	13.5	20.3

It will be seen that thorium nitrate exerted a depressing influence on buck wheat in a dose of 100 mg. per kilo soil and that even if the quantity is reduced to 10 mg. per kilo soil no stimulating action on buck wheat, in contrary a considerable depression of harvest is obtained.

In order to observe whether plants of other families behave alike, a second experiment was made with a grass, *Panicum frumentaceum*.

In manuring the following doses were applied for each pot of eight kilo soil.

KCl	5 g.
K ₂ SO ₄	7 g.
Na NO ₃	6 g.
(NH ₄) ₂ SO ₄	6 g.
Na ₂ HPO ₄	10 g.

The pots and soil used this time were the same as before and therefore no new doses of thorium nitrate were applied this time. On July 24, the seeds were sown and afterwards the plants were reduced to nine in each pot. During the development of these plants, some action was observed in the case of 100 milligram thorium nitrate per kilo soil. Even in the pot which received 0.08 grams thorium nitrate (10 milligram per kilo soil), the plants developed better than in the control case; unfortunately, however, some plants in this pot were damaged by certain insects. On October 5, the plants were harvested with the following results:

	Control	100 mg Thorium nitrate per kilo soil
Average length.....	100 cm.	101.4 cm.
Weight of grains (air-dried) ..	10.2 g.	12.0 g.
Weight of straw (air-dried)...	14.7 g.	23.7 g.

These differences are not very decisive but a slight stimulating action of thorium on Panicum is probable, while with buckwheat no stimulating effect was noticeable.

Experiment with cerium. Three pots, each holding 2.5 kilo air dry soil were manured as follows:

Na NO ₃	3 g.
(NH ₄) ₂ SO ₄	1 g.
KH ₂ PO ₄	3 g.
K ₂ CO ₃	3 g.

Pot (A) received 10 milligr. ceric sulphate per kilo soil; Pot (B) 100 mg. while Pot (C) served as control. On May 4th, 16 seeds of upland rice steeped previously in water for two days were sown and on July 24, the number of plants was reduced to four of equal size in each pot. Till the beginning

of July, the development of plants in B was far behind that of the control plants and even those in A did not surpass the control plants in height. Here some injurious action was evident. But after the middle of July, the plants in A exceeded the others in height.

	A	B	C
	Ceric sulphate 10 mg per k.	Ceric sulphate 50 mg per k.	Control
	cm.	cm.	cm.
	8.7	8.9	8.0
	8.2	7.3	7.7
	7.4	7.2	7.3
	8.0	6.7	8.2
	—	—	—
Average	8.1	7.5	7.8

The fact that the action of the ceric sulphate was now more favorable than in the beginning would find a simple explanation, if the poisonous ceric sulphate which has strong oxidizing powers was turned gradually by the organic matter in the soil into cerous sulphate, which has not such an oxidizing power, and consequently would be less poisonous. On October 5, the plants were harvested with the following results:

	A	B	C
	Ceric sulphate 10 mg per kilo soil	Ceric sulphate 50 mg per kilo soil	Control
Average length	88.5 cm.	95.5 cm.	89.7 cm.
Weight of grains (air dried).	11.2 g.	12.5 g.	10.5 g.
Weight of straw (air dried).	18.0 g.	18.0 g.	18.2 g.

These differences are but small and leave it rather doubtful whether cerium has any stimulating action.



Can Salts of Zinc, Cobalt and Nickel in High Dilution Exert a Stimulant Action on Agricultural Plants?

BY

M. Nakamura.

Since manganese can promote the growth of plants, I believed it to be of some value to observe also the actions of the related elements zinc, cobalt, and nickel in high dilution. The salts of these elements are known to act poisonously in moderate concentration,¹ but whether these may act as stimulants on agricultural plants when very highly diluted has not yet been the object of observation, except quite recently in the laboratory of Prof. Miyoshi.

The results would be of some interest, especially in regard to zinc, since *zinc pots are frequently made use of in agricultural experiments.*

Zinc becomes easily oxidized in presence of moisture and air and the roots of plants growing in zinc pots may come in contact with the oxydized surfaces, and absorb some zinc oxid.

As *Raulin*² and others have shown that fungus growth may be enhanced by traces of zinc salts, erroneous conclusions may be arrived at if phænogams would behave in like manner. In regard to algae *Ono*

¹ *F. Nöbbe, P. Baessler and H. Will* in *Landw. Vers. Stat Bd.*, XXX and further *J. Baumann* *Ibid.*, Bd. XXXI. This author experimented with water and soil cultures and observed with various plants a different degree of resistance-power, further a considerable difference in the absorptive powers of various soils for zinc. Humus in the soil diminishes the poisonous effects of zinc salts, since they are transformed by it into insoluble compounds.—*Kömi* inferred that waters containing $ZnSO_4$ should in no case serve for irrigation.

² *O. Raulin* applied a solution of 70g. sugar and 4g. tartaric acid in 1500g. water in presence of the necessary mineral nutrients. On addition of $\frac{1}{3000}$ zinc sulphate the fungus-growth was found 3-4 times that in the control case.

has made some interesting observations.¹ He writes: „Bei unseren Versuchen mit Algen wirkte Zinkvitriol nächst Eisenvitriol sehr günstig auf des Wachstum ein schon schon bei Zusatz von einer minimalen Quantität, wie 0,00006% bis 0,0003%. Stieg die Concentration auf 0,0016%, so litten die Algen nicht unerheblich, ohne dass jedoch das Wachstum ganz unterdrückt worden wäre. Unsere Versuche mit Pilzen stimmen mit denjenigen von *Richards* überein.“

„Meine Versuche mit Eisenvitriol zeigen, dass Algen dasselbe in höherer Concentration als andere Schwermetallsalze ertragen. So lag bei *Hormidium* das Optimum etwa bei 0,0005%, und sogar bei einer höheren Concentration wie 0,0126% war der Ertrag noch etwas grösser als bei den Controlpflanzen. Die optimale Dosis von Nickelvitriol lag etwa zwischen 0,00006 und 0,00012%, während von 0,0028% an eine schädigende Wirkung auf Algen eintrat.“²

„Bei Algen scheint auch Cobaltsulfat einen begünstigenden Einfluss auszuüben, Optimum bei etwa 0,00012%.“

For my own experiments I selected an *Allium*, a small variety of *Brassica chinensis*, further barley and pea. Each pot contained 2300g. air dry soil and was manured with 3g. sodium nitrate, 3g. potassium carbonate and 4g. double superphosphate.

Pot No. I received—0,01g. Zn SO₄ (0,01782g. Zn SO₄ + 7aq.)

Pot No. II „ —0,01g. Ni SO₄ (0,01813g. Ni SO₄ + 7aq.)

Pot No. III „ —0,01g. Co(NO₃)₂ (0,01593g. Co(NO₃)₂ + 6aq.)

Pot No. IV served as control.

These mineral salts were incorporated in form of a very diluted solution in order to insure a complete distribution through the soil, which was well stirred after the addition. Pot No. 4 received at the same time as much water, as the others the solutions.

¹ Journal of the College of Science, vol. 13. Über die Wachstumsbeschleunigung einiger Algen und Pilze durch chemische Reize.

² A stimulating effect of zinc sulfate on plaeonogams was recently observed by *Kanda* in the Botanical Institute of this University.

Experiment with Allium.

Fifteen seeds were sown September 25 in each pot, and after the young shoots had reached 3-5cm they were reduced to 5 per pot, all of nearly equal height. Towards middle of December it became evident that the leaves of the zinc, nickel and cobalt plants were larger than those of the control plants. Since the growth became gradually very slow and the leaves assumed a yellowish hue, some ammonium sulphate was applied 0,5g. for each pot, February 1. which showed soon a very favorable effect. On February 27 the following observations were made:

	Average length of leaves (cm.)	Number of branches.
Zn—plants.....	32,6	20
Ni— „	34,0	21
Co— „	33,6	10
Control	31,2	10

The plants were harvested May 1 with the following results:

	Total weight.	Number of branches.	Number of flowers.	Average length.
Zn—plants	155,8 g.	25	5	54 cm
Ni— „	163,5 „	23	5	54,4 „
Co— „	129, „	23	4	52,8 „
Control	123, „	23	2	50,6 „

The result shows indeed some stimulating action of small doses of nickel, cobalt and zink.

Experiment with Brassica Chinensis.

Fifteen seeds were sown September 25 in each pot, and after the young shoots had reached 8-10cm. they were reduced to 4 per pot, all

of nearly equal height. Some time afterwards the nickel plants appeared to be ahead of the others. Towards the middle of November it became evident that the leaves of the cobalt plants were considerably larger than those of the others. Unfortunately some damage by a caterpillar was done to some leaves of the zinc plant before the insect was noticed, hence further observations of this pot were abandoned. On December 5 the pots received 0.5g. ammonium sulphate and 0.5g. monopotassium phosphate highly diluted, since some of the lower leaves had a yellowish appearance. The plants were harvested on December 23 with the following results:

	Number of leaves.	Length of leaves (cm).	Fresh weight, plant mass.	Fresh weight of roots.	Root in percentage of total yield.	Weight of the fresh leaves(g.)
Ni—plants	41	14.9	31.1 (g.)	11.4 (g.)	36.5%	21.25 (g.)
Co— „	37	16.5	34.8 „	11.7 „	33.6 „	22.6 „
Control	43	13.5	30.1 „	13 „	43.2 „	17.4 „

It will be observed from this table that in the case of cobalt some noticeable increase of the total production took place but this increase of weight related only to the leaves while the weight of the root was smaller than in the control case. In the case of nickel an insignificant increase of the total weight is noticed which was due to the leaves but not to the root. What with safety can be concluded from this table is only that a stimulant action on the growth of the leaves by the action of a small quantity of cobalt nitrate had taken place.

Experiment with Hordeum.

After cutting *Bressica Chinensis* the same pots were manured with 3 grams sodium nitrate, 3 grams potassium carbonate and 4 grams double superphosphate. No fresh doses of the above named metallic salts were given and thus only the amount left after harvesting *Brassica* came here into action. Twenty seeds of barley per pot were sown on January 14.

On February 1 the young shoots were reduced to 10 per pot of nearly equal height. In the beginning of March the cobalt plants were taller than the control plants while the zinc and nickel plants were lower. On March 10 the average length was :

	Average length
Zn plants	36 cm.
Ni „	38 „
Co „	40,5 „
Control	39,5 „

On March 12 the ears of the cobalt plants commenced to appear while those of the zinc plants on the 18th, those of nickel on the 15th and those of the control plants on 13th. These plants were harvested on May 21 and left to dry. The result was as follows :

	Total weight.	Weight of ears.	Weight of grains.	Number of grains.	Weight of straw (air dry g.)
Zn—plants	18,6 g.	6,7 g.	5 g.	135	11,9 g.
Nickel „	23,0 „	9,2 „	7,1 „	181	13,8 „
Cobalt „	25,3	10,2 „	8,2 „	202	15,1 „
Control „	24,0	9,4 „	7,3 „	102	14,6 „

It will be noticed that only with cobalt a stimulant action had taken place and that nickel and still more zinc exerted even in the very small doses present an injurious action on the barley.

Experiment with Pisum.

Twenty seeds of Pea were sown February 16 and the young plants were reduced later on to 4 of equal height in all the pots. In the early period of growth, the nickel plants showed some stimulation and at the beginning of May, also the zinc and cobalt plants in comparison to the control plants. The following observations were made on May 7 :

	Average length.
Zn-plants	83,25 cm.
Ni- „	92,5 „
Co „	81,75 „
Control	75,75 „

Growth as well as the flowering period lasted longest with the control plants. The harvest on June 17 yielded the following results:

	Total weight.	Weight of seeds.	Number of branches.	Average length.
Zn-plants	24,5 g.	14 g.	4	120 cm.
Ni- „	27,8 „	14,5 „	4	123,8 „
Co- „	25,5 „	14,3 „	5	118 „
Control	24,5 „	14 „	4	120 „

It will be seen that the stimulating effect of Zn was nil and those of Co and Ni minute and uncertain. In all the 4 cases here described I have observed that in the early period of development the nickel plants showed the most favorable growth and from the middle period of development cobalt plants gained headway while the control plants continued its growing and flowering for a longer time than the others.

As a general result, however, it may be inferred that stimulant actions can be exerted in certain cases by small doses of zinc, nickel and cobalt salts, on agricultural plants, but this effect was not considerable in my experiments.

Can Lithium and Cæsium Salts Exert any Stimulant Action on Phænogams ?

BY

M. Nakamura.

Lithium belongs to those elements which are present in very small quantities in many soils.¹ It was found also in many plant ashes by *Bunsen* and *Kirchhoff*.² *Lippmann* found it in the ash of the sugar beet, *Truchot* in the ash of tobacco (0.44%). *Tschermak* observed that lithium especially accumulates in the leaves, and that certain plant species take up lithium salts much more easily than others. He further denies any relation between the lithium contents and the degree of development. *Nobbe* (1871) had observed that lithium cannot replace potassium in the plants, and that lithium salts act even poisonously upon buckwheat.³ With algæ and fungi no injurious action of lithium salts was observed.⁴ It remained now to be seen whether lithium salts are capable to exert a stimulating action on phænogams when applied in very small doses.⁵ This question seemed to me of sufficient interest to justify some experiments in this direction.

¹ Truchot C. r. 78. 1022.

² Ann, Chem, Pharm. Vol. 118 p. 353.

³ The culture solutions of Nobbe contained 104.2 milligr. Li_2O per liter, in another case 73.8 milligr.

⁴ O. Loew, Ein natürliches System der Giftwirkungen, p. 115. Also Journal prakt. Chem. Bd. 36, S. 284 (1887).

⁵ According to *Ono* lithium nitrate can act as a moderate stimulant of growth on algae and fungi (Journal College of Science, Tokyo, vol. 13 p. 165(1900)) :—Thus LiNO_3 in a dilution of $\frac{1}{25} \times 10^{-4}$ increased the growth of *Protococcus* in 24 days, from 0.010 g. in the control case to 0.020 g. and the growth of *Aspergillus niger* was stimulated by $\frac{1}{16} \times 10^{-2}$ from 0.300 g. in the control flask to 0.408 g. in 17 days. *Richards* observed stimulant action of 1% LiCl in the culture solution on fungi (Pringsheims Jahrb. wiss. Bot. Vol. 30, p. 665 ; 1897).

The soil serving for my experiments came from our College Farm at Komaba and was manured with 1 g. NaNO_3 , 1 g. K_2SO_4 , 0.5 g. KCl , 0.5 g. $(\text{NH}_4)_2\text{SO}_4$ and 1.2 g. double superphosphate per kilo. To one pot was added 10 milligr. lithium carbonate per kilo soil, to the second 100 milligr. per kilo while the third served for the control plants. The pots contained 8 kilo soil each and were kept in the greenhouse.

Experiment with Barley.

Twenty grains of barley were sown January 16 and the young plants reduced later on to five of equal height in all the pots. At the beginning of May some difference in development was observed and the measurements were as follows:

	Average length	Number of branches
100 mg. Li_2CO_3 per kilo soil	72 cm.	24
10 " " "	75 "	24
Control	74 "	22

On May 27 the measurements were as follows:

	Average length	Number of branches
100 mg. Li CO_3 per kilo soil	76.0 cm.	27
10 " " "	81 "	24
Control	75.1 "	23

The plants were cut June 13 with the following result:

	Total weight	Weight of grains	Number of grains	Weight of each 100 grains	Average length of branches	Number of branches
100 mg. per kilo soil	104 g.	44.7 g.	887	5.28 g.	76.3 cm.	27
10 mg. per kilo soil	114 "	43.5 "	808	5.38 "	81.5 "	24
Control	97 "	38.5 "	787	4.89 "	75.3 "	23

Experiment with Pca.

The conditions were here essentially the same as in the first experiment. Twenty grains of pea were sown January 28 and the young plants reduced later on to four of equal height in all the pots. In the early period of development the control plants showed the best growth and also showed some flower several days earlier than the lithium plants.

Measurements were made on May 5 with the following results:

	Average length of branches	Number of branches	Number of flowers
100 mg. Li_2CO_3 per kilo soil	86 cm.	5	1
10 " " "	98.3 "	4	3
Contro	101.5 "	4	6

After that time the lithium plants showed a more vigorous development than the control plants.

The plants were harvested June 15.

Air dry plants.

	Total Weight	Weight of seeds	Number of seeds	Number of branches
100 mg.	65 g.	30.5 g.	147	5
10 mg.	61 „	31.2 „	142	4
Control	56 „	29.5 „	140	4

Both experiments show that *lithium carbonate can exert a slight stimulant action.*

Under the conditions just described an experiment with upland rice was carried out to test the influence of caesium on the development. Pot A received 10 Milligr. caesium chlorid per kilo soil, Pot B 100 Milligr. while Pot C served as control. Young plants of equal height were selected from the seed bed and planted into the pots a five in each, at the beginning of June. At the time of flowering it was evident that the plants in B exceeded the others as to height. The plants were cut October 2. In pot A no branches were developed in B one branch with yet unripe seed, while in C one branch with ripe seed.

The measurements gave the following figures :

A	B	C
100 cm.	109 cm.	109 cm.
101 „	112 „	101 „
103 „	113 „	108 „
105 „	114 „	91 „
105 „	101 „	84 „
	—	—
Average height. 103 „	110 „	99 „

The height of the branch in B was =58 cm; in C=75 cm. The total production of ripe seed, weighed in the air dry state was :

A	B	C
13.1 g.	14.6 g.	13.7 g.

It can therefore be inferred, that caesium chlorid at the rate of 100 mg. per kilo soil had exerted a moderate stimulating effect, especially noticeable in the height of the plants.





On the Stimulating Effect of Iodine and Fluorine Compounds on Agricultural Plants II.

BY

K. Aso and S. Suzuki.

In former Bulletins of this College, a field experiment with radish and pot experiments with oats and pea were described which showed the stimulating effect of small doses of sodium fluorid and potassium iodid. We have continued these investigations, upland rice serving now for the test. Five plots were selected, each of these had ten square meter and received as manure 100 g. double superphosphate, 200 g. ammonium sulfate and 150 g. wood ash, the last mentioned salt being ploughed into the soil one day later than the former. The seed to the amount of 47.5 g. per plot was sown April 30. Plot I received 0.08 g. Na F, II 0.8 g. Na F, III 0.025 g. KI, IV 0.25 g. KI, and V served as control plot. The solutions were applied in high dilution. On June 2, each plot received as top-dressing 50 g. sodium nitrate and 50 g. monopotassium phosphate. The plants on the fluorine plot surpassed gradually the others somewhat in height. A damage done by a typhoon proved to be insignificant.

The plants were cut Sept. 8 and left to dry in the glasshouse. The weight was as follows:

Ratio per ha	Weight of seeds unhusked, g.	Weight of straw air-dry, g.
80 g. Sodium fluorid	2465	1885
800 g. „ „	2090	1800
25 g. Potassium iodid	2300	1900
250 g. „ „	2000	1810
Control	1970	1920

A stimulating effect on the seed production by sodium fluorid and potassium iodid respectively is here evident with the smaller doses of 80 g. sodium fluorid and 25 g. potassium iodid per ha, while the ten times higher doses failed to give a decisive result.

On the Treatment of Crops by Stimulating Compounds.

BY

Oscar Loew.

At the International Congress of Applied Chemistry in Berlin, June 1903, Professor *Gabriel Bertrand* from the Institut *Pasteur* in Paris read a paper: "Les Engrais Complémentaires" in which was pointed out that, as to mineral manures, almost exclusively compounds of potassa, phosphoric acid and nitrogen are paid attention to, while there exist rarer elements which occur only in exceedingly small amounts in the plants but may nevertheless be of a certain physiological signification, leading to an increase of the harvest. *Bertrand* proposes to call such compounds supplementary manures (*engrais complémentaires*).

It may therefore not be out of place to call attention to the fact that professors and graduates of this College have during the last three years studied the influence of small doses of various compounds upon the growth of plants and yield of crops and published a number of papers on this subject.¹ A short survey of the results obtained may here be in order since some observations are of theoretical interest and some promise even to become of practical value. In the first place such elements were considered which occur in small doses widespread in the soil whence they pass into the plants and through them into the animals. These elements are manganese, fluorine and iodine. The manganese content of vegetable and animal organs,

¹ These Bulletins Vol. 5, No. 2 and 4; also this number. A part of the former experiments was also reviewed by the writer in the *Landw. Jahrb.* 1903, Heft. 3.

the fluorine content of bones and teeth, the iodine content of the thyroid gland are well known facts.¹

Although according to *Gautier* and to *Bertrand* slight traces of arsenic also occur in animal organs which must have received it through plants from the soil, we did not take this element into consideration. Arsenious salts in a dilution of 1:300000 are still very poisonous for phænogams (*Nobbe*). Arsenates, it is true, are much weaker poisons for plants, but nevertheless also strong poisons for warmblooded animals. The use of arsenic compounds in any form should not be tolerated in agricultural operations. In the United States poison cases were caused by cabbage that had been dusted with Paris green in order to kill the adhering caterpillars. It is true that most of the stimulating compounds are poisons in higher concentration, but the dangers are nowhere as great as with arsenic compounds.²

In the second place also compounds of such elements were tested which occur occasionally in traces in the soil and of which no occurrence in animal organs is reported, as boron, lithium, rubidium and caesium compounds.³ The high price of the salts of these elements would forbid their practical application.

In the third place compounds of elements were tested which are confined to certain localities of relatively rare occurrence, as zinc, nickel, chromium, cobalt, uranium, thorium, vanadium, cerium. (Bromine was tested on

¹ *Gley* and *Bourcel* found iodine in the blood (0.013—0.112 milligr. per Liter). The occurrence of iodine compounds in mineral springs, in some rocks and minerals, as, e.g., phosphorite and in coal are well known. Traces of them occur also in the Chili salpeter. As to fluorine it was shown by *Tamann* to occur also in eggalbumen, milk and blood (about 1 milligr. in 100 parts of fresh substance). *Nickl's* found traces of fluorine in various animal organs as early as 1856. The origin of the traces of fluorine in the soil is the apatite occurring in various rocks.

Also alumina occurs in plants frequently. A stimulating action of alumina was thus for not distinctly recognized by us, but the experiments will be continued with larger doses of aluminium salts.

² Fungi are not quite so sensitive towards arsenious acid, as green plants. According to *Orlovski* 0.001—0.01% sodium arsenite stimulates growth of *Aspergillus niger*, larger quantities act retarding, still larger as strong poison.

³ It is of some interest to note that *Dieulafoy* has discovered traces of lithium, rubidium and boron in the crude Chili salpeter (*Compt. rend.* 48, p. 1545). Traces of these and of caesium and titan were repeatedly observed in plant ashes; also of vanadium in one case by *O. von Liepmann*.

account of its occurrence in sea weeds and for the sake of comparison with fluorine and iodine). All such compounds are foreign to agricultural soils and should therefore be excluded from practical application as stimulants.

For the convenience of the reader some of the observations made by *Nagaoka, Aso, S. Suzuki, Nakamura* and the writer have been embodied in the following Table :

Stimulating Compound.			Remarks.
Name.	Milligrams per kilo soil.	Amount per hectare.	
Lithium carbonate	10—100		Pot experiment with pea and barley.
Rubidium chlorid	200		Pot experiment with barley.
Cæsium chlorid	100		„ „ rice.
Uranium nitrate	5		„ „ pea and oats.
Manganous sulphate } Mn SO ₄ + 4 aq. }	24		Pot experiment with pea.
„ „ „		77 kilo	Field experiment with rice. [Mn ₂ O ₃ = 25 kilo per ha]
Manganous chlorid } Mn Cl ₂ + 4 aq. }		63 kilo	Field experiment with up land rice.
Borax.....	1—5		Pot experiment with spinach and pea.
Bromid of Potassium	10		Pot experiment with beans and rice.
Iodid of Potassium	0.26		Pot experiment with oats.
„ „ „		25 grams	Field experiment with radish and up-land rice.
Fluorid of Sodium	2.6		Pot experiment with pea.
„ „ „		50—140 grams	Field experiment with radish and up-land rice.

The degree of the poisonous character of compounds does not always correspond to the intensity of the stimulating action, exerted when highly diluted, and also the zone of indifference, i.e. that special degree of dilution

at which the stimulating effect and the injurious influence balance each other, is of very different width, it seems e. g. larger with fluorid of sodium than with borax. Vanadium sulphate, poisonous at 0.1% for plants in water culture, still exerted a depressing influence at a rate of 10 milligrams per kilo soil. Borax acted in this dose also very injuriously but at 1-5 milligrams it exerted a weak stimulating action. Chromic alum exerted a poisonous action in a dilution of 0.1% upon seedling of pea, but had in higher dilutions neither an injurious nor a stimulating effect.¹

Of some interest is the stimulating effect of lithium, rubidium and caesium compounds in doses of 0.01—0.2g per kilo soil, since it recalls the beneficial actions of sodium salts observed by many authors, and studied especially by *Wagner*. According to *Doll*² a maximal yield of barley can only be expected by the joint application of potassium and sodium salts.

The question how the stimulant action is to be explained can at present not be answered positively, although in some cases certain views find some support. It is thus, e. g., very probable that manganese salts act beneficially by enhancing the action of the oxidizing enzymes in changing noxious by-products of metabolism by partial oxidation.³ The fact that potassium bromid, manganese, and uranium compounds exert a stimulating effect on phænogams but not on fungi, while sodium fluorid acts stimulating on both groups of the vegetable kingdom, shows plainly that there exist various causes for the phenomenon of stimulation.

As to zinc salts a stimulating effect was observed with a fungus (*Aspergillus*) as well as with phænogams (*Raulin, Kanda, Nakamura*), but the fungi appear to behave not all alike, since *Coupin* observed no stimulating effect of zinc salts on *Strigmatocytis nigra*.⁴

¹ A soil containing 1.6% chromic oxid occurs in the island of Adaman. The coffee plantations on that soil are reported to be normal. (*J. B. f. Agr. Chem.* 1891).

² *Centralbl. f. Agric. Chem.* 32, p. 13.

³ Cf. These Bulletins, vol. V, No. 2, and *Flora* 1902 p. 264.

⁴ *Compt. rend. Febr.* 1903. In experimenting with mould fungi quite a number of flasks, not only one or two, should be observed, since often widely different weights of fungus growth under apparently equal conditions are obtained.

The reason for testing uranium and thorium salts was their showing radioactive properties. Radioactivity has a powerful influence on animal tissues and on bacteria and some influence was probable to be exerted also on phænogams. Both those compounds, however, differ widely in their effects on plants, uranium salts being highly poisonous, thorium salts not. Uranium salts stimulate further in much smaller doses as do thorium salts. Uranium salts also can produce under the influence of light certain chemical changes, which thorium salts are incapable of. These specific changes are not connected with the reduction of uranic to uranous compounds by organic substances under the influence of light. They consist in splitting off one carboxylgroup from the molecule of a bibasic acid. Oxalic acid becomes thus formic acid; succinic acid changes to propionic, pyrotartaric to isobutyric,¹ glutaric to butyric acid. Mesaconic acid seems to yield crotonic acid. Itaconic, tartaric, citric and suberonic acid are but very slowly changed, if at all. Asparagin and peptone remain also apparently unchanged, while parabanic acid is easily changed to ammonia, formic and carbonic acid. Thus the view of the writer seems to have some support that traces of uranium compounds that had passed into the leaves might enhance the transformation of light into chemical energy.

Several experiments were made with uranium salts. In the successful pot experiments with pea and oats, the highly diluted uranium nitrate was applied as top-dressing in six doses. The increase compared with the control plants was in regard to the seeds 1.27 fold with the pea and 1.25 fold with oats. A field experiment with upland rice however was not successful, the yield being too little above that of the control plot. The uranyl nitrate was here applied together with the manure and not as top-dressing (6 g. for 30 square meter) and thus became, as tertiary phosphate, gradually entirely unavailable for the roots.²

¹ Seelkamp, Ann. Chem. Pharm, vol 133 [1865]. He added to a 5% solution of succinic acid 1 percent uranous succinate and exposed this mixture to the direct sun light.

² An unfavorable circumstance was here probably also the application of potassa as carbonate (woodash).

A joint application of two stimulants proved in some cases advantageous¹ in others, however, not. A report on these experiments will follow later on.

The question might be raised whether the seeds of plants grown under stimulating influence would yield again normal plants. In this regard quite a series of tests were made, all of which answered in the affirmative.

Of all the stimulants observed only compounds of manganese, fluorine and iodine come seriously into consideration for practical agriculture. Besides these also the ferrous compounds deserve attention, which apparently act not only as nutritive material inasmuch they render possible the formation of chlorophyll but also exert some kind of stimulating action. The observation of *Molisch* on the relation between iron and fungi shows that iron is not only concerned in chlorophyll production.² Indeed iron like manganese was found repeatedly in the ash of nucleoproteids (*Stoklasa, U. Suzuki, Aso*) and of oxidizing enzymes (*Bertrand, Lepinois, Sarthou, Spitzer, Sieber*).

The action of ferrous sulphate upon crops has often been an object of observation. Some authors reported a favorable action, some an unfavorable one; others again inferred that there was no influence whatever. The effects depend of course very much upon the quantity applied. In one case fully 0.1% was added to the soil in order to prove its injurious influence. This is a very large dose.³ The effects also depend upon the quantity of easily absorbable iron already present in a soil; a further moderate addition may prove of no avail at all when the soil is rich in easily available iron compounds. Our own observations showed that doses of 25—50 Milligrams ferrous sulphate per kilo soil can exert some stimulating action even on a soil that contains enough easily available iron for the chlorophyll formation

¹ This was, e. g., the case in the joint application of ferrous and manganous sulfates.

² *W. Benecke* observed with a moss (*Lunaria cruciata*) that the formation of rhizoides was at first retarded by solutions containing 0.0004% ferrous sulfate, later on, however, enhanced (*Botan. Zeitg.* 1903, Heft. 2.).

³ The occasional occurrence of ferrous salts or ferrous sulphid in swampy grounds is only a sign of wanting aeration but not the original cause for the inferiority of such soils. *Griffiths* observed very good results by green vitriol at the rate of 60—125 kilo per ha. This amount may be for certain crops too high.

of crops. Whether this effect is due to the state of the monoxid, while in the soil iron was present as sequioxid or whether it is due to the much finer state of division of the applied iron compound we are at present not yet prepared to decide.¹ It may here be mentioned that ferrous sulfate is capable of certain catalytic powers. Thus a slight trace of it suffices to bring hydrogen peroxid into action with potassium iodid, whereby the iodine liberated can at once be recognized by the intense blue color produced with starch paste (*Schönbein's* reaction for hydrogen peroxid). In this way still 0.0000001 grams iron in the state of ferrous salt can be recognized.²

In some of our experiments the effect of small doses of ferrous sulphate was compared in absence and in presence of manganous sulphate. A pot-experiment with oats may here be described. Four pots each holding 2300g. air dry soil and manured each with 3 gram sodium nitrate, 3 g. potassium carbonate and 4.6 g. double superphosphate received 15 seeds on February 21. After the shoots had reached 12—15 cm. their number was reduced to 5 per pot, taking care that these remaining plants were all of equal size. While one pot served as control, the others were watered with highly diluted solutions of ferrous and manganous sulphate six times until the flowering period was passed. All pots were treated alike as to watering, exposure to light, etc. The plants were cut July 6, and left to become air-dry. The final result was:

	Fe SO ₄ = 0.126 g.	Fe SO ₄ = 0.06 g. Mn SO ₄ = 0.06 g.	Fe SO ₄ = 0.012 g. Mn SO ₄ = 0.126 g.	Control
Number of stalks	12	8	15	9
Grains, unhusked	25.7 g.	23.1 g.	27.8 g.	21.4 g.
Straw	48.1 g.	46.4 g.	51.0 g.	45.2 g.

¹ The state of division has a very great influence also with other compounds. Thus precipitated magnesium carbonate (basic) has a much more injurious influence on plant growth than an equal dose of even very finely powdered magnesite. Slaked lime yields a much more finely divided carbonate (in presence of sufficient water), than can be obtained by pulverizing lime stone.

² *O. Meyer*, Chemiker Zeitung 1903, No. 52. See further *Schönbein*, Journ. f. prakt. Chem. 1866, p. 66. Also *Schöne*, Ann. Chem. Pharm. 1879, p. 232; finally *D.*, Chem. Ges. Ber. 1901, p. 2479.

Some beneficial effect of ferrous sulphate was here doubtless exerted, the effect of the same amount manganous sulphate in presence of some ferrous sulphate was, however, more marked.¹

Another experiment with tobacco plants, grown in pots from seed from Java, may here be mentioned. Two plants, each measuring 44 cm. received, Oct. 6th, 0.3 g. manganous sulphate ($\text{Mn SO}_4 + 4 \text{ aq.}$) + 0.2 g. ferrous sulphate ($\text{Fe SO}_4 + 7 \text{ aq.}$) dissolved in 100 cc. water; two other plants of 48 and 47 cm. height, 0.3 g. manganous sulphate alone; two others, each of 43 cm., 0.2 g. ferrous sulphate, while two, of 45 and 43 cm. height, served as control-plants. The longest leaves measured 32-36 cm. The soil had been manured with farmyard manure; each pot received, Oct. 15, a further addition of 0.2 g. ammonium sulphate. During the month of November considerable differences in growth became easily noticeable. On Dec. 23 the plants were photographed; this photograph is reproduced on Plate XV. They were cut the same day with the following result:

	Mn + Fe	Mn	Fe	Control
Height of the plants, cm.	160	155	153	115
	135	134	122	109
Percentage of increase in height, {	260	223	209	155
	207	185	184	153
Number of flowers	15	4	14	—
Number of buds	48	46	41	—
Number of dead leaves	7	7	10	7
	6	9	7	8
Number of living leaves from	17	21	19	17
12 cm. upwards	19	18	16	17
Weight of the fresh leaves	84.2	85.5	75.2	59.5

¹ The application of ferrous sulphate and manganous sulphate on the farms would be very cheap, since both these salts can be directly applied in the raw, unpurified state. Crude manganous chlorid $\text{Mn Cl}_2 + 4 \text{ aq.}$ is obtained as a by-product of the manufacture of bleaching powder and costs in Japan only 3 yen per 100 pounds. In Germany its price is more than double as high (16 Mark).

It will be seen that ferrous as well as manganese sulphate had exerted a stimulating action and that the best effect resulted also here by the joint application of both.

Manganous sulphate and chlorid exert a stimulating effect on various crops,¹ but the degree varies according to circumstances. Not only the mode of application but also the manures used influence the result. Repeated application of highly diluted solutions in the form of top-dressing are more favorable than a single application of the same total amount of manganese salt at the time of manuring the soil, before the seed is sown. All conditions further which change the manganous salt soon into manganic oxid or tertiary manganic phosphate appear to depress also the availability of the manganese for the roots. It seems highly probable that potassa applied as carbonate or in the form of woodash will in this respect act unfavorably while in the form of sulphate not. In pot experiments the application of phosphoric acid in the form of secondary sodium phosphate acted less favorable than in the form of double superphosphate. Thus in a pot-experiment with pea the harvest was increased under the influence of manganese 50% in straw and 25% in seed compared with the equally well manured control pot, while in an experiment with buckwheat made under the unfavorable conditions mentioned, no increase was produced.² A most remarkable result was obtained by Prof. Honda and myself with young *Cryptomeria* trees, which received manganese sulphate as top-dressing in monthly doses, winter excepted, for one year and a half. The organic production was hereby doubled compared with the control trees.

It is further evident that fields which served repeatedly for raising crops under the influence of stimulating agencies would sooner be exhausted than others. Hence a moderate increase of manure will be needed. The following experiment seems to furnish an example: Two plots of 64 sq. meter were manured each with 640 g. double superphosphate, 1000 g. ammonium

¹ The susceptibility of different plant families toward stimulants seems to differ. Further observations will be made here to decide this point.

² Injurious effects of manganese in comparatively large quantities have thus far only be observed with watercultures (Landw. Vers. Stat. vol 8, p. 128 and 73 pp. 69 and 218).

sulphate and 1000 g. wood ash (added 8 days later). Seed of radish was sown Sept. 1. (1902). After the plants had reached a height of 15-25 cm. one plot received a top dressing of 94 g. cryst. manganous sulphate (= 64 g. anhydrous $Mn SO_4$) dissolved in 20 Liters of water. The superfluous plants were removed Oct. 8, leaving on each plot an equal number of equally well developed plants. A storm, however, injured a number of plants on both plots, hence only the best developed roots were compared. The height of the plants at the time of harvesting, Dec. 17, did not show any marked difference. The result was:

	Manganese plot	Control plot
Total plant mass (fresh weight).....	57.68 kilo	51.18 kilo
The 20 largest roots weighed	11.1 „	8.9 „

The same plot served now for a growth of potatoes. Amount and kind of manure was the same as in the former case, but manganese was not applied this time, in order to observe, whether some action would still be exerted by the manganese left from the first application. On March 23 (1903) 120 potatoes of equal size were planted on each plot, 10 potatoes in each row. The soil was cultivated several time and weeds removed. In the beginning of July some damage was done by a storm. The harvest, on July 16 yielded:

	Manganese plot	Control plot
Potatoes	17 kilo	15.6 kilo

It appears therefore that some effect of the manganese left was exerted, but the difference is not of very decisive magnitude.

The plots received now farmyard manure at the rate of 8 tons per ha and cryst. Manganese chlorid at the rate of 10 kilo per ha, and served for a growth of millet, of which 110 g. seed were sown on July 24. The harvest on Oct. 5 yielded:

	Manganese plot	Control plot
Total plant mass air-dry	25.1 kilo	26.0 kilo

Here several circumstances probably united to prevent the stimulating action of the manganese. In the first place the rate of manganese chlorid applied was rather low, in the second place there were less nutrients left after the stimulated crops of radish and potatoes had been grown than in the control plot, and in the third place the manganese was not applied as top-dressing but in conjunction with the general manure. It goes without saying that in the form of top-dressing the same amount of manganese must be more effective under otherwise equal conditions than when the distribution is made uniform through the whole soil. An exceedingly favorable result obtained with mustard on the one hand and a poor result with cabbage on the other hand support this inference.

Experiments with clover and maize have further shown that a favorable action of manganese cannot be expected when the manuring is imperfect. In these cases ammonium sulphate alone at the rate of 500 kilo per ha was applied, since some potassa and phosphate was supposed to be left from previous crop.

The manganese plot that had received manganese sulfate at the rate of 10 kilo per ha yielded here the same poor harvest as the control plot. The cobs of the maize on the manganese plot, however, showed in average a little higher weight than those on the control plot, viz 413 g. versus 391 g.

Soils which year after year are manured with farmyard manure are unvoluntarily gradually enriched with manganese compounds in finely divided condition, since the dung of cattle and horses contains almost the total quantity of manganese contained in the original fodder. Let us calculate for a given case how much manganous oxid would thus be supplied to one hectare manured with 36 tons of farmyard manure. *A. Emmerling* and *R. Wagner*¹ determined in a sample of meadow hay the amount of manganous oxid to 0.2% of the ash; the ash itself amounted to 8.86% of the dry matter of the hay. The farmyard manure (with 80% H₂O) from this hay would contain in 36 tons = 7200 kilo dry matter and in this = 1.26 kilo Mn O hence after 10 years the soil receives 12.6 kilo Mn O corresponding to 32.4 kilo

¹ Wolff's Tables of Plant Ashes, II, p. 28.

crystallized manganous chlorid $MnCl_2 + 4 aq.$ In beets¹ was found 13.95% ash in which 0.025% Mn_2O_3 and 0.048% Fe_2O_3 ; in clover 9.17% ash in which again 0.27% MnO and 1.51% Fe_2O_3 . These numbers, of course, vary according to the chemical composition of the soils but in general it may be inferred that the beneficial action of application of manganese salts will be more decisive where mineral manures are used than on soils that are continuously manured with dung. Various facts render the farmyard manure often superior to mineral manures,² as improvement of the mechanical condition of certain soils, the introduction of a rich bacterial flora into the soil, etc. One of the favorable circumstances may also be the presence of finely divided manganese and iron compounds.

Plants which grow on soils containing much manganese in available condition will hardly be benefited by application of further doses of manganese salts. The manganese content of soils differs considerably. Relatively small differences are shown in the analyses of four soils mentioned in *Wolff's* Tables.³ Concentrated hydrochloric acid extracted at the ordinary temperature from:

	Mn_3O_4	Fe_2O_3
Loamy soil	0.135%	2.096%
Clay ,,	0.180 ,,	3.173 ,,
Sandy ,,	0.083 ,,	1.039 ,,
Humus ,,	0.042 ,,	0.406 ,,

In the fine earth of a soil from Barbados⁴ 0.1% Mn_3O_4 , soluble in hy-

¹ *Ibid.*, p. 43 and 37.

² Recently again *Schneiderwind* reported from the experimental farm at Lauchstädt that the root crops yield a maximal harvest only when farmyard manure is applied in addition to mineral manures. Such observations have been made also in regard to tobacco in America.

³ Vol II, p. 16.

⁴ Report of the Barbados Experiment Station 1901.

drochloric acid was found, corresponding to 38 kilo $Mn_3 O_4$ per ha to the depth of 24 cm.

It was mentioned above that also iodine and fluorine compounds might sometimes be used as stimulants in practical agriculture. Some further remarks are therefore in order. As to iodide of potassium it acts poisonously upon phaeogams in waterculture even in a dilution of 0.002 per cent. *Voelker*¹ observed in a field experiment that a top-dressing at the rate of one half centiweight per acre, corresponding to 62.2 kilo per ha injured wheat and barley. This quantity, however, seems extraordinary great when compared with the amount that caused stimulation in our experiments, namely 25 grams per ha! This quantity can be increased to 250 grams per ha without fear of danger, but a further essential increase should be avoided. Hence a dose of 25 g. per ha may be applied for ten consecutive years; if it is taken into consideration, however, that a part is absorbed by the plants and another passes away in the drainage waters, the number of years might be doubled. But after this period a pause of several years should follow, during which the use of potassium iodid is suspended. The degree of stimulation was in some experiments of *S. Suzuki* considerable¹. The dose at the rate of 25 gram per ha produced an increase in the weight of radish of 67% compared with that obtained on the control plot and with upland rice an increase of 16% in grains. At the rate of 250 g. per ha the increase in the weight of radish was 31% while there was no increase in seed production with rice.

As to fluorine compounds of potassium or sodium it must be kept in mind that these are in certain respects still more poisonous than potassium or sodium iodid. While the latter exerts a high degree of poisonous action on all plants with an acid cell sap and but a weak one on objects with a neutral cell sap, as e. g. certain algae, those fluorids are very poisonous for all kinds of vegetable objects independent of the reaction of the cell sap or culture. A few observations may here be mentioned. Algae, such as *Spirogyra* and *Mesocarpus* are killed within 15 minutes in a 1 per cent solu-

¹ In pot experiments stimulation was observed at 0.26 and 2.6 milligrams per kilo soil with oats and pea, while at 26 milligrams a depression resulted with rice.

tion of sodium fluorid, the chlorophyll bands retract their lobes, the nucleus contracts and soon afterwards the cytoplasm recedes from the cellulose wall. In a solution of 0.1% filaments of algae die within 24 hours. Diatoms and monadines become motionless in a 0.1% solution within 12 minutes. Some monadines recover their power of motion but this is only of short duration, since after 35 minutes no trace of motion reappears. If 1 cc. of a 1 per cent solution of sodium fluorid be added to 99 cc. of culture water containing numerous forms of minute organisms, infusoria and diatoms are killed within 2 hours, while some monadines¹ still were seen alive after this time. At 0.05 per cent sodium fluorid injures the germinating power of seed, while in dilutions of 0.0001% to 0.001% it can stimulate growth of phænogams in water culture.

At 0.001% it prevents the action of lactic acid bacilli (*Effront*) and at 0.15 g. per kilo body weight it proves fatal for animals (*Tappeiner*).

Attention must here be drawn to the so-called *Wiborg's* Phosphate which contains fully 1 percent fluorine and is recommended for agricultural purposes. Since phosphates are often applied in large doses as manures, a highly injurious amount of fluorine would soon be accumulated in the soil. This phosphate is manufactured in Sweden by fusing apatite with sodium hydrate and consists chiefly of a sodium—calcium silico—phosphate.² Calcium phosphate is now-a-days also frequently added to the food of young hogs in order to promote the formation of bone. There occur, however, in commerce phosphatic preparations containing fluorids which have caused the death of the animals, as *Emmerling* has shown recently.³

The stimulating effects of small doses of sodium fluorid, viz 80—140 g. per ha have been quite considerable in the experiments of K. Aso, who has observed further that even a dose of 800 g. per ha does not yet act injurious-

¹ The monadines are also in other regards of an unusual resistance power which thus far is not satisfactorily explained.

² *Wiborg's* phosphate was recently reported in the *Chemiker Zeitung* to have the following composition; P₂O₅ = 22%; Si O₂ = 16%; Mg O + Ca O = 35%; Fe₂ O₃ = 6%; Al₂ O₃ = 2%; K₂ O + Na₂ O = 18%; Fluorine = 1%.

³ *Centrallbl. f. Agr. Chem.*, June 1903.

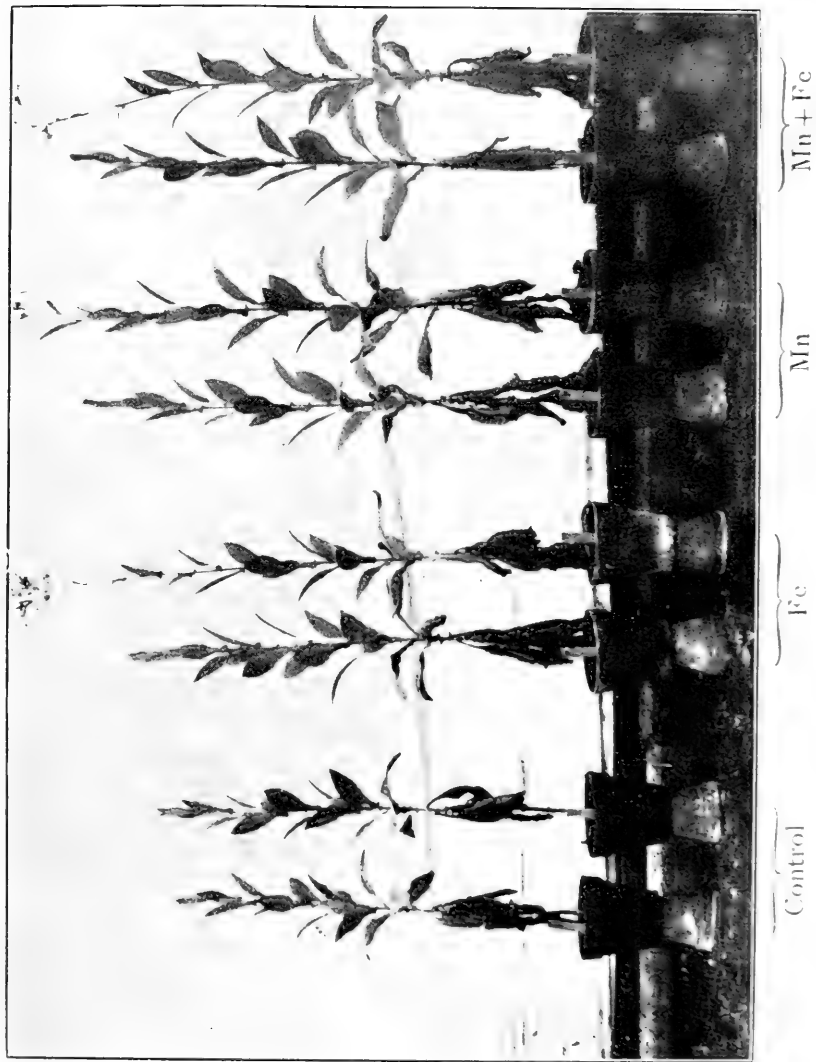
ly, although the stimulating effect has decreased, or almost vanished, showing that a further augmentation of the dose would not be advisable. A part of the sodium fluorid or all may pass into the but little soluble calcium fluorid, which dissolves in 26923 parts of water at 15,° a circumstance that tends to diminish somewhat the evil effects of accumulation. The stimulation of seed production with upland rice amounted to fully 25 percent on a plot that had received sodium fluorid at the rate of 80 g. per ha.

It will be noticed that the application of iodids and fluorids as stimulants requires a certain attention. Since perhaps the majority of farmers are averse to pay sufficient attention to the laws of nature, it may be recommended to restrict the application of stimulants to the manganous salts, since there are no dangers to be feared from their continuous application and any excess gradually turns into not readily available insoluble manganic compounds. They should be applied only in top-dressing—in several doses if possible—at the rate of about 25 kilo per ha—in high dilution and in addition of ferrous sulphate at the rate of about 20 kilo per ha.¹

¹ The ferrous sulphate should be dissolved in cold, not in hot water in order to avoid oxidation and decomposition with formation of basic ferric sulphate.—Manganese as sulphate would be further preferable to the chlorid in many cases.







Tobacco plants under the stimulating action of ferrous and manganous sulphate.

On the Action of Sodium Nitro-prussid upon Plants.

BY

Rana Bahadur,

from Nepal, India.

The highly poisonous character of nitro-prussid of sodium for vertebrate animals was recently demonstrated by *Fonzes-Diacon* and *Carquet*.¹

It seemed of some interest to test whether this salt would be also a strong poison for the lowest animal organisms and for the plants.

I have observed that infusoria, crustacea and worms were dead after one hour and half in a 0.1% solution of sodium nitroprussid; only some monadines were still alive. Diatoms also were killed after a short time in that solution. Filaments of *Nitella* were dead after an hour and half in the 1.0% solution. Young barley and buckwheat plants placed in the 0.1% solution were dead after 18 hours, the leaves having completely lost their turgor and being partially dried up. Leaves of radish and mulberry and branches of *Polygonum aviculare* were also found much affected in that time, the leaves being more or less withered. The leaves of cherry looked more or less brown when held against the light, while the control leaves were perfectly normal.

Since, however, the solution of nitro-prussid of sodium decomposes in direct sunlight and more slowly also in diffused daylight, with the production of prussian blue and prussic acid,² it may be objected that it is the prussic acid formed by this decomposition which killed the organisms.

¹ Chem-Centrl. 1903, p. 519.

² The characteristic odor of this acid is very soon noticed with these solutions.

I have observed that one of the products of decomposition of this salt by sunlight is nitrous acid. Hence the decomposition of nitro prussid of sodium might be represented by the following equation:—



On account of this decomposition all further experiments were now carried on in darkness. Young barley plants were placed in 0.01%, 0.1%, 1% solutions respectively, and kept in darkness. In the 0.01% solution of sodium nitro-prussid, the plants were not killed even after three days; while the plants in 0.1% solution commenced after twenty two hours to dry up slowly from the tips downwards. (This is quite different from the observation when the barley was kept in daylight, in which latter case the plant completely lost its turgor and dried up within eighteen hours, and finally the plants in 1% solution were killed within twenty two hours).

Further to a culture water containing numerous lower organisms 0.01% and 0.1% respectively, of sodium nitro-prussid was added and the flasks kept also in darkness. The microscopical examination of the sediment after twenty-three hours showed that diatoms, flagellata and infusoria were still alive, also the ostracodes and small nematodes were seen in their usual motions. Hence it may be concluded that nitro prussid of sodium is in a dilution of 0.1% not injurious for lower organisms, provided the daylight be excluded and thus the production of prussic acid from the salt be avoided.

Some experiments were also made with mould fungi and bacteria. In the case of mould fungi, sodium acetate 0.6% was the organic nutrient while ammonium sulphate, monopotassium phosphate, and magnesium sulphate each 0.1%, formed the mineral nutrients. In the experiment with bacteria, meat extract served as nutrient. In the former case 1% in the latter 0.5% of nitroprussid were added and the solutions after infection with *Penicillium glaucum* and *Bac. pyocaneus* respectively, were placed in darkness. After about one week *Penicillium* was developed about equally well as in the control case. As to the bacteria, the growth was noticed one day sooner in the control case than in the chief flasks which, however, showed after one week, a luxuriant development.

Conclusion.

Nitroprussid of sodium is a comparatively weak poison for lower animal organisms and green plants, and no poison for fungi, provided the daylight is excluded.

Daylight decomposes the salt with the production of prussic acid and nitrous acid. Such a decomposition probably takes place also in the higher animal organism which would account for the highly poisonous character of that salt for the vertebrate animals.





On the Behavior of Guanidine to Plants.

BY

I. Kawakita.

1. Can guanidine serve as nutrient for fungi?

It has been asserted in a paper published some years ago that guanidine may serve as source of carbon in the development of *Aspergillus niger*. This seemed however exceedingly improbable since guanidine yields by hydrolysis carbondioxid and ammonia and there is no hydrogen atom in direct combination with the carbon. It is most closely related to urea which also is incapable to serve as source of carbon in the nutrition of fungi. Both compounds, however, are good sources of nitrogen for various fungi. In order to test whether really guanidine can serve not merely as source of nitrogen but also as source of carbon, I prepared the following solution :

Water	100 c.c
Guanidine hydrochlorid.....	1. gr
Monopotassium phosphate	0.1 gr
Magnesium sulphate	0.1 gr

Two flasks containing this solution were after sterilization infected with spores of *Aspergillus niger*. For comparison a control flask containing glycocoll in place of guanidine was also infected. After 4 weeks there was no trace of development in the guanidine flasks, while in the control flask there was growth. The same result was obtained with spores of *Penicillium glaucum*. Even *Bacillus methylicus*, which can utilize such poor nutrients as sodium formiate, refused to grow in the above solution after they were neutralised. Hence guanidine can not serve as a source of carbon for fungi. However after addition o-

0.1% glucose, development of various fungi, as *Aspergillus niger* and *Bacillus methylicus* took place readily.

2. Can guanidine serve as source of nitrogen for *Phenogams*?

Since Sawa¹ had observed that urea in moderate quantity can exert a poisonous action upon green plants, it was desirable to compare guanidine and biuret in this respect. The following solution was prepared,

Water.....	1000 c.c
Calcium sulphate	1 gr
Magnesium sulphate	0.5 gr
Monopotassium phosphate.....	1 gr
Ferrous sulphate	0.1 gr

This solution was divided into 4 parts, (*a*) received guanidine hydrochlorid 0.5gr, (*b*) ammonium chlorid in equivalent quantity (0.28gr), (*c*) sodium nitrate in equivalent quantity (0.44gr), (*d*) biuret in equivalent quantity (0.464gr). Young barley plants 12. c.m. high were placed in these solutions, but after 3 days already a very poisonous action of biuret and guanidine was observed, the plants having withered almost completely, while the control plants *b* and *c* were perfectly healthy.

The amount of guanidine and of biuret were now reduced to one tenth of the former quantity and plants of the same size placed into these solutions on November 26th.

On Dec. 11. the guanidine plant was dead, but not yet the biuret plant. This however did not show any further development, the larger leaves all bleached and died off one after the other and on January 20 only the youngest leaf was still green.

¹ The urea even in the high dilution of 0.5 per mille injured the plants after a time. It is probable that the urea is readily split up into ammonia and carbonic acid in the cells and the nascent ammonia killed the chlorophyll bodies. Cf. Bul. of the College of Agr., Tokyo, Vol. IV, 413.

Conclusions.

1. Chlorophyll bearing plants are injured by guanidine even in a ditution of 0.1 per mille. Biuret is somewhat less poisonous.
2. Fungi cannot utilize guanidine as a source of carbon, but only as a source of nitrogen.





Physiological Observations on *Bacillus Methylicus*.

BY

T. Katayama.

Some time ago¹ the writer has communicated a note on the general occurrence of *Bacillus methylicus* in various soils of Japan. He has however paid also attention to the physiological properties of this microbe which will be treated upon in the following lines. It was already mentioned that a perfectly colorless variety of the reddish *Bac. methylicus* occurs frequently in soils, which behaves essentially like that of a weak reddish color. Since I had observed that in the nourishing solution with sodium formate as exclusive organic material besides the *Bac. methylicus* also some other microbes² frequently developed, although only in minute quantities, when the solutions had been inoculated from soils, special care in the preparation of pure cultures was necessary and I repeated therefore several times cultures in formate solutions from the colonies obtained on gelatin plates. Thus pure cultures were obtained which served for the following tests.

As regards my observations on the appearance of the colonies they agree essentially with those of *Loew*, nevertheless they may be especially mentioned.

The cells grown in the formate solution are 0.8-1 μ . thick and 2-2.5 μ . long, but they are smaller and shorter when cultured on agar or gelatin plate for 1 or 2 days, being only 0.5-0.8 μ . thick and 1-1.5 μ . long. It has no motion and is not colored after *Gram*. The formate

¹ These Bul. Vol. V. No. 2.

² Also traces of a red yeast and a mycelium fungus were noticed sometimes.

culture solution becomes gradually alkaline, and required after 5 weeks 2.8 c.c. of a 1.5% sulphuric acid for neutralization for 100 c.c.

Bouillon: becomes turbid after 24 hours (20°C.), and a film is formed after a few weeks which sinks to the bottom on shaking. No growth takes place anaerobically (after *Buchner's* method) in bouillon, not even on addition of sodium formate.

Sugar bouillon: Development more rapid, ring on the surface, sediment after 3 days but no gas.

On gelatin plate: White round colonies, but when the gelatin is gradually liquefied the margin of the colony becomes irregular and radiated lines from the center are formed.

On agar plate: Colony is round, milky white and somewhat elevate.

Agar streak: White featherlike colony along the streak, porcelain-like luster, margin irregular.

Agar stab culture: White colony and on the surface only.

On potato: White thin stratum in the beginning of this culture, later on a little elevated.

In sodium formate solution at 20°C: Turbidity, gradual formation of films of very weak reddish color or white.

In milk: The rim on the surface is colored light yellowish, no coagulation after two weeks, the reaction not at all acid, in contrary weak alkaline, odor very weak rancid, but not putrid.

Indol reaction is not obtained from old bouillon culture.

Against higher temperature, our microbe has but little resistance power. In a bouillon culture, it is killed after 5 minutes at 60°C, while it remains still alive at 50°C.

Behavior to Nitrate. In a culture solution containing 0.3% sodium acetate as the only organic material, further 0.2% KNO_3 , 0.2% K_2HPO_4 , 0.02% MgSO_4 , *Bac. methylicus* develops very well in the form of white films and flocculi. This solution gave after 3 weeks a decided reaction for nitrous acid after *Gries*. Ammonia was not formed by the reduction of nitrate.

In a similar solution in which the nitrogen was applied as potassium nitrite (0.1%), the growth was much weaker than in nitrate; no gas was developed.

Behavior to atmospheric nitrogen. A culture solution, 100 cc, containing 0.3% sodium acetate, 0.2% K_2HPO_4 and 0.02% $MgSO_4$, contained in a large *Erlenmeyer's* flask of 500 c.c capacity was inoculated with *Bac. methylicus* and kept in the incubator at 20°C. Slight opalescence becoming a little stronger after several weeks was noticeable. No farther development took place.

Behavior to urea. Culture solutions containing as sole organic matter 0.5% urea showed when infected with *Bac. Methylicus* after several weeks a slight turbidity, and faint reactions for ammonia, while the control flasks without infection remained unchanged. I hope to decide soon whether a faint impurity in the urea has enabled the minute bacterial growth.

Behavior to pepton. A culture solution containing 0.5% pepton, 0.2% K_2HPO_4 and 0.02% $MgSO_4$ developed a good growth of *Bac. methylicus*, but there was no trace of putrefaction noticeable. The reaction remained almost neutral and the biuret reaction was still obtained after 3 weeks. There was some ammonia produced.

Behavior to ammonium humate. With regard to the presence of humic acid and of *Bac. methylicus* in the soil it seemed of special interest to observe the behavior of this microbe towards humus. A culture solution containing 0.2% ammonium humate as the only organic food showed after two weeks a thick film and a bacterial sediment. In the control solution containing besides ammonium humate 0.3% sodium acetate the growth was, however, much more luxuriant.

Behavior to starch, cane sugar, and glycose. In culture solution in which the only organic matter was starch in form of a 0.2% starch paste, no hydrolysis of starch was noticed; bacterial growth was rather insignificant.

In neutral culture solution containing 0.5% cane sugar as the only organic material growth took place but no inversion of cane sugar was noticed after several weeks.

In culture solution containing 0.5% glycose a good growth was noticed,

but the production of acidity by oxidation was only very minute, 100cc. requiring after two weeks only 1.8 c.c. of a 1% baryta water.

Behavior to mannitol. In a culture solution containing 0.5% mannitol, a trace of acidity but no reducing sugar was observed; the acid is produced probably by oxidation; 100 c.c. required after 3 weeks only 3.1 c.c. of a 1% baryta water. The growth was good but not so luxuriant as with glucose.

Summary.

Bacillus methylicus cannot utilize the free nitrogen of the air.

Bac. methylicus forms no enzymes which can hydrolyse starch, cane sugar or proteins; it can not produce either phenomena of putrefaction or of fermentation. It cannot grow in absence of air.

Bac. methylicus can utilize humic acid as food.

Difference between Bac. methylicus and Bacterium formicicum.

After this investigation was finished an article of *W. Omelianski* appeared on the decomposition of formic acid by microbes.¹ He isolated a bacillus from horse excrements which was able to decompose anaerobically formates with development of hydrogen and carbonic acid, but the presence of pepton or bouillon was necessary.

He believes that his bacillus which he called *Bacterium formicicum* has some resemblance to *Bacillus methylicus* of Loew but states that his microbe cannot subsist upon formates or methylalcohol as food. Several farther essential differences will be seen from the following table:

¹ Cent. 1. Bakt. XI page 177.

	<i>Bac. methylicus</i> Loew	<i>Bacterium formicum</i> Omeliansky †
Pepton or Bouillon + Sodium formate	Anaerobic : no growth at all.	Anaerobic : growth and development of gases.
	Aerobic : growth but no gas development.	Aerobic : growth with development of gas.
	No putrid odor.	Putrid odor.
Milk	No coagulation after 10 days.	Cogulation after one day.
On potato	White colonies, potato itself also not colored.	Yellowish brown colonies, potato becomes brown.
Gelatine	Slowly liquified.	Not liquified.
	Not motile.	Motile.
	Absolute aerobic.	Facultatively anaerobic.

† From this authors own notes.



On the General Occurrence of *Bacillus Methylicus* II.

BY

T. Katayama.

Former observations of *Loew* had demonstrated the occurrence of *Bac. methylicus* in the air of Europe and of Japan. The writer¹ had then proved its general occurrence in the surface soil from different parts of Japan.

It seemed to be of some interest to observe further to what depth it occurs in soils, and whether it is found also in the water of rivers and the ocean.

I procured in November by means of a boring stick samples of soils from various depth from a mulberry plantation, from a bare field and a forest. The surface consisted of loamy humus soil, the subsoil was clayey. The procedure was the same as described in my first communication: Infection in formate culture solution, preparing then the gelatin plate and from there colonies on potato and agar; further microscopical comparisons. The inoculation into the formate solution gave the following result after 2 weeks:

Depth.	Soil of Mulberry plantation.	Bare field.	Forest.
25 cm.	Soon a dense film	Dense film	Dense film
55 cm.	" " " "	" "	" "
65 cm.	Slow development	Thin film	Thin film
85 cm.	No growth	No growth	No growth
95 cm.	" "	" "	" "

¹ These Bulletins, V, No. 2.

The general occurrence of this microbe in the dust of the air made it very probable that it also occurs in all putrid liquids which are in contact with air, although this was doubted by *Omelianski*. The writer has infected sterilized formate culture solution repeatedly from putrid farmyard manure as well as from peptone solution that had become putrid on exposure to air and has thus obtained a growth of *Bac. methylicus*. The doubts of *Omelianski* are therefore not justified.

In order to decide whether this microbe occurs also in rivers the writer has collected in sterilized flasks water from the Sumida river near Akabane, above Tokyo from 10, 30 and 60 cm. depth and added to this water 0.5% sodium formate, 0.2% dipotassium phosphate and diammoniumphosphate and 0.02% magnesium sulphate, previously sterilized. After two weeks at 24°C. a thick film of *Bac. methylicus* had developed in the three flasks.

In order to test for the presence of *Bac. methylicus* in the water of the ocean, the writer has collected water near Yokosuka about 1½ miles from the coast and from a depth of 30 cm. in a sterilized flask of one litre capacity. There are no rivers anywhere in the vicinity emptying into the ocean. To this litre water were added the sterilized nutrients just mentioned. After a few days at 24°C. a turbidity and a white film along the rim of the surface was developing. From this film inoculation in my former sodium formate culture solution was made and from there the characteristic colonies on potato and agar were produced. All these tests as well as the microscopic examination proved the identity of the bacillus in question with the colorless variety of the *Bacillus methylicus*. A further control test was made in order to observe whether the *Bac. methylicus* isolated from soil would also grow in the formate culture solution in presence of 3% Na Cl. Indeed a good growth was soon obtained. Hence *Bacillus methylicus* can not only utilize the most various organic compounds from the peptone and sugars down to methyl-alcohol and formic acid, but also this lowest of the fatty acids under rather unfavorable conditions.

Various investigations on the bacteria in the oceans have been made by *Russel* in 1891, by *Fischer* in 1894 and by *Gran* in 1902. *Russel* found at a depth of 50 meters in the gulf of Naples in 1 cc. 121 bacteria, at 500 meters depth 22, while in the mud of that gulf at that depth 12500. Accord-

ing to *Fischer* most of the species found differ from those on land and are motile; he observed still many bacteria in the water from a depth of 1100 meter. *Gran* observed a certain species that can liquify agar which is of special interest, as galactan is a constituent of many marine algae and agar itself is a galactan obtained from this source. All those authors have overlooked the occurrence of *Bacillus methylicus* in sea water.

Conclusion.

Bacillus methylicus belongs to the widest spread microbes. It occurs not only in the dust of the air, in the soils to a depth of 65 cm. and in rivers, but also in the water of the ocean. Being obligate aerobic it fulfills doubtless an important function in oxidizing organic matter wherever it occurs, in the soil as well as in the waters.





On the Influence of Liming upon the Action of Phosphatic Manures.

BY

M. Nagaoka.

Although much has been published on the favorable effect of the liming of the soils, the subject is by no means exhausted, as shown by the recent communications of *O. Kellner* and *O. Böttcher* „Untersuchung über die Düngewirkung der Knochenmehlphosphorsäure.“ These authors showed that the application of calcium carbonate on soils manured with bone meal had a depressing action on the availability of phosphoric acid in this form.¹ The experiment of these authors also explained the results obtained by *Wagner* and *Märker* with bonemeal. They continued their experiments² thus confirming again their former observation.

F. W. Dafert on the other hand could not observe a close relation between the lime content of a soil and the availability of phosphoric acid for the plants.

Since this object is of great importance for Japanese agriculture, where liming of paddy fields is often practiced in an extensive and detrimental degree, I have undertaken a series of experiments. The conditions in the paddy fields are naturally somewhat different from those of dry fields and the crops require a different management.

My experiment was carried out in wooden frames having an area of 3 square shaku=0.82645 square metres.

The frames, 57 in number, were placed at a distance of one metre from

¹ Deutsche Landw. Presse 1900, No. 52 and 1901, No. 23-24.

² Ibid 1901, No. 28. Rye, mustard and oats had served for experiments of *Kellner* and *Böttcher*. These results were confirmed recently by *G. Söderbaum* (Centrbl. f. Agr. Chem. 1903, p. 737).

each other, sunk about sixty centimetres in the leveled paddy field and projecting about ten centimetres above the level of the field.

The soil came from a paddy field which had been specially exhausted by raising crops for several years without using manures.

The soil was a sandy loam of a fine texture, rather rich in humus (10-11%) and containing 0.9% lime, soluble in conc. hot hydrochloric acid. Towards end of May 1901, shortly before the application of lime, the soil in the frames was carefully agitated with an addition of sufficient water to form a state of mud and sifted in order to remove all rootlets of the former crops.

On May 31, pure caustic lime in a state of fine powder was applied at the rate of 400 kilograms per hectare which makes 333.3 grams per frame. After the addition of the lime, the muddy soil was again stirred to a depth of one foot in order to facilitate the distribution of the lime applied, whilst the frames which did not receive any lime were left untouched.

The double superphosphate and organic manures, which were used in this experiment, were analysed by myself with the following result, as to the quantity of total phosphoric acid:—

In % of the air dry samples.

Kind of manures	Total P ₂ O ₅
Double superphosphate	44.629%
Shimekasu (Sardine)	4.176 „
Shimekasu (Herring).....	3.972 „
Arakasu (a fish bone manure)	10.762 „
Steamed bone meal (exceedingly fine, imported from India)	20.195 „
Rice bran.....	2.703 „
Rape cake	1.699 „
Sesamum cake... ..	4.039 „
Soy bean cake (produced in North China)	1.336 „

On June 14th, the special phosphatic manures were applied to each frame, and on the 19th, as the general manures, ammonium sulphate at the

rate of 100 kilograms nitrogen per ha and potassium sulphate also at the rate of 100 kilograms potassa, per ha.

The quantities of the special phosphatic manures per frame will be seen from the following table :—

Number of frames			Kinds of the special manures	Quantity of P_2O_5 and Ca O per tan, Kilogram		Quantity of the special manures per frame, Gram
				P_2O_5	Ca O	
1	20	39	Double superphosphate	5	0	9.335
2	21	40	Shimekasu (Sardine)	5	0	99.751
3	22	41		5	400	99.751
4	23	42	Shimekasu (Herring)	5	0	104.880
5	24	43		5	400	104.880
6	25	44	Arakasu (fish bone)	5	0	38.710
7	26	45		5	400	38.710
8	27	46	Steamed bone meal	5	0	20.628
9	28	47		5	400	20.628
10	29	48	Rice bran	5	0	154.120
11	30	49		5	400	154.120
12	31	50	Rape cake	5	0	245.200
13	32	51		5	400	245.200
14	33	52	Sesamum cake	5	0	103.140
15	34	53		5	400	103.140
16	35	54	Soy bean cake	5	0	311.820
17	36	55		5	400	311.820
18	37	56	No P_2O_5	0	0	0
19	38	57		0	400	0

As to the young rice plants which served for the experiment, I add here a few words. These plants had been carefully raised in a special seed bed.

The variety was that known as "Satsuma" whose origin is in the western part of Japan. It is noted for its resistance power towards winds, fungi, insects, etc.

The transplantation took place on the 20th of June, after the soil in the frames had been well stirred once more for the purpose of aeration and the most uniform distribution of the manuring compounds. The number of the young rice plants which were then 40 days old was 192 per frame. They were planted in 16 bundles containing 12 healthy individuals of equal size.

During the season the weather was favorable and neither diseases nor damages by insects occurred. Already after a fortnight a very marked difference was observed. The plants which had received double superphosphate and the manure of animal origin exhibited a bright green color and a healthy appearance while those that had received the vegetable manure as well as those that had not been supplied with phosphoric acid were deficient in color and strength. A still greater difference was observed between the plants supplied with lime and those without lime, the latter plants being quite vigorous and much better developed than the former which exhibited a pale, weak condition. Gradually these differences increased, proving that the lime had caused much injury. The photographs reproduced in Plates I-III. will not fail to convince the reader on this point.

On the 8th of November the rice plants were harvested and left to dry. The general result was as follows (the details of the weights are contained in the Appendix):—

No of frames	Kind of manures	Average yield per frame (gram)			
		Straw	Full grain	Empty grain	Total crop
1, 20, 39.	Double superphosphate	570.0	500.7	6.2	1076.9
2, 21, 40.	Shimekasu (Sardine)	566.0	508.0	7.7	1081.7
3, 22, 41.	„ + Ca O	282.0	194.3	4.1	480.4
4, 23, 42.	Shimekasu (Herring)	528.3	438.3	6.8	973.4
5, 24, 43.	„ + Ca O	264.7	182.3	3.9	450.9
6, 25, 44.	Arakasu (fish bone)	588.0	520.3	8.2	1116.5
7, 26, 45.	„ + Ca O	387.0	313.0	4.0	704.1
8, 27, 46.	Steamed bone dust	563.0	489.3	6.5	1058.8
9, 28, 47.	„ „ „ + Ca O	268.3	196.0	4.3	468.6
10, 29, 48.	Rice bran	365.0	309.0	4.0	678.0
11, 30, 49.	„ „ + Ca O	278.3	200.7	4.6	483.6
12, 31, 50.	Rape cake	348.3	235.7	4.2	588.2
13, 32, 51.	„ „ + Ca O	213.0	139.0	3.6	355.6
14, 33, 52.	Sesamum cake	325.3	255.3	4.7	585.3
15, 34, 53.	„ „ + Ca O	254.0	176.0	3.6	433.6
16, 35, 54.	Soy bean cake	338.0	236.0	4.5	578.5
17, 36, 55.	„ „ „ + Ca O	242.0	131.7	4.3	378.0
18, 37, 56.	No P ₂ O ₅	185.3	155.4	4.6	345.3
19, 38, 57.	„ „ + Ca O	179.0	132.0	3.5	314.0

It will be observed from this table that phosphatic manure had a very great influence upon the production of the rice plants proving that our soil was rather poor in available phosphoric acid.

As a whole, double superphosphate and the manures of animal origin

without the addition of lime gave the highest produce, much higher than the manures of vegetable origin.

For the sake of obtaining a still clearer information as to the relative efficacy of the various manures and also, as to the effect of lime which has mainly affected the action of phosphoric acid of the manures, I have calculated from the above table the following numbers taking the total yield of the double superphosphate to be as 100:—

Kind of manures	Relative yield	
	Without Ca O	With Ca O
Double superphosphate	100.	
Shimekasu (Sardine)	100.0	44.6
Shimekasu (Herring)	90.3	41.9
Arakasu (fish bone)	103.8	65.5
Steamed bone meal	98.3	43.5
Rice bran	63.0	44.9
Rape cake	54.6	33.1
Sesamum cake	54.3	40.3
Soy bean cake	53.8	35.1

Before entering into the discussion of these results, we may consider here the proportion of phosphoric acid absorbed by the rice plants from the soil and manures. The chemical analysis of the crops was made by myself in the usual way.

Kind of manures	Phosphoric acid in the manures, gram	Phosphoric acid in the whole crop, gram	Phosphoric acid absorbed from the manures	
			gram	per cent of the P_2O_5 applied
Double superphosphate	4.167	2.948	2.092	50.2
Shimekasu (Sardine).....	„	2.308	1.452	34.5
„ +Ca O	„	1.345	0.585	14.0
Shimekasu (Herring)	„	2.483	1.627	39.0
„ +Ca O	„	1.100	0.340	8.2
Arakasu (Fish bone).....	„	2.709	1.853	44.5
„ +Ca O	„	1.764	1.004	24.1
Steamed bone meal	„	2.469	1.613	38.7
„ „ „ +Ca O	„	1.070	0.310	7.4
Rice bran	„	1.660	0.804	19.3
„ „ +Ca O	„	1.175	0.415	10.0
Rape cake	„	1.418	0.562	13.5
„ „ +Ca O	„	0.866	0.106	2.6
Sesamum cake	„	1.739	0.883	21.2
„ „ +Ca O	„	1.060	0.300	7.2
Soy bean cake	„	1.389	0.533	12.8
„ „ „ +Ca O	„	0.866	0.106	2.8
No P_2O_5	0	0.856	0	0
„ „ +Ca O	0	0.760	0	0

Again, when the above number for the percentage of double superphosphate, that is 50.2, assumed to be 100, the relative availability of P_2O_5 in the other manures will be found as follows:—

Kind of manures	Relative availability of phosphoric acid	
	Without Ca O	With Ca O
Double superphosphate	100.	
Shimekasu (Sardine).....	68.7	28.7
Shimekasu (Herring)	77.5	16.3
Arakasu (fish bone)	88.6	48.0
Steamed bone meal	77.1	14.7
Rice bran	38.4	19.9
Rape cake	26.9	5.2
Sesamum cake	42.3	14.3
Soy bean cake	25.5	5.2

From the results of the above calculation, it will be clearly noticed that the application of lime to the paddy field supplied with organic manures, has caused an extraordinary diminution of the absorption of phosphoric acid and has consequently lessened the yield to an extensive degree.

Here I add for the purpose of clearing up these points more fully, the following calculations from the above data. From these calculations, it will be seen how much the relative diminution of the rice crop amounts to, and how much the lime depressed the availability of the phosphates.

Kind of manures	Relative diminution of yield	Relative action on the decrease of assimilability of P_2O_5	Quotient of diminution	
Animal manures {	Shimekasu (Sardine)	55.4	40.0	47.7
	Shimekasu (Herring)	48.4	61.2	54.8
	Arakasu	38.3	40.6	39.3
	Steamed bone meal	54.8	62.4	58.6
	Average	49.2	51.1	50.2

Vegetable manures	Rice bran	18.1	18.5	18.3
	Rape cake	21.5	21.7	21.6
	Sesamum cake	14.0	28.0	21.0
	Soy bean cake	18.7	20.3	19.5
	Average	18.1	22.1	20.1

These numbers show, with but a few exceptions, namely for Shimekasu (Herring) and sesamum cake, a singular coincidence in the relative diminution on the yield and the relative decrease of the availability of phosphoric acid; and this coincidence appears still more striking in the averages. The average of these two relative series supplies a quotient of the retarding action of lime upon the phosphoric acid contained in various organic manures, suited for the rice plant under the condition similar to that of our experimental field in regard to soil and climate.

Now, when a comparison is made in regard to the above numbers, it is observed, in a general sense, that the consumption of phosphoric acid and the yield of the rice plant supplied with manures of animal origin, were affected to an extensive degree on account of lime application, while in the case of vegetable manures, the depressing action of lime was not so great.

The respective averages of the diminution quotients of both kinds of manures, that is 50.2 for the animal manures and 20.1 for the vegetable manure, show the relative difference between these two manures; and these two numbers will be practically of a great value in regard to a paddy field rich in lime or over limed, especially to a field which is freshly manured with lime in a large dose.

Now what is the cause which brought on this great difference in the results of these two kinds of manures? This may to a certain extent depend upon the relative amount of organic matter in the manure and the relative degree of its decomposition.¹ The humus exerts an important influence upon the availability of phosphoric acid from soils and manures. Thus it has been

¹ The opinions as to the action of lime on humus and humification process differ. Kossowitsch (1902) infers from his experiments that lime retards the humus formation.

demonstrated in the application of basic slag or other phosphatic manures rich in lime that the result was more satisfactory in the presence of much humus than in its absence, what it probably due to the formation of organic acids formed in the humus. By the application of lime the original humus in my experimental soil had been rendered inactive.

This fact was well proved by the unfavorable condition of the rice plants in the limed frames which had not recieved phosphoric acid, when a comparison was made with that of the control frames.

In all the limed frames, this deficiency or want of humus action in the soil is counteracted to a certain extent by the application of the above mentioned organic manures and the notable fluctuation, which was brought upon the harvest as well as the rate of phosphoric acid consumption, is due chiefly to the quantity and nature of the organic matter contained in the manures.

The manures of animal origin containing less organic matter and being naturally more readily decomposed, their humification in the soil was not sufficient enough to bring the insoluble phosphoric acid into a more available state.

The acidity of the rice roots which exerts a solvent action upon the phosphates also seems to have been weakened by the liming. And finally the ratio between the amounts of lime and magnesia in the soil may have become unfavorable to rice by the liming, although the amount of lime was not large enough to exert in this regard much influence.

The above discussion will receive some further support by the results of the after-effects of this experiment.

With special reference to the steamed bone meal, I may quote here the results obtained by *Kellner* and *Böttcher*. These authors obtained on an average of ten trials with different samples of bone meal a relative retarding quotient of 47 with rye,¹ and of 50 in the next experiments with white mustard² while in the experiment of the writer the retarding quotient was as high as 54.8. It is very satisfactory to say that without considering the

¹ Deutsch, Landw. Presse, 1900, No. 52 and 1901, No. 23-24.

² Deutsch, Landw. Presse, 1901, No. 28.

great difference in climate, in the condition of soil and in the character of the crops there is a very close resemblance between their results and mine. Nevertheless when a strict comparison is made between both results, it will be seen that in my own, the action of lime was more powerful what may most probably be due to the fact that *Kellner* and *Böttcher* used calcium carbonate while in the experiments of the writer caustic lime had served.¹

It may be of some interest to add here the relative manurial effects of the phosphoric acid of the various manures most commonly used in the paddy fields of Japan. As to these determinations compare the Bul. Vol. I of this College. In the following table the recompiled numbers from the above pages are added:—

Kind of manures.		Relative action on the plus yield of total crop over the frame without P ₂ O ₅ .	Relative assimilability of P ₂ O ₅ .	Relative manuring value.
Double superphosphate		100	100	100
Animal manures	Shimekasu (Sardine)	100.7	68.7	85
	Shimekasu (Herring)	85.9	77.5	82
	Arakasu (Fish bone)	105.4	88.6	97
	Steamed bone meal.....	97.5	77.1	87
	Average	—	—	88
Vegetable manures	Rice bran	45.5	38.9	42
	Rape cake	33.2	26.9	30
	Sesamum cake	32.8	42.3	38
	Soy bean cake	31.2	25.5	28
	Average	—	—	35

From a glance upon these figures, it will be seen that the relative manuring effects of the manures of animal origin upon rice plants show, but

¹ If sodium nitrate in place of ammonium sulphate would have been applied, the depression by liming might have been less. But we have to consider, that nitrates are not a suitable source of nitrogen for rice.

with the exception of the arakasu, a satisfactory coincidence, and also the manures of vegetable origin, resemble each other in their effect.

It is worthy of notice however, that the phosphoric acid in manures of animal origin attained such a high rate of manurial efficacy; and even the steamed bone meal, which has been hitherto proved by P. Wagner¹ and others to be one of the difficultly assimilable manures in Europe, has shown here such an unexpected and remarkably high rate. Our former experiment in paddy fields has proved it to be only 56.²

This result may likely be due to the favorable conditions of our paddy field favoring an easy digestion of animal manures. On the other hand, the manurial efficacy of the phosphoric and in vegetable manures amounts only to $1/3$ to $1/2$ of that of animal manures.

The manurial effects of the animal and vegetable manures varie, as it seen from the above table, within certain limits and the definite conclusion as to these differences must be left for still further investigation; but, as a fact, the experiments of this kind are not yet performed in Japan, and accordingly the figures given in the table may serve, for a time, as a standard for the calculation of values and quantities of various vegetable and animal manures applied in rice cultivation in countries similar to our own with regard to soil and climate.

After-effect of the preceding Experiment.

This experiment was tried in order to ascertain the further actions of the lime upon the residual phosphoric acid from the preceding year; and at the same time, to observe the manurial value of the unrecovered phosphoric acid of the various organic manures without lime application, the conditions, the weather excepted, were the same as in the first year. After the harvest, the soil in the frames was left untouched till the spring of the following year (1902). On the 28th of May, the soil of all the frames was cautiously ploughed to a depth of about 30 centimetres, and was exposed to the air for a few days: then all the frames were irrigated with sufficient

¹ Wagner's Thomas phosphate powder, 1887, p. 23.

² Bull. College of Agriculture, Tokio; Vol. I, No. 10, p. 25.

water and the soil was stirred to a fine mud as usual. On the 20th of June, each frame received nitrogen and potassa (as potassium sulphate) at the rate of 100 kilograms per hectare. On July 7, the soil was again agitated after the application of much water and the young rice plants 55 days old were planted in the usual manner.

The weather was very unfavorable that year. The harvest, cut Nov. 6, yielded (in the air dry state) the following numbers. (The details per frame are given in the appendix).

No of frames.	Kind of manures.	Average yield per frame (gram).			
		Straw.	Full grain.	Empty grain.	Total crop.
1, 20, 39.	Double superphosphate	250.2	182.6	4.7	437.5
2, 21, 40.	Shimekasu (Sardine)	214.5	156.7	4.1	375.3
3, 22, 41.	„ +Ca O	305.0	223.3	6.4	534.7
4, 23, 42.	Shimekasu (Herring)	248.5	188.6	4.5	441.6
5, 24, 43.	• „ +Ca O	297.7	216.6	7.2	521.5
6, 25, 44.	Arakasu (fish bone)	237.8	202.3	4.8	444.9
7, 26, 45.	„ +Ca O	265.3	189.3	4.7	459.3
8, 27, 46.	Steamed bone dust	240.0	154.1	5.7	399.8
9, 28, 47.	„ „ „ +Ca O.....	306.0	227.1	5.5	538.6
10, 29, 48.	Rice bran	212.1	134.5	3.3	349.9
11, 30, 49.	„ „ +Ca O	242.1	166.9	4.8	413.8
12, 31, 50.	Rape cake	228.3	161.3	3.7	393.3
13, 32, 51.	„ „ +Ca O.....	303.0	215.6	5.1	523.7
14, 33, 52.	Sesamum cake	262.7	163.6	3.9	430.2
15, 34, 53.	„ „ +Ca O.....	272.0	188.1	5.7	465.8
16, 35, 54.	Soy bean cake	218.0	155.1	3.8	376.9
17, 36, 55.	„ „ „ +Ca O.....	276.3	208.8	5.5	490.6
18, 37, 56.	No P ₂ O ₅	180.0	125.3	2.8	308.1
19, 38, 57.	„ „ +Ca O	242.3	161.5	4.3	408.7

It is thus seen that in all the trials, the residual phosphoric and from the preceding year increased the yield of straw and grain to a certain extent over the yield of the frames without phosphoric acid and lime.

The differences of the harvested crop between the limed and unlimed frames will be best seen by the following results of calculation, when the total yield of double superphosphate is taken as 100:—

Kind of manures.	Total yield of double superphosphate = 100.	Relative increase over the unlimed frame.
Double superphosphate	100	—
Shimekasu (Sardine).....	85.8	36.4
„ + Ca O.....	122.2	
Shimekasu (Herring)	100.9	18.3
„ + Ca O.....	119.2	
Arakasu (fish bone)	101.7	3.3
„ + Ca O	105.0	
Steamed bone meal	91.4	34.0
„ „ „ + Ca O.....	125.4	
Rice bran	80.0	14.6
„ „ + Ca O.....	94.6	
Rape cake	89.5	30.2
„ „ + Ca O	119.7	
Sesamum cake.....	98.3	8.2
„ „ + Ca O.....	106.5	
Soy bean cake	86.2	25.9
„ „ „ + Ca O.....	112.1	

These results evidently show that the unfavorable action of the lime upon the phosphatic manures extended even to the second year to some degree according to the nature of manure applied. The increase of the crop in the limed frames may have been caused by the further decomposition of the manures by long exposure of the soil during winter time, but for the main source, I would ascribe the increase to the organic matter in the soil from the preceding crops (roots).

The surplus yield of each manure over that of the frames not supplied with any phosphoric acid, has some interest concerning the judgment on the efficacy of the phosphoric acid. The numbers obtained in the first year for the relative value of the various manures are for the sake of comparison, entered again here.

Kind of manures.	Unrecovered phosphoric acid, grams.	Plus yield over the frames not supplied with P ₂ O ₅ grams.	Relative action on the increase of total crop.	
			1st year.	2nd year.
Double superphosphate	2.174	129.4	100	100
Shimekasu (Sardine).....	2.715	67.2	100.7	51.9
Shimekasu (Herring)	2.536	133.5	85.9	103.9
Arakasu (fish bone)	2.314	136.8	105.4	107.1
Steamed bone meal	2.554	91.7	97.5	70.8
Rice bran	3.363	41.8	45.5	32.3
Rape cake	3.553	85.2	33.2	65.9
Sesamum cake	3.282	122.1	32.8	94.3
Soy bean cake	3.634	68.8	30.2	53.2

Thus it will be seen that in all the trials the unrecovered phosphoric acid had an influence on the following crop, but when we take the quantity of the unrecovered phosphoric acid into consideration, the effect of the latter was far from being anticipated, and there was no case that the crop has attained as much as that produced by means of a fresh application of phosphoric acid.

As to the relative increase of the total crop for the second year, the

figures for both, animal and vegetable manures show a wide variation. These differences seem to be most probably due to the different forms of phosphoric acid contained in the manures and not to the quantity left from the preceding year.

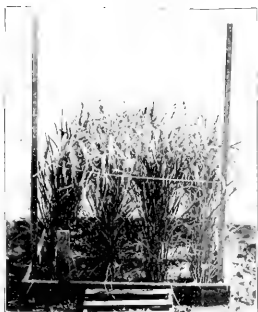
The results of the two years successive experiments show that the phosphoric acid of animal manures was more readily available to the rice plant than the phosphoric acid compounds of manures of vegetable origin, although the majority of the latter manures have displayed a comparatively better effect in the second year.

Conclusions.

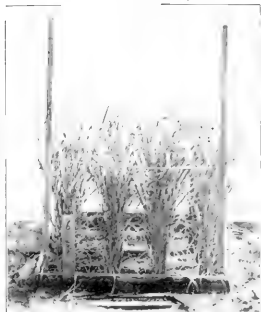
1. Lime exerts a retarding and unfavorable influence upon the availability of phosphoric acid of various organic manures.
2. This injurious action is about twice as powerful with animal manures than with manures of vegetable origin.
3. The action of organic matter as well as humus in manures diminishes the unfavorable effect of lime to a certain extent.
4. The retarding rate found for animal manures exceeds even that observed by *Kellner* and *Böttcher* with steamed bonemeal and confirms therefore the claims of these authors.
5. The unfavorable effect of lime extends even to the second year as the return of yield over the loss of the preceding year was not satisfactory.
6. The relative manurial action of the phosphoric acid compounds in the animal manures exceeds almost double that of the phosphoric and compounds in the manures of vegetable origin in the first year.
7. In the second year the relative action of the vegetable manures increased to a certain extent, yet it remained still behind that of the animal manures.



Shimekazu I (Sardine) without CaO



Shimekazu II (Herring) with CaO



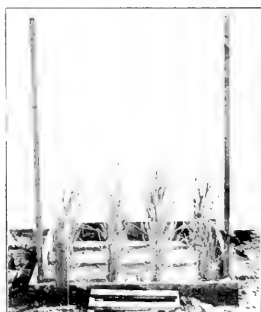
Arakazu I (Sardine) without CaO



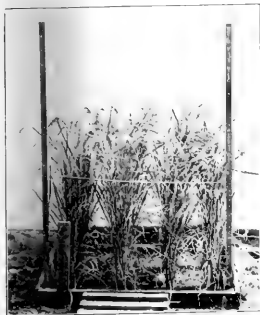
Shimekazu I (Sardine) with CaO



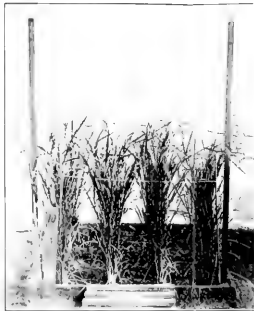
Shimekazu II (Herring) with CaO



Arakazu (Fish-bone) with CaO



Steamed bone meal without CaO.



Rice bran without CaO.



Rape cake without CaO.



Steamed bone meal with CaO.



Rice bran with CaO.



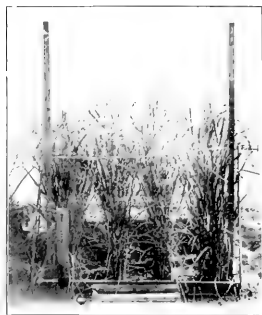
Rape cake with CaO.



Sesamum Cake without CaO



Sesamum Cake without CaO



Sesamum Cake with CaO



Sesamum Cake with CaO



Soy-bean Cake with CaO

Appendix.

YIELD OF THE SINGLE FRAMES (1st year).

No of frames,	Kind of manures,	Straw, grams.	Full grain, grams.	Empty grain, grams.
1	Double superphosphate	558.0	488.0	5.6
20	" "	565.0	490.0	6.1
39	" "	583.0	524.0	7.1
2	Shimekasu (Sardine)	599.0	529.0	9.7
21	"	540.0	472.0	7.5
40	"	559.0	523.0	6.0
3	" + Ca O	239.0	156.0	2.8
22	" " "	212.0	123.0	4.1
41	" " "	395.0	304.0	5.3
4	Shimekasu (Herring)	525.0	458.0	6.6
23	"	550.0	456.0	7.2
42	"	510.0	401.0	6.7
5	" + Ca O	217.0	141.0	2.7
24	" " "	254.0	168.0	4.1
43	" " "	323.0	236.0	4.8
6	Arakasu (fish bone)	531.0	454.0	6.1
25	"	627.0	560.0	11.1
44	"	606.0	547.0	7.5
7	" + Ca O	(110.0)	(56.0)	(1.9)
26	" " "	394.0	330.0	3.9
45	" " "	380.0	296.0	4.1
8	Steamed bone meal	476.0	442.0	7.7
27	" " "	632.0	519.0	6.8
46	" " "	581.0	507.0	5.1
9	" " " + Ca O	269.0	183.0	4.7
28	" " " " "	234.0	153.0	3.2
47	" " " " "	302.0	252.0	4.9

YIELD OF THE SINGLE FRAMES. (1st year.)

No of frames.	Kind of manures.	Straw, grams.	Full grain, grams.	Empty grain, grams.
10	Rice bran	369.0	312.0	4.0
29	" "	312.0	257.0	3.6
48	" "	414.0	358.0	4.4
11	" " +Ca O	225.0	145.0	5.3
30	" " " "	303.0	234.0	4.6
49	" " " "	307.0	223.0	3.9
12	Rape cake	306.0	233.0	4.7
31	" "	317.0	238.0	3.5
50	" "	422.0	236.0	4.5
13	" " +Ca O	198.0	118.0	3.3
32	" " " "	175.0	(84.0)	(3.6)
51	" " " "	266.0	160.0	3.8
14	Sesamum cake	253.0	179.0	3.8
33	" "	420.0	357.0	6.6
52	" "	303.0	230.0	3.8
15	" " +Ca O	200.0	134.0	2.1
34	" " " "	296.0	218.0	3.7
53	" " " "	266.0	176.0	5.0
16	Soy bean cake	276.0	175.0	2.7
35	" " "	331.0	234.0	5.7
54	" " "	410.0	299.0	5.0
17	" " " +Ca O	209.0	96.0	2.8
36	" " " " "	262.0	152.0	5.1
55	" " " " "	255.0	147.0	5.0
18	No P ₂ O ₅	173.0	148.0	5.1
37	" "	200.0	163.0	4.2
56	" "	182.0	155.0	4.5
19	" " +Ca O	196.0	140.0	3.5
38	" " " "	176.0	128.0	3.3
57	" " " "	165.0	128.0	3.7

YIELD OF THE SINGLE FRAMES. (2nd year.)

No of frames.	Kind of manures.	Straw, grams.	Full grain, grams.	Empty grain, grams.
1	Double superphosphate	279.0	210.6	5.0
20	" "	234.0	170.6	4.6
39	" "	237.5	166.6	4.6
2	Shimekasu (Sardine)	220.5	163.6	4.6
21	"	181.0	129.6	3.0
40	"	242.0	177.1	4.8
3	" + Ca O	306.0	216.6	7.3
22	" " "	284.5	199.6	6.9
41	" " "	324.5	253.6	5.0
4	Shimekasu (Herring)	257.5	185.6	4.4
23	"	214.5	156.1	4.9
42	"	273.5	224.1	4.2
5	" + Ca O	284.0	199.6	8.5
24	" " "	295.0	218.6	6.2
43	" " "	314.2	231.6	6.9
6	Arakasu (fish bone)	261.5	188.6	4.4
25	"	298.0	219.6	5.9
44	"	254.0	198.6	4.2
7	" + Ca O	308.0	195.6	5.3
26	" " "	242.0	181.1	4.6
45	" " "	246.0	191.1	4.1
8	Steamed bone meal	227.0	103.6	10.1
27	" " "	261.0	181.6	3.8
46	" " "	232.0	177.1	3.5
9	" " " + Ca O	293.5	212.1	5.6
28	" " " " "	361.5	268.6	6.7
47	" " " " "	263.0	200.6	4.2

YIELD OF THE SINGLE FRAMES. (2nd year.)

No of frames.	Kind of manures.	Straw, grams.	Full grain, grams.	Empty grain, grams.
10	Rice bran	212.1	152.7	3.4
29	" "	190.1	133.7	3.5
48	" "	234.1	117.1	3.0
11	" " + Ca O	254.8	167.6	6.1
30	" " " "	236.0	170.6	3.7
49	" " " "	235.5	162.6	4.6
12	Rape cake	201.0	141.6	3.6
31	" "	240.0	161.6	4.3
50	" "	244.0	180.6	3.3
13	" " + Ca O	291.0	198.6	5.7
32	" " " "	314.0	222.6	4.8
51	" " " "	304.0	225.6	4.7
14	Sesamum cake	241.5	156.1	4.9
33	" "	310.0	147.1	2.8
52	" "	236.5	187.6	3.9
15	" " + Ca O	264.0	179.1	4.9
34	" " " "	253.0	178.6	5.8
53	" " " "	299.0	206.6	6.3
16	Soy bean cake	178.0	114.1	3.6
35	" " "	258.0	194.6	4.7
54	" " "	218.0	156.6	3.1
17	" " " + Ca O	295.0	223.1	6.6
36	" " " " "	265.0	199.1	5.4
55	" " " " "	269.0	204.1	4.4
18	No P ₂ O ₅	188.0	134.6	2.7
37	" "	170.0	115.6	2.9
56	" "	182.0	125.6	2.9
19	" " + Ca O	240.0	160.6	3.9
38	" " " "	245.0	161.1	4.2
57	" " " "	242.0	163.6	4.8

On the Action of Various Insoluble Phosphates upon Rice Plants.

BY

M. Nagaoka.

The phosphoric acid of the soil occurs partly in a soluble form while the larger part is present as compounds insoluble in water. Among the insoluble forms the phosphates of iron, aluminium and calcium predominate. Besides these mineral forms some phosphoric acid exists also in form of an organic compound, thus far not very closely studied.

As to the origin of the insoluble mineral phosphates, there are two sources, one being the mineral particles of rocks which always contain more or less phosphate of calcium, aluminium and iron in the form of phosphorite, vivianite and wavellite respectively, the other source being the soluble phosphoric acid in the manure, being transformed in the soil to the aluminium, iron and calcium phosphate.

Since the iron and aluminium phosphate are insoluble in dilute acids their manurial effects upon crops thus far observed are but of small value. Since, however, the paddy fields for the cultivation of rice are irrigated for the greater part of the year, their soil conditions are entirely different from those of the dry fields. Among these conditions, the feature of the decomposition of organic matter is most noteworthy. Thus the decomposition or putrefaction of organic matter easily takes place in paddy fields, with the result that an acid humus is produced, since the oxygen does not penetrate sufficiently into the wet soil to lead to a complete oxidation. Paddy fields have a more or less acid nature according to the amount of humus present. This acid reaction must naturally have a beneficial action, since the insoluble phosphates become thus more easily available for the roots.

This led me to carry out some experiments with the intention of obtaining some informations on the effect of various insoluble phosphates upon rice plants.

I. Series.

These experiments were performed in wooden frames (3 square shaku each) which were, after the usual manner, placed in an uniformly agitated and levelled paddy field, the soil of the latter having been exhausted for four years by the continuous cultivation of rice crops without manures. After the careful management of the soil in each frame, general manures were given. Thus, on the 23rd of June 1898, the potassium sulphate was added at the rate of 100 kilograms of potash per hectare and four days afterwards, the same rate of nitrogen was applied in the form of ammonium sulphate. On the next day, the special phosphates were given. The quantity of the phosphoric acid employed and the amount of the corresponding phosphates will be seen in the following table.

No of frames.	Kind of phosphates.	Quantity of phosphoric acid.		Quantity of the phosphates used per frame, grams.
		per hectare kilograms.	per frame grams.	
1, 40, 79.	No phosphoric acid.	0	0	0
4, 43, 82.	Ferric phosphate.	25	2.083	8.711
5, 44, 83.		50	4.166	17.422
6, 45, 84.		100	8.333	34.844
13, 52, 91.	Ferrous phosphate.	25	2.083	7.299
14, 53, 92.		50	4.166	14.598
15, 54, 93.		100	8.333	29.196
22, 61, 100.	Aluminium phosphate.	25	2.083	6.001
23, 62, 101.		50	4.166	12.002
24, 63, 102.		100	8.333	24.004
31, 70, 109.	Calcium phosphate.	25	2.083	5.039
32, 71, 110.		50	4.166	10.078
33, 72, 111.		100	8.333	20.156

The above quantities of the four phosphates were calculated from the following analytical results :—

In percent of the air dry samples.

1. Ferric phosphate	40,123
2. Ferrous phosphate	34,825
3. Aluminium phosphate	52,001
4. Calcium phosphate	50,123

With the exception of the calcium phosphate, these numbers show close coincidence with their theoretical content in phosphoric acid. The calcium phosphate applied was guaranteed to be a pure tricalcium phosphate; but according to the above analysis it showed a somewhat higher content of phosphoric acid, making it probable that the sample contained some dicalcium phosphate. On the 29th of June, the young rice specially grown for the purpose and 52 days old was transplanted to each frame. Each frame received 16 bundles of twelve healthy individuals. The irrigation was at once commenced in the usual manner, but was discontinued at the end of August.

However, according to the system of the common practice of rice culture, the frames were again irrigated for three days during the full bloom of the rice plants.

Referring to the report of the meteorological observatory of Tokio, the weather, during the whole vegetation of the rice plants, showed fewer clear days than usual, but temperature and rainfall were as favourable as in other good rice years.

On the 29th of November, the crops were harvested and exposed to the sun for about ten days. Thus, the crops having been completely air dried, the grains were carefully isolated from the panicles. The average yields¹ of three equally treated frames were as follows :—

¹ The yield per frame is left to the appendix.

No of frames.	Kind of phosphates.	P ₂ O ₅ applied per ha, Kilograms.	Average yield per frame.			
			Straw, grams.	Full grain, grams.	Empty grain, grams.	Total crop, grams.
1, 40, 79.	o	o	341.0	289.3	7.4	638.0
4, 43, 82.	Ferric phosphate	25	488.0	451.3	12.7	952.0
5, 44, 83.		50	668.0	573.5	23.2	1265.0
6, 45, 84.		100	816.7	636.0	20.7	1473.0
13, 52, 91.	Ferrous phosphate	25	476.3	408.6	12.8	898.0
14, 53, 92.		50	552.3	458.6	13.6	1025.0
15, 54, 93.		100	632.3	529.7	19.1	1171.0
26, 61, 100.	Aluminium phosphate	25	457.0	366.7	12.0	836.0
27, 62, 101.		50	570.0	465.3	14.2	1050.0
28, 63, 102.		100	649.7	554.7	18.6	1223.0
31, 70, 109.	Calcium phosphate	25	500.0	446.7	11.0	958.0
32, 71, 110.		50	618.3	495.7	18.8	1132.0
33, 72, 111.		100	715.3	588.7	22.8	1327.0

It will be seen from the above figures that all the series of experiments showed a prominent influence of the phosphoric acid of the various insoluble phosphates upon the yield of the grain, as well as of the straw of the rice plants, and in every case it will be further seen, that the greater the amount of the phosphoric acid was, the larger was the yield.

The increasing rate upon the yield by the action of different doses of the various phosphates is still more clearly explained in the following calculations. Thus, when the plus yield of the smallest doses of the respective phosphates over the frame not supplied with phosphoric acid, is assumed to be 100, the other plus yields will have the following ratios.

Kind of phosphates.	Phosphoric acid applied per ha, kilograms.	Plus yield over the frame not supplied with phosphoric acid, grams.	Relative increase caused by different doses.
Ferric phosphate	25	314	100
	50	627	200
	100	835	266
Ferrous phosphate	25	260	100
	50	387	149
	100	533	205
Aluminium phosphate	25	198	100
	50	412	208
	100	585	296
Calcium phosphate	25	320	100
	50	495	155
	100	689	215

It will be observed from the above figures that the relative increase caused by the medium doses of the ferric and aluminium phosphates is nearly the same, while the increase by ferrous phosphate and calcium phosphate showed also a close coincidence, thus the medium doses of the former phosphates increased equally the yield 100% more over the yield of the smaller doses and the latter phosphates gave likewise almost 50% more. The relative increasing yields of the largest doses of the respective phosphates were not insignificant and there is, excepting the ferric phosphate, still almost the same climax of the yield over that of the medium doses, as that which between the smallest and medium doses.

In fact, the crop produced by the largest amount of the phosphoric acid was considerably higher than that obtained with the smaller quantity, and this proves that the smaller and the medium doses of the phosphoric acid displayed throughout all trials their full actions and that the increase obtained with the medium doses over the frame without phosphoric acid,

gives a reliable measure of the relative value of the various phosphates applied.

In order to compare the effects of the various insoluble phosphates with that of soluble phosphoric acid, a special trial was made with double superphosphate on a part of the same field in a similar manner; 5 kilograms of the phosphoric acid of the double superphosphate gave on average 581 grams of straw, 491 grams of full grain and 13 grams of empty grains which makes 1085 grams of total yield per frame. Taking, now, the plus yield of the double superphosphate over the frame without phosphoric acid to be as 100, the relation of the increase with the application of 5 kilograms of the phosphoric acid of each phosphate is calculated with the following figures:—

Kind of phosphates.	Surplus over the frame without phosphoric acid, grams.	Relation of the increase. Surplus of the double superphosphate = 100
Double superphosphate	447	100
Ferric phosphate.....	626	140
Ferrous phosphate.....	387	87
Aluminium phosphate.....	412	92
Calcium phosphate.....	495	117

Before entering into a discussion on these results we may further consider the proportions of the phosphoric acid consumed by the rice plants from the soil and the respective phosphate, as found by the chemical analysis made by myself.

Kind of phosphates.	P ₂ O ₅ applied.		P ₂ O ₅ in the total crop, grams.	P ₂ O ₅ consumed from the phosphate.	
	Per ha. kilograms.	Per frame grams.		grams.	% of the P ₂ O ₅ applied.
Without phosphoric acid	0	0	1.369	—	—
Double superphosphate	50	4.166	2.812	1.443	34.6
Ferric phosphate	25	2.083	2.170	0.801	38.5
" "	50	4.166	2.930	1.561	37.5
" "	100	8.333	3.953	2.584	31.0
Ferrous phosphate	25	2.083	1.947	0.578	27.7
" "	50	4.166	2.301	0.932	22.4
" "	100	8.333	2.705	1.336	16.0
Aluminium phosphate	25	2.083	1.761	0.392	18.8
" "	50	4.166	2.138	0.769	18.5
" "	100	8.333	2.557	1.188	14.3
Calcium phosphate	25	2.083	2.026	0.657	31.5
" "	50	4.166	2.459	1.090	26.3
" "	100	8.333	3.083	1.614	19.4

It is thus seen in all trials, that from the larger dose of the phosphoric acid applied, considerably more of this phosphoric acid was consumed by the rice plants than from the smaller dose; and it will be most reasonable to say that the results obtained with the medium doses constitute again a reliable information on the availability of the phosphoric acid of the various insoluble phosphates.

Assuming, now, the availability of the double superphosphate (34.6) to be 100, and calculating, on this basis, the relative availability of the other forms of phosphoric acid, the following numbers are obtained, and when an average is made of these numbers as to the relative action on the increase of total yield, the results will express the manurial value of the various phosphates for the first year.

Kind of phosphates.	Relative assimilability.	Relative action on the increase of total crop.	Relative manurial value in the first year.
Double superphosphate	100	100	100
Ferric phosphate	108.4	140	124
Ferrous phosphate	64.8	87	76
Aluminium phosphate.....	53.5	92	73
Calcium phosphate	76.1	117	97

As all the precipitated phosphates are naturally insoluble in water and as they are not easily distributed through the soil, I did not expect such a considerable good effect and rapid influence upon rice plants, and the results obtained by the experiments of the first year sufficiently prove that the insoluble phosphates which have been hitherto believed to be of very low value, have a fair action upon the rice plants cultivated in a soil of a certain character.

The condition which brought out these unexpected good results in the present experiment, is most probably due to the presence of much acid humus in our soil, the acidity of which having played a most important rôle upon the solution of the insoluble phosphates. The further discussion on this point is left for the later pages.

II. Series.

After-effects of the insoluble phosphates.

(Second year).

In order to determine the further action and the value of the various phosphates, the series of the preceding experiments were continued for the second year (1899).

All the frames after the harvest of the first crop, were left untouched till the end of May, but the soils in the frames were exposed to the air during the whole winter in a sufficiently dry state.

On the 20th of June, after the soil had been previously ploughed, all the frames equally received nitrogen and potash respectively at the rate

of 100 kilograms per hectare and in the form of sulphate. On the 25th, the young rice plants of the same variety as of the previous year were transplanted in the same manner; also the treatments of the plants was not essentially different. The weather was most favourable for rice plants. No parasites were observed.

On the 21st of November, the crops were harvested with the following yields; the numbers are the average of three equally treated frames:—

No. of frames.	Kind of phosphates.	P ₂ O ₅ applied in the first year per. ha. kilo-grams.	P ₂ O ₅ left from the preceding crop. per frame. grams.	Average yield per frame.			
				Straw. grams.	Full grain. grams.	Empty grain. grams.	Total crop. grams.
1, 40, 79.	Without phosphoric acid.	0	—	293.2	213.7	5.5	512
4, 43, 82.	Ferric phosphate	25	1.282	335.5	250.7	5.7	592
5, 44, 83.	„ „	50	2.605	355.3	275.8	6.2	637
6, 45, 84.	„ „	100	5.749	453.1	279.7	7.0	840
13, 52, 91.	Ferrous phosphate	25	1.505	310.1	230.7	5.9	547
14, 53, 92.	„ „	50	3.234	332.4	252.0	5.5	590
15, 54, 93.	„ „	100	6.997	414.8	332.5	6.4	754
22, 61, 100.	Aluminium phosphate	25	1.691	320.5	194.8	6.2	522
23, 62, 101.	„ „	50	3.397	362.8	271.7	6.2	641
24, 63, 102.	„ „	100	7.145	383.8	306.4	6.8	697
31, 70, 109.	Calcium phosphate	25	1.426	316.0	228.7	6.3	551
32, 71, 110.	„ „	50	3.076	346.5	255.5	7.6	610
33, 72, 111.	„ „	100	6.719	347.9	274.7	5.8	628
Specially tried	Double super-phosphate	50	2.723	340.5	254.0	6.5	601

In all the experiments, it was observed that the unrecovered phosphoric acid had an influence to a certain extent on the second crop. However, there was no case that the crops of the second year exceeded that of the first

year. Further, the greater was the amount of phosphoric acid left from the preceding year, the larger was the harvest.

In order to arrive at a definite idea, as to the influence upon the increase of the total crops, I calculated from the above results of the medium doses, assuming the plus yield of the double superphosphate over the frame not supplied with phosphoric acid to be 100, the following numbers; and for the sake of comparison, I add here the results of the first year.

Kind of phosphates.	Plus yield over the frame without P_2O_5 , grams.		Relation of the increase surplus of the double superphosphate = 100.	
	1st year	2nd year	1st year	2nd year
Double superphosphate	447	89	100	100
Ferric phosphate.....	626	125	140	141
Ferrous phosphate	387	78	87	88
Aluminium phosphate	412	129	92	145
Calcium phosphate.....	495	89	117	110

The two series of figures on the relative increase coincide tolerably well with the one exception of that obtained by the aluminium phosphate which seemed to be relatively more effective, owing to its late and gradual decomposition in the soil.

Before entering into a full discussion of the above results, I may here consider the amount of the phosphoric acid absorbed by the plants from the various phosphates in the second year. The chemical analysis of the crops of the second year gave the following figures:—

Kind of phosphates.	P ₂ O ₅ applied in the first year. Per ha. kilograms.	Unrecovered P ₂ O ₅ Per frame, grams.	P ₂ O ₅ in the total crop of second year. grams.	Consumed from the unrecovered P ₂ O ₅ .	
				Per frame grams.	% of the unrecovered P ₂ O ₅
Without phosphoric acid.	0	—	1.105	—	—
Double superphosphate	50	2.723	1.406	0.301	11.1
Ferric phosphate	25	1.282	1.308	0.203	15.8
" "	50	2.605	1.520	0.415	15.6
" "	100	5.749	1.955	0.850	14.8
Ferrous phosphate	25	1.505	1.237	0.132	8.8
" "	50	3.234	1.362	0.257	7.9
" "	100	6.997	1.816	0.711	11.6
Aluminium phosphate	25	1.691	1.257	0.152	9.0
" "	50	3.397	1.470	0.375	11.0
" "	100	7.145	1.638	0.533	7.5
Calcium phosphate	25	1.426	1.260	0.155	12.3
" "	50	3.076	1.349	0.244	7.9
" "	100	6.719	1.439	0.334	5.0

Assuming the availability of the residual phosphoric acid of the double superphosphate (11.1%) to be 100, the relative assimilability of the other kinds of phosphates will be as follows:—

Kind of phosphate.	Relative assimilability.	
	In the first year.	In the second year.
Double superphosphate	100	100
Ferric phosphate	108	142
Ferrous phosphate	65	71
Aluminium phosphate	54	99
Calcium phosphate	76	71

Thus it is seen, that the rates of the relative assimilability in the second year are, but with the exception of the calcium phosphate, greater than that of the first year in every trial, and this may be due to the nature of the gradual efficacy of the various insoluble phosphates.

As in the first year, I calculate the manurial value of the various phosphates for the second year, making an average of the two series of results, that is the relative increase on the yield and the relative assimilability of the residual phosphoric acid.

Kind of phosphate.	Relative manurial value.		Average.
	1st year.	Second year.	
Double superphosphate	120	100	100
Ferric phosphate	124.2	141.5	133
Ferrous phosphate	75.9	79.5	78
Aluminium phosphate.....	72.8	122.0	97
Calcium phosphate	96.6	90.5	94

Thus two years experiments have shown a most reliable relative value of the various insoluble phosphates. The best effect was displayed by the ferric phosphate in both years; however this phosphate being the most insoluble among all the phosphates experimented on, I did not anticipate such an extraordinary result. The real cause for this phenomenon is left to future researches. (Acidity of humus?)

Next to the ferric phosphate, the calcium phosphate has acted pretty well in the first year, but its action was somewhat decreased in the second season.

The ferrous phosphate and aluminium phosphate had less value in the first year, but the latter phosphate has shown a prominent action compared to the other phosphates in the second year; still the yield of the second year did not exceed that of the first year.

III. Series.

The after-effect of the residual phosphoric acid in the 3rd and 4th year. (1900 and 1901).

The former series of the experiments have been carried on as far as the 4th year in order to estimate the action of the residual phosphoric acid left by the successive rice crops.

In each year, the frames were treated just in the same manner as in the second year, and every year they received, in the middle of June, only nitrogen and potash as general manures, in the form of sulphate and at the rate of 100 kilograms per hectare respectively.

In 1900, the crops were harvested on November 20 and in 1901, the 4th crops on November 23.

The average yields of the three equally managed frames are, for both years, as follows :—

THE YIELD OF THE 3RD YEAR (1900).

No of frames.	Kind of phosphates.	P ₂ O ₅ applied in the 1st year per ha kilo.	Average yield per frames.				Surplus over the frame without P ₂ O ₅ grams.
			Straw grams.	Full grain grams.	Empty grain grams.	Total crop grams.	
1, 40, 79	Without phosphoric acid	0	278.0	242.0	2.8	522.8	
4, 43, 82	Ferric phosphate	25	281.0	243.5	3.4	527.5	4.7
5, 44, 83	" "	50	340.0	287.7	2.5	631.2	108.4
6, 45, 84	" "	100	390.6	360.0	3.4	754.0	231.2
13, 52, 91	Ferrous phosphate	25	286.7	237.0	4.0	527.7	4.9
14, 53, 92	" "	50	312.3	260.1	3.2	575.6	52.8
15, 54, 93	" "	100	308.6	267.8	3.7	580.1	57.3
22, 61, 100	Aluminium phosphate	25	340.4	278.1	4.6	623.1	100.3
23, 62, 101	" "	50	357.7	301.0	4.0	662.7	139.9
24, 63, 102	" "	100	372.3	313.8	4.1	690.2	167.4
31, 70, 109	Calcium phosphate	25	288.8	231.8	2.8	523.4	0.6
32, 71, 110	" "	50	314.5	248.3	3.8	566.6	43.8
33, 72, 111	" "	100	376.2	297.3	4.1	677.6	54.8
Specially tried	Double superphosphate	50	310.0	237.0	3.0	550.0	27.2

THE YIELD OF THE 4TH YEAR (1901).

No of frames.	Kind of phosphates.	P ₂ O ₅ applied in the 1st year per ha kilo.	Average yield per frames.				Surplus over the frame without P ₂ O ₅ grams.
			Straw grams.	Full grain grams.	Empty grain grams.	Total crop grams.	
1, 40, 79	Without phosphoric acid	0	188.3	167.8	2.5	358.6	
4, 43, 82	Ferric phosphate	25	214.0	167.7	2.4	384.1	25.5
5, 44, 83	" "	50	246.0	198.2	2.8	447.0	88.4
6, 45, 84	" "	100	336.3	283.7	3.7	623.7	265.1
13, 52, 91	Ferrous phosphate	25	229.0	175.0	2.8	406.8	48.2
14, 53, 92	" "	50	235.0	186.7	3.6	425.3	66.6
15, 54, 93	" "	100	275.0	233.2	3.4	511.6	153.0
22, 61, 100	Aluminium phosphate	25	250.0	205.5	3.0	458.5	99.9
23, 62, 101	" "	50	285.0	240.0	3.7	528.7	170.1
24, 63, 102	" "	100	311.7	270.3	3.2	585.2	226.6
31, 70, 109	Calcium phosphate	25	265.0	206.0	3.0	474.0	115.4
32, 71, 110	" "	50	292.3	243.7	2.9	538.9	180.3
33, 72, 111	" "	100	316.3	265.7	3.3	585.3	226.7
Specially tried	Double superphosphate	50	285.0	226.0	3.0	511.0	152.4

According to the above results, it will be clearly observed that the phosphoric acid applied in 1898 had still a distinct effect on the crops up to three as well as four years, but when the sums of the actual yield of the above table are compared with these of the preceding two years (1st and second years) a gradual decrease was obvious, showing that the phosphoric acid given in the first year was exhausted to a great extent, or was converted in a difficulty soluble form in the soil.

The surplus yields of the 4th year over the frame not supplied with any phosphoric acid, were, in all series, greater than those of the 3rd year and it might seem that the residual phosphoric acid in the 4th year had displayed a better action than in the 3rd year, but when we take into consideration the total yield in the frame without phosphoric acid in both years, it will be clearly noticed that the apparent plus yield of the fourth year is not due to efficacy of residual phosphoric acid but to the

diminution of harvest in the frame not supplied with phosphoric acid, the latter frame being exhausted of its phosphatic nutriment in the fourth year to a considerable extent.

Now assuming the third and fourth year's plus yield of the double superphosphate to be 100 respectively, the relative increase caused by the respective medium doses of the other phosphates will be found as follows.

	First year.	Second year.	Third year.	Fourth year.
Double superphosphate	100	100	100	100
Ferric phosphate	140	141	399	58
Ferrous phosphate	87	88	194	44
Aluminium phosphate.....	92	145	514	112
Calcium phosphate	117	110	161	118

These figures show that the effects of the residual phosphoric acid of the various phosphates in the third and fourth year varie within wide limits.

In the third year, the best action was displayed by the aluminium phosphate and ferric phosphate, while in the fourth year, the aluminium and calcium phosphates were best.

The remarkable effects of the aluminium aud also ferric phosphates in the third year will most probably be due to the action of humus left from the second year upon the residual phosphoric acid, because, in the second year, both phosphates gave a comparatively higher produce than others and consequently the organic matter, as roots and stubbles, left in the soil, were also larger than in the other frames.

IV. Series.

The action of caustic lime and calcium carbonate upon the effect of various insoluble phosphates.

In 1891, Dr. Kellner¹ then professor in this college, had observed that

¹ Bulletin, College Agr., Imperial University, Tokio Vol. 1 No. 9. p. 23.

basic ferric phosphate treated with lime water in presence of much carbon dioxide is decomposed and more than 14% of the total phosphoric acid passed into the filtrate. From this observation, he inferred that "such a process will, of course, also take place in soils between the basic phosphates of iron and freshly applied lime, which will display its action there also in both forms, as hydrate and bicarbonate; as a result the crop will be benefited as to their nutrition with phosphoric acid." Further he added that similar processes are certainly accomplished still more easily between calcium compounds and ferrous phosphate.

In order to confirm practically his observation and to get further informations on the actions of lime compounds towards various insoluble precipitated phosphates, these series of experiments were carried out in conjunction with the preceding experiments.

These experiments have also been progressing since 1898 and were still continued up to 1901, in order to study the after-effects of the various phosphates under the influence of lime compounds.

The methods in these trials were not essentially different from those followed previously, but the calcium compounds were applied in the first year as early as 14 days before the special phosphates. The calcium compounds used were pure freshly burnt lime and pure precipitated calcium carbonate; these were applied to the soil at the rate of 4000 kilograms of CaO per hectare, which quantity is the usual dose for most of the Japanese paddy fields. The general plan of these experiments will be seen from the following table :-

No of frames.			Kind of the phosphate.	Phosphoric acid applied per ha, kilograms.	Kind of lime compounds.
2,	41,	80.	No phosphoric acid	0	With CaO
3,	42,	81.	" " "	0	With Ca CO ₃
7,	46,	85.	Ferric phosphate	25	With Ca O
8,	47,	86.	" "	50	" "
9,	48,	87.	" "	100	" "
10,	49,	88.	" "	25	" Ca CO ₃
11,	50,	89.	" "	50	" "
12,	51,	90.	" "	100	" "
16,	55,	94.	Ferrous phosphate	25	With Ca O
17,	56,	95.	" "	50	" "
18,	57,	96.	" "	100	" "
19,	58,	97.	" "	25	" Ca CO ₃
20,	59,	98.	" "	50	" "
21,	60,	99.	" "	100	" "
25,	64,	103.	Aluminium phosphate	25	With Ca O
26,	65,	104.	" "	50	" "
27,	66,	105.	" "	100	" "
28,	67,	106.	" "	25	" Ca CO ₂
29,	68,	107.	" "	50	" "
30,	69,	108.	" "	100	" "
34,	73,	112.	Calcium phosphate	25	With Ca O
35,	74,	113.	" "	50	" "
36,	75,	114.	" "	100	" "
37,	76,	115.	" "	25	" Ca CO ₃
38,	77,	116.	" "	50	" "
39,	78,	117.	" "	100	" "

Already two weeks after the transplantation of the young rice some distinct differences were observed upon the limed and unlimed soil. All

limed plants showed a somewhat unhealthy condition while those not limed appeared throughout vigorous and healthy; in the course of time these differences decreased to a certain extent, yet at the approach of ripening an unfavorable condition of the limed plants was still distinctly noticeable by the comparatively short and somewhat thin stalks.

The crops harvested on the 29th of November, yielded the following numbers.¹

No of frames.	Kind of manures.	Phosphoric acid per ha, kilograms.	Average per frame.			
			Straw grams.	Full grain grams.	Empty grain grams.	Total crop grams.
2, 41, 80.	No phosphoric acid with Ca O	0	294.0	227.3	6.3	527.6
3, 42, 81.	" " " " Ca CO ₃	0	317.7	265.0	5.6	588.3
7, 46, 85.	Ferric phosphate with Ca O	25	449.6	345.8	9.9	805.3
8, 47, 86.	" " " "	50	457.6	407.0	12.9	877.5
9, 48, 87.	" " " "	100	639.3	570.7	17.4	1227.4
10, 49, 88.	" " " Ca CO ₃	25	445.3	380.6	11.0	836.9
11, 50, 89.	" " " "	50	609.0	511.6	21.2	1141.8
12, 51, 90.	" " " "	100	698.6	542.0	21.1	1261.7
16, 55, 94.	Ferrous phosphate with Ca O	25	330.0	281.7	7.1	618.8
17, 56, 95.	" " " "	50	369.0	319.0	7.7	695.7
18, 57, 96.	" " " "	100	504.7	400.0	8.9	913.6
19, 58, 97.	" " " Ca CO ₃	25	412.7	349.0	9.7	771.4
20, 59, 98.	" " " "	50	539.0	445.0	13.3	997.3
21, 60, 99.	" " " "	100	623.7	532.7	15.7	1162.1
25, 64, 103.	Aluminium phosphate with Ca O	25	357.3	286.3	8.7	652.3
26, 65, 104.	" " " "	50	393.3	321.7	7.5	722.5
27, 66, 105.	" " " "	100	454.0	403.3	10.4	867.7
28, 67, 106.	" " " Ca CO ₃	25	410.0	450.5	7.2	867.7
29, 68, 107.	" " " "	50	460.0	411.0	9.7	880.7
30, 69, 100.	" " " "	100	578.0	514.7	13.9	1106.6
34, 73, 112.	Calcium phosphate with Ca O	25	408.3	312.0	9.2	729.5
35, 74, 113.	" " " "	50	446.7	351.0	13.7	811.4
36, 75, 114.	" " " "	100	510.0	421.0	12.5	943.5
37, 76, 115.	" " " Ca CO ₃	25	509.0	433.3	13.3	955.6
38, 77, 116.	" " " "	50	571.7	485.7	14.2	1071.6
39, 78, 117.	" " " "	100	640.7	561.0	14.3	1216.0

Before entering into the discussion of these results, I may here calculate the surplus yield over the frames not supplied with phosphoric acid and

¹ The yield per frame is left to the appendix.

lime, and for the sake of comparison the plus yields of the unlimed frames are added in the last column.

Kind of phosphates.	Phosphoric acid applied per ha, kilograms.	Plus yield over the frame not supplied with phosphoric acid and lime.		
		With Ca O.	With Ca CO ₃ .	Without lime.
Without phosphoric acid	0	(-) 110.4	(-) 49.7	---
Ferric phosphate	25	167.3	198.9	314.0
" "	50	239.5	503.8	627.0
" "	100	589.4	623.7	835.0
Ferrous phosphate	25	(-) 19.2	133.4	260.0
" "	50	57.7	359.3	387.0
" "	100	275.6	524.1	533.0
Aluminium phosphate	25	14.3	229.7	198.0
" "	50	84.5	242.7	412.0
" "	100	229.7	468.6	587.0
Calcium phosphate	25	91.5	313.6	320.0
" "	50	173.4	433.6	495.0
" "	100	395.5	578.0	689.0

Thus we see that in all trials, excepting the frame without phosphoric acid, and also the smallest dose of the ferrous phosphate, the phosphoric acid applied in the form of the precipitated phosphate had again a remarkable action upon the rice production, but when a close comparison is made to the surplus yield of each unlimed frame, some significant decrease in the respective series was plainly observed with but the exception of the smallest dose of the calcium phosphate with the calcium carbonate which, on the contrary, gave a still higher plus yield than the control unlimed frame.

These diminutions were, without doubt, caused by a prompt action of the lime upon the various phosphates and it was further observed that even

in the frames not supplied with any phosphoric acid, the yield diminished to a high extent in consequence of the lime application.

In order to clear up more fully the injurious action of the lime, the following calculations were made in which the surplus yield of the unlimed frames are assumed respectively to be 100.

Kind of phosphates.	Phosphoric acid applied per ha, Kilograms.	Relative diminution of the plus yield.	
		With Ca O.	With Ca CO ₃ .
Ferric phosphate	25	46.7	36.7
.. ..	50	61.8	19.6
.. ..	100	29.4	25.3
Ferrous phosphate	25	—	48.7
.. ..	50	85.1	7.2
.. ..	100	48.3	2.0
Aluminium phosphate	25	92.8	(+) 16.0
.. ..	50	79.5	41.1
.. ..	100	60.9	19.9
Calcium phosphate	25	71.4	2.0
.. ..	50	65.0	12.4
.. ..	100	55.9	16.1

In these figures, the injurious action of both, caustic lime and calcium carbonate, upon the availability of various phosphates is plainly disclosed; it becomes further evident that the action of the caustic lime was far much more marked than that of the calcium carbonate throughout all the trials.

Besides, some injurious effect of both the lime compounds were even displayed upon the ordinary phosphoric acid of the experimental soil. When the diminution of the yield in the limed frame is compared to the yield in the unlimed frame, the percentage diminution amounts to as much as 17%

for the frame with the caustic lime and 8% for that with the calcium carbonate.

The diminution by the calcium carbonate for different doses of the various phosphates shows a great irregularity, and this may, most probably, be due to unequal distribution of the carbonate in the soil.

From the results of the chemical analysis, I may quote here the proportion of the phosphoric acid consumed from the various phosphates and the soil; the results are as follows.

Kind of phosphates.	Phosphoric acid applied.		With Ca O.			With Ca CO ₃ .			Without lime compound.
	per ha Kilo-grams.	per frame grams.	P ₂ O ₅ in the total crop	P ₂ O ₅ consumed from the phosphate		P ₂ O ₅ in the total crop	P ₂ O ₅ consumed from the phosphate		% of the phosphoric acid applied.
			grams.	grams.	% of the applied.	grams.	grams.	% of the applied.	
Without P ₂ O ₅	0	0	1.135			1.337			
Ferric phosphate	25	2.083	1.711	0.576	27.7	1.998	0.661	31.3	38.5
	50	4.166	2.007	0.872	20.9	2.881	1.544	37.1	37.5
	100	8.333	2.670	1.535	18.4	2.962	1.625	19.5	31.0
Ferrous phosphate	25	2.083	1.539	0.404	19.4	1.783	0.446	21.0	27.7
	50	4.166	1.813	0.678	16.3	2.117	0.780	18.7	22.4
	100	8.333	2.192	1.057	12.7	3.047	1.710	20.5	16.0
Aluminium phosphate	25	2.083	1.487	0.352	16.9	1.707	0.370	17.8	18.0
	50	4.166	1.688	0.552	13.3	2.040	0.703	16.9	18.5
	100	8.333	2.182	0.947	11.3	2.564	1.227	14.7	14.3
Calcium phosphate	25	2.083	1.596	0.461	22.1	1.896	0.559	26.8	31.5
	50	4.166	1.754	0.621	14.9	2.193	0.856	20.6	26.3
	100	8.333	2.089	0.954	11.5	2.954	1.617	19.4	10.4

Thus we see, that the application of the lime and calcium carbonate has disturbed the absorption of the phosphoric acid to a great extent, and

that the intensity of this influence of the caustic lime was far greater than that of the calcium carbonate.

These obvious diminutions of the phosphoric acid consumption by the crop in the limed frames have, no doubt, caused also the decrease of the yield.

Although the general features of the assimilation coefficients of the various phosphates were considerably decreased by the lime, yet the action of the calcium carbonate upon the *largest dose* of the phosphates were, excepting that of the ferric phosphate, very trifling, besides, in the ferrous and aluminium phosphates even some increase of the phosphoric acid consumption was observed, but in spite of this increase, the yields of these frames still remained behind those of the respective unlimed frames. This peculiar result may be due to the fact that in the beginning of the growth of the rice plants only little phosphoric acid is needed, while in the later period, as the action of the calcium carbonate decreases, much of this nutriment was absorbed and deposited chiefly in stalks and leaves; the quantity of the yield was not materially affected, for at this later time unfavorable conditions of climate to rice plants already prevailed, preventing the application for the formation of grains.

V. Series.

The after-effects of lime upon the residual phosphates.

(2nd, 3rd and 4th year.)

The cultivation and treatment of these series were just the same as that of the II and III series. Here, at first, the average yields[†] of the three equally treated frames for the second year are given:—

[†] The yields per frame are contained in the appendix.

No of frame.	Kind of manures.	P ₂ O ₅ applied in the 1st year per ha, kilo.	P ₂ O ₅ left from the preceding year per frame, grams.	Average yield per frame.			
				Straw, grams.	Full grain, grams.	Empty grain, grams.	Total Crop, grams.
1, 40, 79.	No P ₂ O ₅ without lime.	0		293.2	213.7	5.5	512.0
2, 41, 80.	No P ₂ O ₅ with Ca O	0		342.7	248.3	5.9	596.9
3, 42, 81.	" " " Ca CO ₃	0		306.6	225.7	5.8	538.1
7, 46, 85.	Ferric phos. with Ca O	25	1.507	392.1	298.0	6.3	696.4
8, 47, 86.	" " " "	50	3.294	424.1	335.6	7.5	767.2
9, 48, 87.	" " " "	100	6.798	494.3	397.3	8.3	899.9
10, 49, 88.	" " " Ca CO ₃	25	1.422	322.1	237.3	5.6	565.0
11, 50, 89.	" " " "	50	2.622	383.8	289.0	6.8	679.6
12, 51, 90.	" " " "	100	6.708	455.7	333.5	8.5	797.7
16, 55, 94.	Ferrous phos. with Ca O	25	1.679	396.4	307.3	7.7	711.4
17, 56, 95.	" " " "	50	3.488	413.8	313.0	7.8	734.6
18, 57, 96.	" " " "	100	7.276	407.9	317.7	7.3	732.9
19, 58, 97.	" " " Ca CO ₃	25	1.637	315.6	225.4	5.1	540.1
20, 59, 98.	" " " "	50	3.386	316.0	237.3	5.2	558.5
21, 60, 99.	" " " "	100	6.623	407.1	317.7	7.3	732.1
25, 64, 103.	Aluminium phos. with Ca O	25	1.731	378.8	287.5	7.0	673.3
26, 65, 104.	" " " "	50	3.613	331.9	291.0	7.2	630.1
27, 66, 105.	" " " "	100	7.386	446.7	352.5	8.1	807.3
28, 67, 106.	" " " Ca CO ₃	25	1.713	298.1	215.0	6.3	519.4
29, 68, 107.	" " " "	50	3.463	337.6	251.7	6.5	595.8
30, 69, 108.	" " " "	100	7.166	403.4	310.0	7.0	721.3
34, 73, 112.	Calcium phos. with Ca O	25	1.622	323.8	241.3	5.9	571.0
35, 74, 113.	" " " "	50	3.545	357.4	254.7	6.0	618.7
36, 75, 114.	" " " "	100	7.379	403.2	305.7	7.6	718.5
37, 76, 115.	" " " Ca CO ₃	25	1.524	323.9	227.3	6.4	557.6
38, 77, 116.	" " " "	50	3.310	329.3	242.0	6.6	577.9
39, 78, 117.	" " " "	100	6.716	371.5	281.7	7.8	661.0

The above figures show that in nearly all the trials, the larger the amount of phosphoric acid, that was left from the preceding crop, the greater was the yield, and that the effect of these residual quantities of phosphates was greater than those of the unlimed frames. In order to demonstrate these points more fully, the actual surplus yield over the frame without phosphoric acid and lime compounds was calculated.

Kind of phosphates.	P ₂ O ₅ applied in the first year per ha, kilograms.	Surplus yield over the frame without phosphoric acid and lime compounds. (grams.)			Surplus yield not limed frame = 100.	
		with Ca O.	with Ca CO ₃ .	not limed.	with Ca O.	with Ca CO ₃ .
No P ₂ O ₅	0	84.9	26.1			
Ferric phosphate.	25	184.4	53.0	80.0	(+) 130.5	(-) 37.7
	50	255.2	167.6	125.0	(+) 104.2	(+) 34.1
	100	387.9	285.7	328.0	(+) 15.5	(-) 12.9
Ferrous phosphate.	25	199.4	34.1	35.0	(+) 82.4	(-) 2.6
	50	222.6	46.5	78.0	(+) 185.4	(-) 40.6
	100	220.9	220.1	242.0	(-) 8.7	(-) 9.1
Aluminium phosphate.	25	161.3	7.4	10.0	(+) 151.0	(+) 26.0
	50	118.1	83.8	129.0	(-) 8.4	(-) 35.1
	100	295.3	209.3	185.0	(+) 59.6	(+) 13.1
Calcium phosphate.	25	59.0	45.6	39.0	(+) 51.3	(+) 17.0
	50	106.7	65.9	98.0	(+) 8.7	(-) 32.5
	100	206.5	149.0	116.0	(+) 78.0	(+) 28.4

Before entering into a discussion of these results we shall consider the proportion of the phosphoric acid absorbed by the plants from the unrecovered phosphoric acid for the second year.

Kind of phosphates.	P ₂ O ₅ applied in the 1st year per ha, kilo-grams.	With Ca O				With Ca CO ₃			
		Unre- coverd P ₂ O ₅ per frame, grams.	P ₂ O ₅ in the total crop per frame, grams.	Consumed from unrecovered P ₂ O ₅ .		Unre- coverd P ₂ O ₅ per frame, grams.	P ₂ O ₅ in the total crop per frame, grams.	Consumed from unrecovered P ₂ O ₅ .	
				grams.	% of the enre- coverd P ₂ O ₅ .			grams.	% of the unre- coverd P ₂ O ₅ .
No P ₂ O ₅	0		1.325				1.211		
Ferric phosphate.	25	1.507	1.641	0.316	21.0	1.422	1.270	0.069	4.9
	50	3.294	1.926	0.601	18.6	2.622	1.630	0.419	16.0
	100	6.798	2.093	0.778	11.4	6.708	1.854	0.643	9.9
Ferrous phosphate.	25	1.679	1.736	0.411	24.5	1.637	1.249	0.038	2.3
	50	3.488	1.734	0.409	11.7	3.386	1.314	0.103	3.0
	100	7.276	1.791	0.476	6.5	6.623	1.781	0.570	8.6
Aluminium phosphate.	25	1.731	1.558	0.233	13.5	1.713	1.242	0.031	1.8
	50	3.613	1.607	0.282	7.8	3.463	1.351	0.140	4.0
	100	7.386	1.933	0.608	8.2	7.106	1.672	0.461	6.5
Calcium phosphate.	25	1.622	1.393	0.068	4.2	1.524	1.281	0.070	4.6
	59	3.545	1.401	0.076	2.1	3.310	1.346	0.135	4.1
	100	7.379	1.657	0.332	4.5	6.716	1.531	0.320	4.8

The figures of the two above tables decidedly prove, that in all the frames which received caustic lime in the first year, there were, with but few exceptions, apparently larger yields and also larger amounts of the residual phosphoric acid absorbed, than in the cases of the unlimed frames, but when a reference is made to the unfavorable results of the limed frames in the *first* year, this increase of the yield, was, in general, far from being sufficient to recover the diminution caused by the caustic lime in the first year. These results, therefore, prove that the action of the caustic lime has, by no means, favorably effected the assimilability of the phosphates.

As to the frames supplied with calcium carbonate, it is seen that a slight increase of the crop was observed but in few frames, while in the majority, on the one side, a decrease and also a diminution of phosphoric acid consumption took place.

As we have already seen from the results of the first year's trials, the retarding action of the calcium carbonate was not so extensive as that of the caustic lime, but here, for the second year, we notice the assimilation of the residual phosphoric acid was interfered with considerably by further effects of the calcium carbonate left also from the preceeding year.

With a view of obtaining still further data regarding the behavior of the residual phosphates to lime the series of the present investigation were continued for two years more.

Leaving the details of the yield per frame for the appendix, the average of the equally treated frames are, for the third and fourth year given in the following two tables:—

THE YIELDS OF THE THIRD YEAR.

No of frames.	Kind of manures.	P ₂ O ₅ applied in the 1st year per ha, kilo-grams.	P ₂ O ₅ left from second year per frame, grams.	Average yield per frame.				Surplus over the frame without P ₂ O ₅ and CaO, grams.
				Straw grams.	Full grain, grams.	Empty grain, grams.	Total crop, grams.	
1, 40, 79.	No P ₂ O ₅ without lime	0		278.0	242.0	2.8	522.8	
2, 41, 80.	No P ₂ O ₅ with Ca O	0		321.3	271.3	4.0	596.6	73.8
3, 42, 81.	" " " Ca CO ₃	0		276.7	228.2	2.5	507.4	(-) 15.4
7, 46, 85.	Ferric phos. with Ca O	25	1.191	399.3	340.1	3.9	743.3	220.5
8, 47, 86.	" " " "	50	2.693	398.3	353.3	3.9	755.5	232.7
9, 48, 87.	" " " "	100	1.010	416.7	361.3	5.3	783.3	260.5
10, 49, 88.	" " " Ca CO ₃	25	1.353	293.3	240.3	3.3	536.9	14.1
11, 50, 89.	" " " "	50	2.203	295.7	255.2	3.1	554.0	31.2
12, 51, 90.	" " " "	100	6.065	394.2	347.0	5.6	746.8	224.0
16, 55, 94.	Ferrous phos. with Ca O	25	1.268	326.7	290.7	4.0	621.4	98.6
17, 56, 95.	" " " "	50	3.079	358.3	311.8	4.1	674.2	151.4
18, 57, 96.	" " " "	100	6.800	408.7	359.5	4.3	772.5	249.7
19, 58, 97.	" " " Ca CO ₃	25	1.599	284.1	243.0	3.1	530.2	7.4
20, 59, 98.	" " " "	50	3.283	339.3	296.2	3.7	639.2	116.4
21, 60, 99.	" " " "	100	6.053	343.4	308.7	4.2	656.3	133.5
25, 64, 103.	Aluminium phos. with Ca O	25	1.498	340.8	303.2	3.5	647.5	124.7
26, 65, 104.	" " " "	50	3.331	327.8	286.0	5.2	619.0	96.2
27, 66, 105.	" " " "	100	6.778	388.5	350.2	5.1	743.8	221.0
28, 67, 106.	" " " Ca CO ₃	25	1.682	301.7	256.5	3.5	561.7	38.9
29, 68, 107.	" " " "	50	3.323	306.3	270.2	3.7	580.2	57.4
30, 69, 108.	" " " "	100	6.645	331.3	260.8	3.8	595.9	73.1
34, 73, 112.	Calcium phos. with Ca O	25	1.554	352.6	287.0	3.6	643.2	120.4
35, 74, 113.	" " " "	50	3.469	378.0	282.7	3.1	663.8	141.0
36, 75, 114.	" " " "	100	7.047	415.9	375.9	4.2	796.0	273.2
37, 76, 115.	" " " Ca CO ₃	25	1.454	304.3	227.5	4.1	535.9	13.1
38, 77, 116.	" " " "	50	3.175	246.7	307.2	3.3	557.2	34.4
39, 78, 117.	" " " "	100	6.396	330.5	281.0	3.9	605.4	82.6

THE YIELDS OF THE FOURTH YEAR.

No of frames.	Kind of manures.	P ₂ O ₅ applied in the 1st year per ha, kilo-grams.	Average yield per frame.				Surplus over the frame without P ₂ O ₅ and CaO grams.
			Straw, grams.	Full grain, grams.	Empty grain, grams.	Total crop, grams.	
1, 40, 79.	No P ₂ O ₅ without lime.	0	188.3	167.8	2.5	358.6	
2, 41, 80.	No P ₂ O ₅ with Ca O	0	236.3	161.3	3.6	401.2	42.6
3, 42, 81.	" " " Ca CO ₃	0	196.0	148.7	2.6	347.3	(-) 8.7
7, 46, 85.	Ferrie phos. with Ca O	25	286.0	226.0	3.0	515.0	156.4
8, 47, 86.	" " " "	50	282.3	225.0	3.4	504.6	146.0
9, 48, 87.	" " " "	100	300.0	250.3	3.6	553.9	195.3
10, 49, 88.	" " " Ca CO ₃	25	267.0	207.3	3.1	477.4	118.8
11, 50, 89.	" " " "	50	296.0	241.5	3.5	541.0	182.4
12, 51, 90.	" " " "	100	308.0	260.3	3.8	572.1	213.5
16, 55, 94.	Ferrous phos. with Ca O	25	286.7	244.3	3.4	534.4	175.8
17, 56, 95.	" " " "	50	315.3	260.0	3.7	579.0	220.4
18, 57, 96.	" " " "	100	300.3	252.0	3.3	555.6	197.0
19, 58, 97.	" " " Ca CO ₃	25	260.7	206.3	2.9	469.9	111.3
20, 59, 98.	" " " "	50	294.7	247.0	3.1	544.8	186.2
21, 60, 99.	" " " "	100	281.0	224.2	3.4	508.6	150.0
25, 64, 103.	Aluminium phos. with Ca O	25	289.3	243.8	3.3	536.4	177.8
26, 65, 104.	" " " "	50	300.0	242.2	3.6	545.8	187.2
27, 66, 105.	" " " "	100	327.3	282.5	3.6	613.4	254.8
28, 67, 106.	" " " Ca CO ₃	25	246.0	186.7	3.3	436.0	77.4
29, 68, 107.	" " " "	50	243.3	189.7	3.2	436.2	77.6
30, 69, 108.	" " " "	100	332.7	274.0	3.9	610.6	252.0
34, 73, 112.	Calcium phos. with Ca O	25	298.0	238.0	3.3	539.3	180.7
35, 74, 113.	" " " "	50	327.3	274.7	3.7	602.7	244.1
36, 75, 114.	" " " "	100	338.0	289.3	3.8	631.1	272.5
37, 76, 115.	" " " Ca CO ₃	25	312.7	253.7	3.6	570.0	211.4
38, 77, 116.	" " " "	50	320.7	253.0	3.5	577.2	218.6
39, 78, 117.	" " " "	100	315.7	259.7	3.5	578.9	220.3

The figures contained in these two tables obviously demonstrate, in spite of the presence of the residual lime, still some favorable effects of the unrecovered phosphoric acid in the third and even up to the fourth year, but in the course of time, the variation of the yields between the three different doses of each phosphate, became gradually narrower, although the frames which were manured more with the phosphates in the first year left also more of the phosphates for the successive crops.

As to the after-effects of the lime it is seen from the frames without phosphoric acid that the caustic lime caused in both (3rd and 4th) years a gradation of the yield to a noted degree, while the calcium carbonate, on the other hand, caused some diminution of the crop when a comparison is made to that of the unlimed frame. The same fact is, in most cases, also observed in other frames which received the various phosphates and also two forms of the lime. This fact tends to prove that, although the calcium carbonate had a much weaker retarding action upon the effects of the phosphates in the first year than the caustic lime, a gradual and unfavorable influence of the former lasted even to the 4th year. This opposite action which exists between the caustic lime and calcium carbonate may be due to the natural character of both compounds. Thus caustic lime being endowed with stronger alkalinity than calcium carbonate, neutralizes more effectively the acid humus which is one of the most important factors in the solution of insoluble phosphates.

The Carbonic acid which also plays an important role in the solution of the phosphates is further absorbed by the caustic lime.

Caustic lime, when freshly applied, certainly neutralizes also the acid juice of roots decreasing thus also the dissolving actions of the roots.

These are the main reasons for the injurious effects of caustic lime in the first year.

On the other hand, the calcium carbonate had not such highly injurious action in the first year as the caustic lime had, but its actions were slower in the beginning and lasted longer.

The calcium carbonate further is less soluble in the irrigating water than caustic lime, hence more is left for the following years.

Before a general review of my results will be given, recent investigations by *F. Sutherst*¹ will be mentioned.

This author has studied the action of caustic lime and calcium carbonate upon ferrous, ferric and aluminium phosphates and observed the following results.

By the action of caustic lime, in presence of sufficient water upon ferrous, ferric and aluminium phosphates respectively for 1, 2 and 3 days, the solubility, in weak citric acid, of the phosphoric acid in these phosphates had extraordinarily increased in proportion of the lime applied; he has calculated the following percentage of the dissolved phosphoric acid.

Hours.	Ferrous phosphate.	Ferric phosphate.	Aluminium phosphate.
24.	75.42%	94.45%	64.33%
48.	85.45 „	96.38 „	69.31 „
72.	85.88 „	96.55 „	72.00 „

In the same manner he has also tested the action of calcium carbonate, but the results were totally negative. Hence he infers: "From the above results it will be seen that it is essential that the lime applied in practice should be in the form of hydrate, the carbonate being of no value whatever." The observations of *Sutherst* relate to trials in flasks, but while these were confirmed by myself, it is different with soils.

K. Kawashima, formerly one of my students has carried out a trial under my direction on the series of the soils of my experimental frames which had received the various phosphates at the rate of 50 kilograms per hectare, that is the medium dose that we had applied.

On the 12th of October, 1898, he took samples of the soil from the respective destined frames of my first years trials above mentioned in a careful manner.

He determined in each sample the phosphoric acid soluble in neutral ammonium citrate and also in a 5% acetic acid and obtained the following figures calculated in % of the dry samples.

¹ Chemical News, Vol. LXXXV, No. 2210, p. 157.

No of frames from which the samples were taken.	Kind of manures.	% of the dry samples.	
		Phosphoric acid soluble in neutral ammonium citrate.	Phosphoric acid soluble in the 5% of acetic acid.
40.	No phosphoric acid	—	0,00224
41.	" " " with Ca O	0,04608	0,00214
42.	" " " " Ca CO ₃	0,05162	0,00291
44.	Ferric phosphate	0,08578	0,00315
47.	" " " with Ca O	0,05896	0,00199
50.	" " " " Ca CO ₃	0,08377	0,00327
14.	Ferrous phosphate	0,07315	0,00491
17.	" " " with Ca O	0,04807	0,00305
20.	" " " " Ca CO ₃	0,06149	0,00402
23.	Aluminium phosphate	0,07312	0,00422
26.	" " " with Ca O	0,05447	0,00270
29.	" " " " Ca CO ₃	0,06977	0,00478
32.	Calcium phosphate	0,07604	0,00263
35.	" " " with Ca O	0,06623	0,00289
38.	" " " " Ca CO ₃	0,07261	—

Thus it is clearly seen that in no case a favorable action of the caustic lime upon the solubility of the insoluble phosphates had taken place, but on the contrary, the caustic lime, in all cases, diminished to a great extent the solubility of the phosphoric acid in both reagents, while the action of the carbonate was much weaker.

This result sufficiently coincides with the actual yields in both, limed and unlimed frames and in the first year; hence it may be concluded that the caustic lime in soils has no beneficial action upon the insoluble phosphates whatever, within a short time.¹

¹ This fact was also proved in one of my recent trials (cf. the previous article).

In order to estimate the total effects of the liming the total sums of the four years' crops obtained from the frames supplied with the various phosphates and also with or without lime application, are given in the following table and further, the increase or decrease of the yields of the limed frames to those unlimed.

Kind of manures.	Phosphoric acid applied in the first year, per ha, kilograms.	The total sum of the yield of four years. (grams.)			Retation of increase or decrease. The respective yield of without lime = 1000.	
		without lime.	with Ca O	with Ca CO ₃	with Ca O	with Ca CO ₃
No phosphoric acid.	0	2031.4	2122.3	1981.1	(+) 44	(-) 25
Ferric phosphate.	25	2455.6	2760.0	2416.2	(+) 124	(-) 16
	50	2980.2	2914.8	2916.4	(-) 22	(-) 29
	100	3685.7	3463.5	3378.3	(-) 60	(-) 83
Ferrous phosphate.	25	2379.5	2486.0	2417.6	(+) 44	(+) 11
	50	2795.9	2683.5	2739.8	(-) 40	(-) 20
	100	3016.7	2974.6	3059.1	(-) 14	(+) 14
Aluminium phosphate.	25	2439.6	2509.5	2384.8	(+) 29	(-) 23
	50	2882.4	2517.4	2492.9	(-) 127	(-) 135
	100	3195.4	3032.2	3033.8	(-) 51	(-) 47
Calcium phosphate.	25	2506.4	2482.0	2619.1	(-) 10	(+) 45
	50	2848.5	2696.6	2783.9	(-) 53	(-) 23
	100	3217.9	3089.1	3061.3	(-) 40	(-) 49

From these figures it may be seen that the application of the caustic lime has caused but a slight increase of the rice crop in the frames without phosphoric acid and also in each of the smallest doses of the various phosphates, excepting but that of the calcium phosphate.

As to the action of the calcium carbonate, the results were quite irregular and no definite conclusion can be drawn here.

In an aqueous mixture containing an excess of calcium hydrate the facts of Sutherst can be observed, whilst in soil the case is different.

The caustic lime, before it can act upon the insoluble phosphate, can be absorbed mainly by the humus and partly be transformed into carbonate and silicate.

Liming of the soils can therefore not be recommended for the culture of rice in presence of such conditions as in our paddy field. Finally I should recommend to the practical farmers an application of organic manures such as farm yard manures, acid cakes, peat &c., into over limed or freshly limed soils, as it would facilitate the absorption of insoluble phosphates by the plants and thus lead to a satisfactory harvest.



Note to the Preceding Paper.

BY

Oscar Loew.

Soon after the conclusion of these highly valuable and laborious investigations Prof. *Nagaoka* left for Europe and was not aware of the results Prof. *Aso* had obtained with rice grown in soil limed in various degrees and described in our Bul. VI, No. 2. Since the soil was quite of the same character and derived from the same locality, it may be permitted to add a few remarks. *Aso* has shown that for rice the best ratio of lime to magnesia is =1 or nearly so, what agrees with the behavior of other cereals. Any increase either of lime or of magnesia beyond that proportion depressed the yield under otherwise equal conditions. Now the soil in question contains already lime and magnesia in nearly equal quantities, namely lime =0.9% magnesia =0.7%, hence the liming of that soil produced in *Nagaoka's* case essentially the same results as in *Aso's* trial. The effects of liming, however, would differ on soils that contain a considerable surplus of magnesia over the lime content.

Appendix

YIELDS PER FRAME, FIRST YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
No P ₂ O ₅ and without lime.				46	420.0	351.0	8.0
1	267.0	243.0	5.0	85	485.0	405.0	12.5
40	330.0	266.0	6.7	8	414.0	370.0	13.0
79	426.0	359.0	10.5	47	442.0	382.0	12.3
No P ₂ O ₅ with Ca O.				86	517.0	469.0	13.5
2	262.0	205.0	6.0	9	657.0	581.0	22.8
41	246.0	174.0	4.7	48	667.0	608.0	16.8
80	374.0	303.0	8.2	87	594.0	523.0	12.5
No P ₂ O ₅ with Ca CO ₃ .				Ferric phosphate with Ca CO ₃ .			
3	260.0	212.0	5.2	10	408.0	357.0	14.2
42	265.0	196.0	5.0	49	406.0	309.0	9.0
81	428.0	386.0	6.7	88	522.0	476.0	9.7
Ferric phosphate.				11	640.0	482.0	25.7
4	417.0	393.0	12.7	50	552.0	494.0	14.0
43	530.0	492.0	12.0	89	635.0	559.0	24.0
82	517.0	469.0	13.5	12	663.0	510.0	24.5
5	659.0	579.0	18.0	51	670.0	503.0	22.0
44	683.0	579.5	34.0	90	763.0	613.0	16.8
83	662.0	562.0	17.5	Ferrous phosphate.			
6	770.0	593.0	16.8	13	429.0	412.0	10.7
45	897.0	682.0	32.5	52	494.0	405.0	12.5
84	782.0	633.0	12.7	91	506.0	409.0	15.2
Ferric phosphate with Ca O.				14	411.0	400.0	9.7
7	344.0	281.5	9.3	53	644.0	473.0	13.0
				92	602.0	503.0	18.0
				15	585.0	513.0	16.0

YIELDS PER FRAME, FIRST YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
54	616.0	490.0	27.5	100	548.0	459.0	14.5
93	696.0	586.0	13.7	23	521.0	412.0	14.3
Ferrous phosphate with Ca O.				62	597.0	481.0	16.8
16	253.0	212.0	4.5	101	592.0	503.0	11.5
55	309.0	263.0	7.7	24	647.0	588.0	16.7
94	428.0	370.0	9.0	63	567.0	516.0	16.0
17	310.0	256.0	6.7	102	735.0	560.0	23.0
56	383.0	349.0	7.5	Aluminium phosphate with Ca O.			
95	414.0	352.0	9.0	25	(197.0)	(133.0)	(4.2)
18	383.0	342.0	6.7	64	333.0	286.0	7.3
57	497.0	432.0	10.0	103	542.0	440.0	14.7
96	534.0	426.0	10.0	26	264.0	191.0	4.0
Ferrous phosphate with Ca CO ₃ .				65	364.0	306.0	7.2
19	298.0	251.0	6.0	104	552.0	468.0	11.2
58	462.0	387.0	13.2	27	430.0	403.0	11.7
97	478.0	409.0	10.0	66	407.0	340.0	8.0
20	520.0	401.0	12.7	105	525.0	467.0	11.5
59	522.0	434.0	14.7	Aluminium phosphate with Ca CO ₃ .			
98	575.0	500.0	12.5	28	318.0	267.0	5.8
21	609.0	541.0	13.7	67	406.0	344.5	7.2
60	619.0	555.0	17.3	106	506.0	440.0	8.7
99	643.0	522.0	16.0	29	361.0	312.0	9.2
Aluminium phosphate.				68	447.0	403.0	6.5
22	374.0	312.0	9.0	107	572.0	518.0	13.5
61	449.0	329.0	12.5	30	618.0	553.0	14.0
				69	506.0	448.0	15.0

YIELDS PER FRAME, FIRST YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
108	610.0	543.0	12.7	35	302.0	232.0	6.5
Calcium phosphate.				74	372.0	283.0	13.7
31	387.0	345.0	7.0	113	666.0	538.0	21.0
70	546.0	475.0	14.0	36	445.0	367.0	9.8
109	567.0	520.0	12.0	75	500.0	428.0	11.7
32	655.0	555.0	17.7	114	585.0	468.0	16.0
71	521.0	425.0	19.0	Calcium phosphate with Ca CO ₃ .			
110	679.0	507.0	19.7	37	437.0	390.0	11.5
33	755.0	653.0	25.2	76	452.0	408.0	9.2
72	663.0	510.0	24.5	115	638.0	492.0	19.2
111	728.0	603.0	18.7	38	547.0	481.0	15.2
Calcium phosphate with Ca O.				77	556.0	465.0	12.5
34	340.0	280.0	6.5	116	612.0	511.0	15.0
73	354.0	258.0	9.0	39	620.0	577.0	16.7
112	531.0	398.0	12.2	78	580.0	546.0	13.5
				117	722.0	560.0	12.8

YIELDS PER FRAME, SECOND YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
No P ₂ O ₅ no lime.				46	391.3	293.0	5.7
1	250.5	216.0	7.0	85	423.7	319.5	7.5
40	324.7	226.0	5.8	8	383.3	320.0	5.0
79	304.3	199.0	3.7	47	423.3	325.0	6.5
No P ₂ O ₅ with Ca O.				86	465.8	361.5	10.9
2	258.5	191.0	4.0	9	471.3	368.0	7.7
41	363.2	284.0	4.5	48	498.7	406.0	7.0
80	406.3	270.0	9.2	87	512.8	418.0	10.2
No P ₂ O ₅ with Ca CO ₃ .				Ferric phosphate with Ca CO ₃ .			
3	251.7	182.0	4.2	10	296.8	208.0	3.9
42	311.7	218.0	4.7	49	304.8	215.0	5.7
81	356.3	277.0	8.5	88	364.8	289.0	7.1
Ferric phosphate.				11	327.8	235.0	5.0
4	248.8	181.0	3.4	50	381.8	289.0	7.5
43	347.8	265.5	4.8	89	441.8	343.0	8.0
82	409.8	305.5	9.0	12	400.7	356.0	5.5
5	324.8	262.5	5.0	51	380.7	308.0	7.4
44	344.5	263.0	4.5	90	585.7	336.5	12.7
83	396.7	302.0	9.0	Ferrous phosphate.			
6	396.7	346.0	6.2	13	252.8	187.0	3.5
45	463.3	385.5	7.5	52	326.3	236.0	7.7
84	499.3	407.5	7.4	91	351.3	269.0	6.5
Ferric phosphate with Ca O.				14	265.7	204.0	4.1
7	361.3	281.5	5.7	53	363.8	261.0	6.0
				92	367.8	291.0	6.5
				15	353.8	297.5	4.9

YIELDS PER FRAME, SECOND YEAR.

No of frames,	Straw grams,	Full grains grams,	Empty grains grams,	No of frames,	Straw grams,	Full grains grams,	Empty grains grams,
54	454.8	347.0	7.9	100	342.3	240.0	8.0
93	431.8	353.0	6.5	23	340.3	240.0	4.7
Ferrous phosphate with Ca O.				62	403.0	312.0	8.2
16	341.8	260.0	6.5	101	345.0	263.0	5.8
55	434.7	334.0	9.6	24	374.8	317.0	5.2
94	412.7	328.0	6.9	63	406.0	299.0	9.0
17	342.8	261.5	5.7	102	370.5	303.3	6.1
56	440.8	321.5	8.2	Aluminium phosphate with Ca O.			
95	457.8	356.0	9.4	25	320.3	249.0	4.9
18	340.6	267.0	5.7	64	434.3	307.0	10.0
57	429.0	324.0	9.4	103	381.8	306.5	6.2
96	454.0	362.0	6.9	26	342.8	269.0	5.2
Ferrous phosphate with Ca CO ₃ .				65	419.8	319.0	8.2
19	222.8	154.7	3.2	104	333.2	285.0	8.1
58	346.0	256.2	5.5	27	460.3	356.0	6.5
99	378.0	265.2	6.5	66	453.5	342.5	8.4
20	249.5	188.0	3.4	105	426.3	359.0	9.5
59	337.8	253.0	6.2	Aluminium phosphate with Ca CO ₃ .			
98	360.8	271.0	6.0	28	260.7	186.0	3.5
21	377.3	311.0	5.7	67	303.8	219.0	7.3
60	427.0	322.0	9.2	106	329.7	240.0	8.0
99	417.0	320.0	7.0	29	278.8	204.0	4.4
Aluminium phosphate,				68	368.0	276.0	6.9
22	262.5	177.0	4.0	107	366.0	275.0	8.2
61	356.7	167.5	6.5	30	370.5	309.0	5.1
				69	446.8	334.0	9.9

YIELDS PER FRAME, SECOND YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
108	392.8	287.0	9.5	35	339.3	245.0	4.7
Calcium phosphate.				74	364.5	240.0	7.2
31	242.8	174.0	3.7	113	368.5	279.0	7.9
70	356.8	262.0	7.4	36	377.3	284.0	6.5
109	348.3	250.0	7.9	75	444.3	333.0	7.5
32	307.8	235.5	4.7	114	394.0	300.0	8.7
71	369.8	278.0	8.2	Calcium phosphate with Ca CO ₃ .			
110	361.8	253.0	10.0	37	296.3	203.0	4.5
33	322.7	266.0	4.5	76	361.1	252.0	7.7
72	396.0	312.0	6.0	115	314.3	227.0	7.0
111	325.0	246.0	6.9	38	324.5	234.0	5.2
Calcium phosphate with Ca O.				77	314.5	234.0	6.5
34	312.5	232.0	5.5	116	348.8	258.0	8.2
73	352.5	269.0	6.4	39	319.3	227.0	4.4
112	306.5	223.0	5.7	78	442.8	319.0	10.5
				117	352.3	269.0	8.5

YIELDS PER FRAME, THIRD YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
No P ₂ O ₅ no lime.				46	423.0	349.5	3.9
1	211.0	164.0	2.5	85	438.0	379.0	3.9
40	326.0	294.0	2.9	8	306.0	279.0	3.4
79	297.0	268.0	2.9	47	439.0	382.0	4.0
No P ₂ O ₅ with Ca O.				86	450.0	399.0	4.2
2	252.0	207.0	5.5	9	352.0	321.0	4.5
41	353.0	295.0	2.9	48	470.0	377.0	5.9
80	359.0	312.0	3.5	87	428.0	386.0	5.4
No P ₂ O ₅ with Ca CO ₃ .				Ferric phosphate with Ca CO ₃ .			
3	216.0	185.0	2.4	10	217.0	160.0	3.0
42	348.0	275.0	2.9	49	326.0	261.0	3.4
81	266.0	224.5	2.2	88	337.0	300.0	3.4
Ferric phosphate.				11	187.0	122.0	2.2
4	252.0	222.0	3.7	50	306.0	303.5	3.0
43	273.0	244.0	4.2	89	394.0	340.0	4.2
82	318.0	264.5	2.2	12	364.0	297.0	6.2
5	247.0	211.7	3.5	51	321.5	315.0	5.7
44	387.0	306.0	3.5	90	497.0	429.0	4.9
83	386.0	345.5	3.5	Ferrous phosphate.			
6	347.0	338.0	3.9	13	248.0	221.0	3.5
45	398.0	355.0	3.2	52	247.0	206.0	4.5
84	426.7	387.0	3.2	91	365.0	284.0	4.0
Ferric phosphate with Ca ₂ O.				14	279.0	243.0	3.4
7	337.0	291.7	3.7	53	295.5	233.5	3.2
				92	362.5	304.0	2.9
				15	265.0	194.0	3.7

YIELDS PER FRAME, THIRD YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
54	309.7	298.7	3.9	100	384.7	304.7	4.5
93	351.0	310.7	3.5	23	347.0	286.0	4.2
Ferrous phosphate with Ca O.				62	380.0	325.0	4.2
16	238.0	201.0	3.5	101	346.0	292.0	3.5
55	347.0	330.0	3.7	24	316.0	240.0	3.7
94	395.0	341.0	4.7	63	388.0	343.5	4.4
17	243.0	212.0	4.0	102	413.0	358.0	4.2
56	374.0	344.0	4.4	Aluminium phosphate with Ca O.			
95	458.0	379.5	4.0	25	273.0	212.0	3.2
18	407.0	361.5	4.7	64	373.0	354.5	3.5
57	368.0	350.0	4.4	103	376.5	343.0	3.7
96	451.0	367.0	3.7	26	227.5	193.5	3.2
Ferrous phosphate with Ca CO ₃ .				65	388.0	358.5	3.7
19	261.5	238.0	2.4	104	368.0	306.0	8.7
58	278.5	229.5	3.2	27	364.5	340.0	5.0
97	312.5	261.5	3.7	66	348.0	320.5	5.0
20	317.0	284.7	4.2	105	453.0	390.0	4.5
59	328.0	296.0	3.9	Aluminium phosphate with Ca CO ₃ .			
98	373.0	308.0	3.0	28	309.5	262.5	3.7
21	315.7	269.7	4.5	67	306.0	266.5	3.4
60	325.0	315.5	4.0	106	289.5	240.5	3.5
99	389.5	341.0	4.0	29	265.0	222.0	4.4
Aluminium phosphate.				68	283.0	251.5	3.2
22	334.0	281.0	3.4	107	371.0	337.0	3.4
61	302.5	248.5	5.5	30	327.0	193.0	4.0
				69	325.0	283.0	3.7

YIELDS PER FRAME, THIRD YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
108	342.0	306.5	3.7	35	400.0	223.0	4.5
Calcium phosphate.				74	393.5	355.5	3.5
31	271.0	225.0	2.5	113	367.5	299.5	4.4
70	281.0	215.0	2.5	36	444.0	383.0	4.7
109	314.5	255.5	3.5	75	366.7	359.7	4.2
32	306.0	211.0	4.2	114	437.0	385.0	3.7
71	324.0	260.0	2.7	Calcium phosphate with Ca CO ₃ .			
110	313.5	244.0	4.4	37	292.0	210.5	2.9
33	395.5	328.5	5.4	76	233.0	209.0	4.5
72	364.0	291.5	3.9	115	378.0	263.0	5.0
111	367.0	272.0	3.0	38	265.7	256.0	3.7
Calcium phosphate with Ca O.				77	336.5	305.5	3.5
34	369.5	311.0	3.2	116	438.0	360.0	2.7
73	365.5	300.5	4.2	39	240.0	190.0	2.9
112	323.0	249.5	3.4	78	335.5	325.0	3.9
				117	392.0	328.0	5.0

YIELDS PER FRAME, FOURTH YEAR.

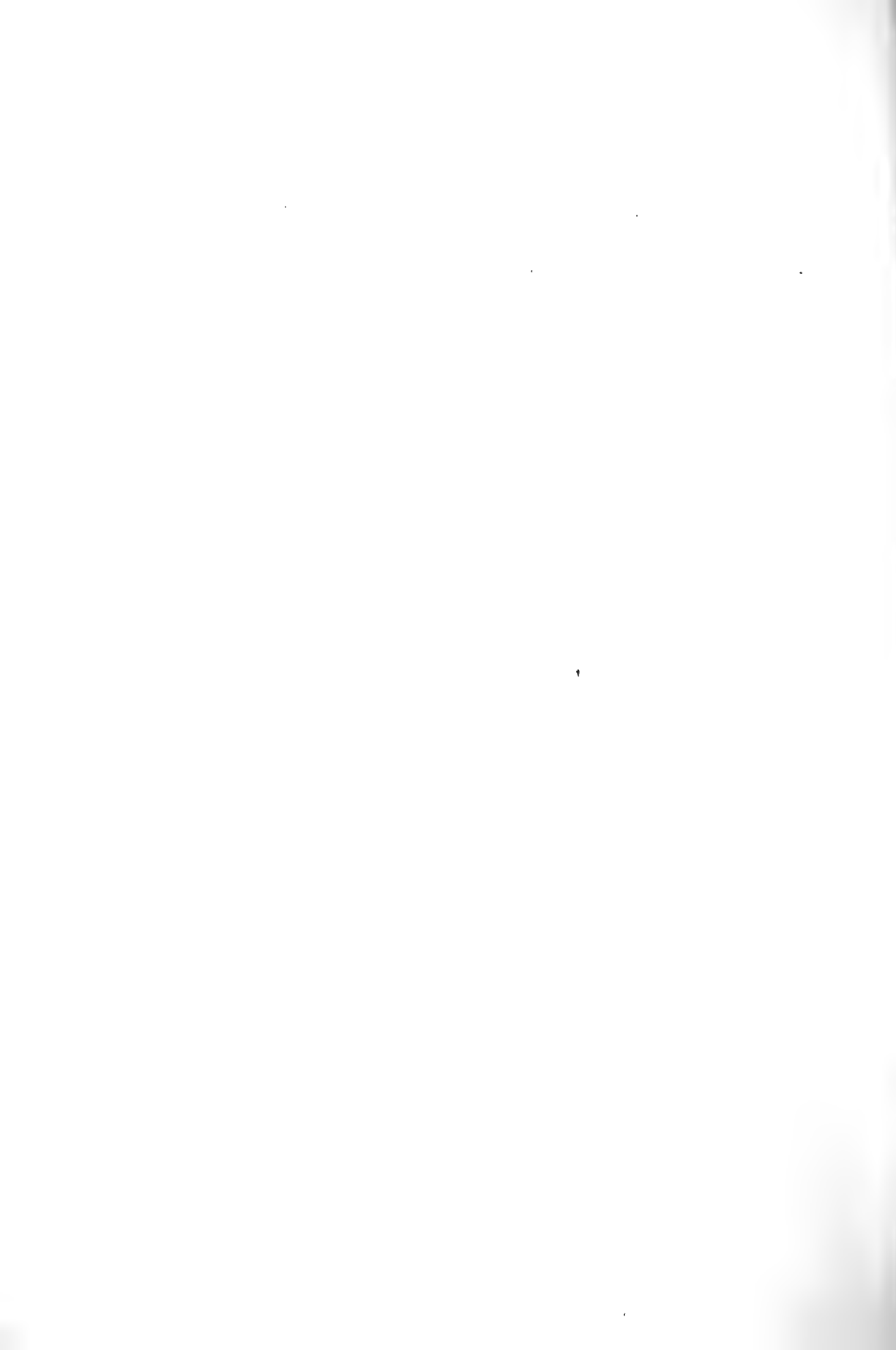
No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
No P ₂ O ₅ , no lime.				46	290.0	216.0	3.1
1	206.0	207.6	2.0	85	282.0	224.0	3.7
40	137.0	95.5	2.1	8	264.0	210.0	3.0
79	222.0	200.2	3.5	47	289.0	212.0	3.7
No P ₂ O ₅ with Ca O.				86	294.0	253.0	3.4
2	272.0	200.0	3.5	9	294.0	253.0	2.4
41	225.0	159.0	3.0	48	299.0	239.0	3.9
80	212.0	155.0	4.0	87	307.0	259.0	4.5
No P ₂ O ₅ with Ca CO ₃ .				Ferric phosphate with Ca CO ₃ .			
3	183.0	143.0	1.7	10	306.0	240.0	2.4
42	140.0	87.0	2.0	49	218.0	164.0	2.9
81	268.0	216.0	4.1	88	277.0	218.0	3.9
Ferric phosphate.				11	257.0	205.0	2.2
4	215.0	169.0	1.7	50	399.0	319.0	4.6
43	146.0	98.0	2.2	89	332.0	200.0	3.7
82	281.0	236.0	3.2	12	286.0	235.0	2.7
5	225.0	176.0	2.4	51	356.0	319.0	4.4
44	235.0	175.7	2.1	90	282.0	227.0	4.2
83	278.0	243.0	4.0	Ferrous phosphate.			
6	337.0	298.0	2.9	13	158.0	94.0	1.9
45	372.0	291.0	3.5	52	262.0	211.0	2.8
84	300.0	262.0	4.8	91	267.0	220.0	3.7
Ferric phosphate with Ca O.				14	241.0	145.0	4.9
7	298.0	238.0	2.1	53	254.0	186.0	3.0
				92	210.0	229.0	3.0
				15	282.0	235.6	2.4

YIELDS PER FRAME, FOURTH YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
54	252.0	211.0	3.7	100	217.0	165.0	3.7
93	291.0	253.0	4.2	23	274.0	228.0	2.2
Ferrous phosphate with Ca O.				62	282.0	229.0	4.7
16	294.0	243.5	2.4	101	299.0	262.0	4.2
55	317.0	258.6	4.4	24	315.0	280.0	2.4
94	249.0	231.0	3.4	63	330.0	255.0	3.4
17	318.0	266.0	2.2	102	290.0	276.0	3.8
56	301.0	225.0	5.8	Aluminium phosphate with Ca O.			
95	327.0	289.0	3.2	25	323.0	214.5	2.5
18	260.0	214.0	2.4	64	350.0	260.0	4.0
57	355.0	303.0	3.5	103	295.0	257.0	3.4
96	286.0	239.0	4.0	26	312.0	253.7	2.7
Ferrous phosphate with Ca CO ₃ .				65	313.0	262.0	3.6
19	277.0	225.0	2.5	104	275.0	211.0	4.5
58	272.0	222.0	3.0	27	327.0	265.5	2.9
97	233.0	172.0	3.2	66	357.0	301.0	3.8
20	296.0	245.0	1.9	105	298.0	281.0	4.1
59	268.0	222.0	4.0	Aluminium phosphate with Ca CO ₃ .			
98	320.0	274.0	3.5	28	281.0	231.0	2.5
21	220.0	155.5	2.7	67	250.0	177.0	3.5
60	293.0	245.0	3.2	106	207.0	152.0	3.8
99	330.0	272.0	4.1	29	206.0	150.0	2.4
Aluminium phosphate.				68	224.0	166.0	3.7
22	274.0	231.5	2.3	107	300.0	253.0	3.4
61	259.0	220.0	2.9	30	311.0	261.0	3.2
				69	361.0	310.0	3.9

YIELDS PER FRAME, FOURTH YEAR.

No of frames.	Straw grams.	Full grains grams.	Empty grains grams.	No of frames.	Straw grams.	Full grains grams.	Empty grains grams.
108	326.0	251.0	4.5	35	271.0	211.0	3.0
Calcium phosphate.				74	355.0	305.0	4.0
31	276.0	219.0	2.7	113	347.0	308.0	3.7
70	220.0	178.0	2.5	36	274.0	217.0	3.4
109	299.0	218.0	3.9	75	388.0	335.0	4.4
32	301.0	242.0	2.1	114	352.0	316.0	3.5
71	361.0	329.0	3.4	Calcium phosphate with Ca CO ₃ .			
110	215.0	160.0	3.1	37	254.0	194.0	2.2
33	320.0	273.0	2.1	76	317.0	240.0	4.5
72	359.0	303.0	4.7	115	367.0	327.0	4.0
111	270.0	221.0	3.2	38	323.0	261.0	2.4
Calcium phosphate with Ca O.				77	353.0	261.0	4.5
34	200.0	136.0	2.8	116	286.0	237.0	3.7
73	385.0	319.0	3.8	39	314.0	249.0	2.5
112	309.0	259.0	3.4	78	310.0	258.0	4.9
				117	323.0	272.0	3.2



On the Effects of Soil Ignition upon the Availability of Phosphoric acid for Rice Culture in Paddy Fields.

BY

M. Nagaoka.

My earlier experiments carried out in conjunction with my colleagues, proved the fact that in the paddy field of our college the application of soluble phosphoric acid in the form of sodium phosphate and superphosphate often gave a rather inferior yield than precipitated calcium phosphate and further it was observed that the action of those soluble phosphates soon faded away, although the residual phosphoric acid, proved from the analytical data, to be present in such a quantity that it could produce still a full crop of rice. At first we supposed that this might be due to the transformation of the soluble phosphoric acid into iron and aluminium phosphates.

However, my later trials on the manurial action of the various phosphates, such as ferric, ferrous, aluminium phosphate & upon rice plants proved, contrary to my anticipation, that, in the first season, such insoluble phosphates displayed a most satisfactory good action upon the plants, the yield of the ferric phosphhate being even larger, and also these of ferrous and aluminium phosphate being less only 24 % and 27 % respectively than that of the double superphosphate, and, in the second year, their residual phosphoric acids gave still comparatively better results as also compared to that of the double superphosphate. These facts sufficiently show that our former assumption was not quite correct. There must exist another circumstance that renders the phosphoric acid insoluble and unavailable. I suspected that the rich humus content (11%) of our soil has something to do with that phenomenon.

As to the original phosphoric acid of our paddy soil, we have often found it to be as much as 0.49% in the dry samples, which quantity exceeds

that present generally in Japanese paddy fields, and would according to our calculations, be quite sufficient to produce a medium rice crop every year for more than two hundred years without any new supply of phosphoric acid. Nevertheless our practical investigations have always shown results entirely contradictory, since manuring with nitrogen and potash alone—leaving out a fresh supply of phosphoric acid to this soil, gave, as a rule, a very unsatisfactory crop—resembling that obtained on entirely unmanured soil.

The supposition above expressed led me to investigate the behavior of ignited paddy soil (1899). It was important to determine how much phosphoric acid might be rendered available by the process of ignition-or incineration of its humus content. I determined in both original and burnt soil the phosphoric acid soluble in water, in neutral ammonium citrate, in 5 % acetic acid, in 1 % citric acid, in 1 % oxalic acid and in saturated carbonic acid solution, and further the total phosphoric acid extracted by a hot hydrochloric acid of 1.15 sp. gr.

The sample of the soil, which served for these trials was taken from a part of our experimental paddy field. The well dried soil was sifted through a sieve with meshes of 1/2 millimeter. The burnt soil was prepared from a part of the same sample, by incineration for fifteen minutes to faint redness, by which process the loss of ignition was 21.123 % of which 10.92 % was that of the genuine humus.

In each trial, I took 50 grams of the original soil and 39.439 grams from the burnt soil, which quantities corresponded to 50 grams of the original sample.

The digestion of the weighed soils lasted for seventy two hours stirring from time to time and in the respective filtrate, the phosphoric acid was determined in the usual way.

The results will be seen in the following table:—

Kind of solvents.	Dissolved phosphoric acid in % of the dry soil.		The rate of increase.
	Original soil.	Burnt soil.	
Hydrochloric acid (sp. gr. 1.15).....	0.475	0.499	5.1
Distilled water	trace	trace	—
Ammonium citrate.....	0.046	0.076	63.9
Acetic acid (5%).....	0.145	0.167	15.2
Citric acid (1%).....	0.125	0.165	32.0
Oxalic acid (1%).....	0.132	0.145	9.9
Carbonic acid (saturated solution)	0.010	0.013	30.0

Thus it is seen that from the ignited soil, in all cases, *more phosphoric acid was dissolved by the same reagent*. It may be that the destruction of the organic matter in a soil rich in humus, by incineration would also with other soils increase the availability of the phosphoric acid present.

The fact that some phosphoric acid exists in soils under some organic forms, has already been noticed by several authors. *Eggerty* and *Nilson*¹ pointed out from their study on peat soil that some phosphoric acid exists in such soil in organic combination. Later *Schmoeger*² found that a part of the phosphoric acid in peaty soils is present in the form of nuclein.

*G. Nannes*³ observed with swampy soils that the ether alcohol extract contained but very little phosphoric acid, indicating the presence of an only insignificant amount of lecithin in the soil.

*C. Schreiber*⁴ inferred from his experiment on humus soils that the phosphoric acid combined with the humus moor soils, which is readily soluble in alkaline ammonium citrate, is almost useless for vegetation; he farther concluded that under certain circumstances, humus exerts an influence analogous to carbonate of lime on assimilable phosphoric acid In 1898,

¹ Biedermann's Central Blatt f. Agricul. Chemie XVIII 1889.

² Landw. Jahrb. Bd. XXV 1896 and Bd XXXVI 1897.

³ Journal Landw. Bd. 47, 1899.

⁴ Rev. Agron. Louvain. p.4 No 1 1895.

*K. Aso*¹ carried out several investigations in this line. After treating the soil by the same methods which were undertaken by *Schmoeger* he arrived essentially at the same results that were obtained by this author. Besides, he proved the presence of lecithin in our soil, and determined in the dry sample its quantity to 0.0493 %.

In short, his trials sufficiently proved the fact that the paddy soil of our college, contains much phosphoric acid in the form of the organic combinations especially in form of nuclein. Although exhaustive determinations in this respect have not yet been carried out yet we have some good reasons to infer from our numerous trials that the phosphoric acid in our cultivated paddy field assumes the following three principal forms.

1. As mineral phosphates, a very small part of which is soluble in water.

2. As combinations with humus, which are produced chiefly from phosphoric acid of the manures.

3. As well defined organic compounds, such as nuclein, and lecithin which are mostly accumulated in soil from the residues of the former crops, also from the vegetable manures applied, and also perhaps formed by bacterial growth.

It was therefore important to determine how much phosphoric acid may become available by the destruction of the organic compounds.

1. Series of experiments.

This series of the experiments was performed with 15 porcelain pots of 25 centimetres in diameter. Before the soil was put in, each pot received about 4 kilograms of small pebbles so as to make the weight of all the pots equal, just 11 Kilograms each.

The soil used in these experiments, was taken from one of our experimental fields, which has been cultivated without manures for two years. The sufficiently dried soil was sifted through a sieve of small meshes and then ignited in a kiln of our gas factory. An iron pan containing the soil was put upon red hot coles for 15 minutes and left to cool for two days.

¹ The paper of the author follows this.

On June 21, 1900, all the pots received the soils in the following proportions :

No. of pots.	Kind and quantity of the soils applied per pot.	General manures per ha. Kg.
1, 18, 35.	8 Kg. original soil.	O
2, 19, 36.	„ „ „ „	150 Kg. of N. and 100 Kg. K ₂ O
3, 20, 37.	8 Kg. burnt soil	„ „ „ „ „ „ „ „
4, 21, 38.	4 „ „ „ and 4 Kg. original soil.	„ „ „ „ „ „ „ „
5, 22, 39.	2 „ „ „ „ 6 „ „ „	„ „ „ „ „ „ „ „

On the 25th, after a sufficient application of distilled water so as to give the soils a state of mud, the general manures in the form of sulphates, were added and the soils then agitated thoroughly.

On the 30th, the young rice plants (Variety Satsuma) were transplanted in such a manner that each pot received 3 bundles, each consisting of three healthy individuals of equal size. After the transplantation, the same level of water was procured for all pots, a supply of some distilled water from time to time being procured, but after the blossom of the plants the application of water was diminished, so that the soils maintained only sufficient humidity for the ripening of the seeds.

On the 15th of November the rice plants were harvested when they had attained the state of milk ripeness, and left to become air-dry.

The yields per pot and also the averages of the three parallel pots were as follows :—

No. of pots.	Kind of soils.	No. of panicles.	Straw. gr.	Grains. gr.	Average per pot.		
					Straw. gr.	Grains. gr.	Total crop. gr.
1	Original soil with no manures.	12	17.0	10.5	16.17	10.63	26.80
18		14	16.2	11.7			
35		15	15.3	9.7			
2	Original soil with manures.	36	38.2	23.7	39.07	21.70	60.77
19		29	37.1	19.9			
36		30	38.9	21.5			
3	8 Kg. burnt soil with manures.	13	11.3	3.0	9.27	2.13	11.40
20		12	6.2	1.2			
37		12	10.3	2.2			
4	4 Kg. burnt soil with manures.	33	45.8	26.7	41.30	23.47	64.77
21		34	42.3	24.7			
38		25	35.8	19.0			
5	2 Kg. burnt soil with manures.	54	68.3	52.9	63.63	48.20	111.83
22		45	64.8	49.2			
39		39	57.8	42.5			

The moderate doses of the burnt soil had therefore a considerable action upon the yield of the rice plants. As all the pots, excepting those of without manures, had received nitrogen and potash in sufficient quantities and as all were treated in a similar manner, the above differences in the yield, must be due, according to the law of minimum, to the influence of the phosphoric acid in the soils.

The effect of the phosphoric acid in the ignited soils will be noticed more conveniently in the following calculation, in which we assume the plus yield of the original soil with manures over the yield of that not manured, as 100.

Kind of soils.	Plus yields.		Ratio of increase.	
	Total crop. gr.	Grains. gr.	Total crop.	Grains.
Original soil with manures.	(+) 33.97	(+) 11.07	100	100
8 Kg burnt soil,	(-) 15.40	(-) 8.50	(-) 45.3	(-) 76.8
4 " " "	(+) 37.97	(+) 12.84	(+) 111.8	(+) 116.0
2 " " "	(+) 85.03	(+) 37.57	(+) 250.3	(+) 339.4

It will be noticed from these figures, that the smallest dose of the ignited soil gave more than the double plus yield over that of the original soil even with the same manures. By increasing the amount of burnt soil to 4 Kilograms per pot the yield decreased, while this decrease was still greater when burnt soil alone served for the crop.

I shall further consider here how much phosphoric acid was extracted by the crop from each pot; the results of the chemical analysis of the crops gave the following figures.

Kind of soils.	Phosphoric acid in the total crop. gr.	Surplus phosphoric acid. gr.	The surplus of the original soil with manures = 100.
Original soil without manure.	0.0682	—	—
" " with manure.	0.1053	(+) 0.0371	100
8 Kg. burnt soil,	0.0341	(-) 0.0341	(-) 91.9
4 " " "	0.1184	(+) 0.0502	(+) 135.3
2 " " "	0.1930	(+) 0.1258	(+) 339.1

Thus it will be attested that the numbers of the last column almost agree with the increase in grains, and this also proves that from the moderate quantity of the burnt soil admixed to the original soil more phosphoric acid is rendered available to the rice plants, and in consequence of which the increase of grams attains almost the same proportion of the phosphoric acid absorbed. But when the quantity of the ignited soil increased to a certain extent over that of the original soil, the availability of the phosphoric was much decreased, beside, with the ignited soil only the phosphoric acid absorption and the yield were both uncomparably

diminished. Hence we may suggest that the economy of the phosphoric acid in burnt soils requires some precautions with special regard to the proportion of a burnt soil to the unburnt, the character of which and contents requiring careful consideration.

It is highly probable that the acid humus contained in the original soil played an important rôle, inasmuch as it acted as a solvent of the phosphoric acid of the burnt soil; this point of view will be further explained later on.

This series of the experiments was carried on for the second year in the hope of obtaining some further informations on the ignited soil. All pots, excepting these without manures, received, on June 21 (1901) the same quantity and the same form of nitrogen and potash as the preceding year. On July 1., the young rice plants were also planted just in the same manner as before. The crops were harvested on the 26th of November with the following figures of the air dry matter. I added in the last column the numbers, resulting from chemical analysis, of the phosphoric acid contained in the total crop per pot.

No. of pots.	Kind of soil.	Straw. gr.	Grains. gr.	Average per pot.			Phosphoric acid in the total crop per pot. gr.
				Straw. gr.	Grains. gr.	Total crop. gr.	
1	Original soil without manure.	15.0	6.0				
18	" " " "	16.0	7.2	15.67	7.10	22.77	0.02782
35	" " " "	16.0	8.1				
2	Original soil with manure.	15.0	9.8				
19	" " " "	14.0	6.3	16.33	8.17	24.50	0.02837
36	" " " "	20.0	8.4				
3	8 Kg. burnt soil with manure.	3.1	0.1				
20	" " " " " "	3.5	0.1	4.03	0.27	4.3	0.00272
37	" " " " " "	5.6	0.6				
4	4 Kg. burnt soil with manure.	31.1	21.6				
21	" " " " " "	48.6	38.1	41.27	31.13	72.40	0.0816
38	" " " " " "	44.1	33.7				
5	2 Kg. burnt soil with manure.	24.0	16.7				
22	" " " " " "	33.5	16.7	30.17	15.97	46.14	0.07096
39	" " " " " "	33.0	14.5				

It is seen, in general, from the above results that the yield, excepting but one (4 kilograms of the burnt soil) and the phosphoric acid consumption more or less actually diminished as compared to the former year's, and this proves the fact that the stock of the phosphoric acid in all pots was already in the first year exhausted to a great extent, and for the coming year but little of the nutriment was left in an assimilable form. Yet when a close comparison is made it is well proved that the ignition of the soil had still a considerable influence upon the rice crop even in the second year, for, the yield of the four kilograms of the burnt soils attained almost to three fold and that of the two kilograms, two fold of the yield of the original soil with sufficient general manures.

As to the pots which were supplied with 4 kilograms of the burnt soil, the phosphoric acid extraction by the plants was not so great as in the first year, but we see, on the contrary, the actual yield was augmented as much as 12% more than that of the first season. This proves the fact that with the proportion of 4 kilograms of the burnt soil to the same quantity of the original one, the phosphoric acid contained in the former soil is rather slowly and less absorbed in the second year but this nutriment is utilized by the rice plants for organic matter production in a most satisfactory manner.

The pots, which received the burnt soil only, exhibited again a most miserable condition and gave quite an insignificant yield in the second year. This is due most probably to the insufficiency of some acid humus and further, to the poor *mechanical condition* caused by the ignition. From these trials with ignited soil we may now safely conclude that, in some soils rich in humus, there exists certainly more or less phosphoric acid in form of organic compounds which can be utilized in practice by ignition to a certain extent.

In 1901, G. Daikuhara¹ and T. Hanai have published some very

¹ Report of the Agricultural Experiment Station, Tokio, Vol. 20.

interesting and useful articles upon the so called "Burnt soil manure."²

The authors have carried out separately some experiment on the said manures which were specially prepared by themselves and they observed that in such gently roasted manures, the solubility of the phosphoric acid in several weak acids had considerably increased and the proportion of this increase was, in average, calculated as much as 37% over the soluble phosphoric acid of the original soils (that is the soils which served as the material of the smouldered manure). Besides, *Daikuhara* has even observed that soil poor in humus can be enriched with soluble phosphoric acid by gently roasting it. These authors have further undertaken several extensive comparative pot experiments with such manures upon rice as well as barley and obtained similar results which proves that the phosphoric acid in the burnt soil manures gave, in general, a much better harvest than that without this manure. *Daikuhara* concluded that the increase of the solubility of the phosphoric acid in a burnt soil manure is partly due to the destruction of some organic phosphatic matter and partly to the influence upon the mineral phosphatic constituents of the original soil.

When we take into consideration the results obtained by the above two authors as compared to those obtained by myself, the part is still further confirmed that the existence of the phosphoric acid in form of some organic combinations in soils rich in humus is possible and ignition or as well as gently roasting (smouldering) of these soils can secure some profit.

II Series.

Are caustic lime, calcium carbonate and potassium carbonate beneficial to the assimilation of the phosphoric acid in a burnt soil?

These series of the trials were performed in accordance with the preceding experiments with the view to determine whether caustic lime, calcium

² The burnt soil manures are, according to *G. Daikuhara*, generally prepared by a very slow smouldering or gently roasting of some soils such as muds from ditches, soils from mountains and plains arable soils from certain cultivated fields etc. and these manures are extensively and particularly used for the cultivation of various agricultural crops in the south western provinces of Japan.

carbonate and potassium carbonate have some useful action or not upon the efficacy of the phosphoric acid in a burnt soil.

The treatment of the rice plants was the same as usual, but all the pots received either caustic lime or calcium carbonate at the rate of 1000 kilograms of CaO per hectare. These lime compounds were applied in the first year only and they were mixed carefully into the weighed soils before the latters were filled into the pots. As to the pots of the potassium carbonate, we had not enough burnt soil to carry out the whole series, so I tried but for 4 kilograms of the ignited soil, the quantity of which being the medium ration of this series.

These pots received, in the first year, besides the general manure 100 kilograms of K_2O per hectare in the form of potassium carbonate, and in the second year, the same rate of the carbonate, but not the potassium sulphate as general manure.

The crops for both, first and second year were likewise harvested the same days as that the first series. To avoid too much complication of the numbers, I shall simply give the averages of the parallel trials in the following table in which the actual quantities of the phosphoric acid absorbed by the rice plants from the soil per pot are also given.

Kind of soils,	First year.				Second year.			
	Average per pot.			Phosphoric acid in the total crop. gr.	Average per pot.			Phosphoric acid in the total crop. gr.
	Straw. gr.	Grains. gr.	Total crop.		Straw. gr.	Grains. gr.	Total crop.	
With caustic lime.								
Original soil with manure.	41.00	19.37	60.37	0.1026	15.10	7.60	22.70	0.01341
8 Kg. burnt soil.	8.07	1.53	9.60	0.0074	4.93	0.20	5.13	not determined
4 " " "	38.00	21.60	59.60	0.0765	41.00	26.40	67.40	0.0725
2 " " "	47.30	34.45	81.75	0.1227	23.00	12.00	35.00	0.0576
With calcium carbonate.								
Original soil with manure.	41.40	17.70	59.10	0.1015	10.50	5.15	15.65	0.01538
8 Kg. burnt soil.	8.40	2.20	10.60	0.0108	2.80	0	2.80	not determined
4 " " "	39.80	20.20	60.00	0.0831	47.50	28.95	76.45	0.1047
" " " "	52.20	36.05	88.25	0.1364	30.25	17.15	47.40	0.04828
With potassium carbonate.								
4 Kg. burnt soil.	22.25	15.60	47.85	0.0516	30.50	25.4	55.90	0.05919

From these figures the part can be learned that, in spite of the presence of the alkalis, the proportions of the increase or decrease between the yields of the burnt and not burnt soils attained almost the same degree as in the first series, which fact proves also that the best action of the phosphoric acid in the soil is obtained by mixing burnt and unburnt soil in the proportion of 1 : 3.

But when these figures are compared to those obtained in the two years of the first series, we have again a most obvious evidence that by the application of some alkaline compounds, the yield in all soils, whether burnt or unburnt, was more or less diminished and the phosphoric acid consumption by the plants was also equally affected.

In order to show this point still more clearly, I calculated in the following table the actual diminutions of yields as well as the diminutions of the phosphoric acid consumption in consequence of the alkali application. Furthermore with the aid of these numbers the percentage diminution in

each pot was calculated when the total crops of the pots not supplied with any alkali were, for the first and second year, assumed respectively to be 100.

Kind of soils.	First year.				Second year.			
	Actual diminution per pot.		Percentage diminution.		Actual diminution per pot.		Percentage diminution.	
	Yield. gr.	O ₂ P ₂ O ₅ . gr.	Yield.	P ₂ O ₅ .	Yield. gr.	P ₂ O ₅ . gr.	Yield.	P ₂ O ₅ .
With caustic lime.								
Original soil.	0.40	0.0027	0.7	2.6	1.80	0.01496	7.3	52.7
8 Kg. burnt soil.	1.80	0.0267	15.8	78.3	(+) 0.87	—	(+) 20.2	—
4 " " "	5.17	0.0419	8.0	35.4	5.00	0.0091	6.9	11.1
2 " " "	30.08	0.0703	18.5	36.4	11.14	0.01336	24.2	18.8
With calcium carbonate.								
Original soil.	1.67	0.0038	2.8	3.6	8.85	0.01298	36.1	45.8
8 Kg. burnt soil.	0.80	0.0233	7.0	68.3	1.15	—	34.8	—
4 " " "	4.77	0.0353	7.4	29.8	(+) 4.05	(+) 0.0231	(+) 1.6	(+) 28.3
2 " " "	23.58	0.0566	21.1	29.3	(+) 1.26	0.02268	(+) 2.7	31.8
With potassium carbonate.								
4 Kg. burnt soil.	16.92	0.0568	26.1	47.1	16.50	0.02244	22.8	27.5

As it is seen in the above figures, the total yields and phosphoric acid consumption in the first year were both affected by the alkali application to a certain extent and the percentage diminution of the phosphoric acid consumption was far more greater than that of the total yields. In the second year this decrease was still observed in some pots, while in others that had received calcium carbonate, there was a slight surplus over the yield of the control (not limed pot), but this surplus was not sufficient to compensate the loss in the preceding year.

In general, some unfavourable action of the alkali still prevailed in the second year in the majority of the pots. As to the difference of the effects of the caustic lime and calcium carbonate, it is seen the former acted more

powerfully than the latter in both years, while the potassium still more so.

It is evidently the neutralization of the acid humus in our paddy soil as well as of the acid juice of the plant roots which diminishes the availability of the phosphoric acid. Since ignited soils have, in general, a rather strong alkaline character, the poor results obtained on burnt soil alone without an addition of unburnt, are also explained. Hence it may be recommended to add to burnt soil a sufficient amount of humus or organic matter yielding humus. Compounds of an alkaline nature are injurious to the rice grown in paddy soils.



I. YEAR
Without Lining



Original Soil (No Manure & No Line), (With Manure) 8 Kg. Burnt Soil, 4 Kg. Burnt Soil, 2 Kg. Burnt Soil

With Caustic Lime.



Original Soil (No Manure & No Line), (With Manure) 8 Kg. Burnt Soil, 4 Kg. Burnt Soil, 2 Kg. Burnt Soil

With Calcium Carbonate.



Original Soil (No Manure & No Line), (With Manure) 8 Kg. Burnt Soil, 4 Kg. Burnt Soil, 2 Kg. Burnt Soil

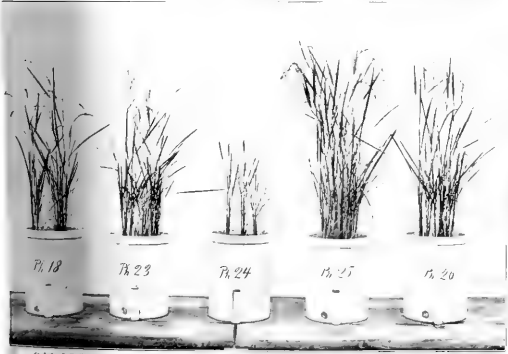


II. YEAR
Without Liming



Original Soil (No Manure) Original Soil (With Manure) S. K. (No Manure) 4 K. (With Manure) 2 K. (With Manure)

With Caustic Lime

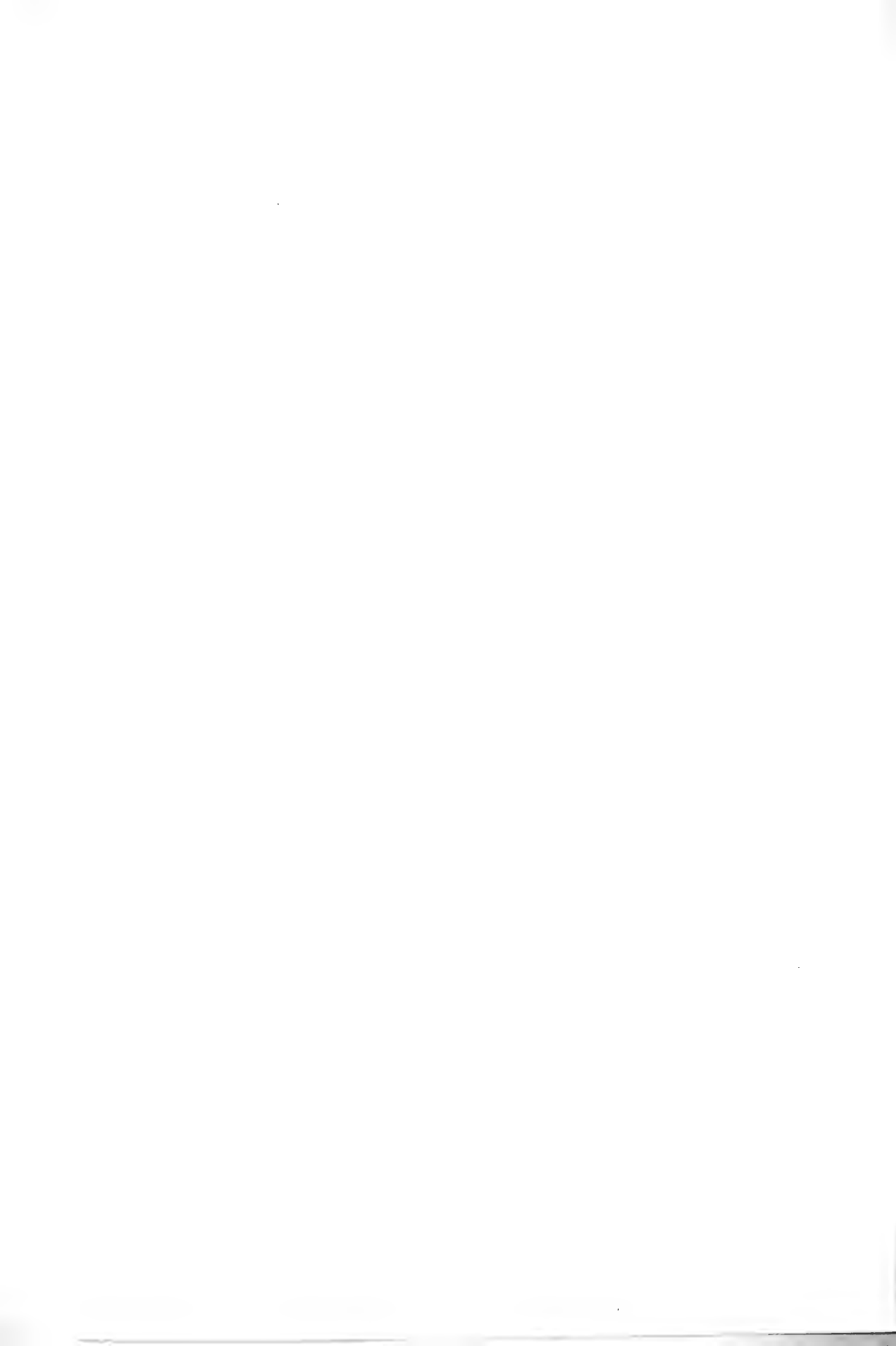


Original Soil (No Manure & No Lime) Original Soil (With Manure) S. K. (No Soil) 4 K. (Partial Soil) 2 K. (Full Soil)

With Calcium Carbonate



Original Soil (No Manure & No Lime) Original Soil (With Manure) S. K. (No Soil) 4 K. (Full Soil) 2 K. (Full Soil)



On Organic Compounds of Phosphoric Acid in the Soil.

BY

K. Aso.

It is a well known fact that peaty soils show much more phosphoric acid soluble in hydrochloric acid after they are ignited than before ignition. *Eggertz*¹ showed that 'Mullkörper' contained 0.15—7.58 % phosphorus and 0.55—2.09 % sulphur. The same author and *Nilson*² pointed out that, in the hydrochloric acid extract of the ignited muck soils, a larger quantity of sulphuric and phosphoric acid is found than in the extract of the original soil by hydrochloric acid, hence it is inferred that phosphorus and sulphur are present in humus in form of an organic combination. Recently, *Schmöger*³ made similar observations and concluded that a certain amount of phosphoric acid is present in such soils in the form of nuclein.

The fact that the soil of our college farm in Komaba contains nearly 11 % of humus of a distinctly acid reaction and that hot concentrated hydrochloric acid does extract about double the amount of sulphuric and phosphoric acid than cold concentrated hydrochloric acid, as *O. Kellner* has observed years ago, induced me to search in what form a part of the phosphoric acid would be present.

The soil serving for my investigation came from the uncultivated part of our fields and gave 27.16 % loss on ignition. It contained 10.9 % humus and 0.65 % total nitrogen in the dry matter. The soil was collected from several holes of a suitable depth after removing the surface layer, spread on mats and dried in the sun. The air-dry soil was then sifted

¹ Biedermann's Centr. Bl. f. Agric. Chem. XVIII, 1889.

² Ibid.

³ Landw. Jahrb. XXV, 1896, and XXXVI, 1897.

through a sieve of 1 mm. meshes. The samples serving for my investigations were :

- I. Raw soil.
- II. Ignited soil.
- III. Soil steamed for three hours under pressure in Koch's steam apparatus commonly used for sterilization.
- VI. Soil steamed under the pressure of three atmospheres in an autoclave for three hours.

1. The raw soil.

a). 50 grams of airdry soil were treated with 100 c.c. of cold hydrochloric acid of sp. gr. 1.15 at 15°C and digested for twenty four hours at the ordinary temperature shaking it from time to time, and then adding 100 c.c. water. 100 c.c. of the filtrate were evaporated to dryness on the water bath. After separating the silicic acid in the usual way with strong hydrochloric acid, the acid solution was filtered and filled in a measure flask of 300 c.c. and 50 or 100 c.c. of this solution were taken for determination of sulphuric or phosphoric acid. Sulphuric acid was determined in the usual way with barium chlorid, and phosphoric acid was determined by the molybdic method, replacing first hydrochloric acid by nitric acid.

b). The quantity of the sample taken and the treatment with hydrochloric acid were quite the same as with a), but after filtering, the residue was washed thoroughly on a filter with water until a few drops of the filtrate did no longer show any reaction with silver nitrate. After evaporating the filtrate to dryness on the water bath, the after treatment was carried on in the manner described above in a).

The result is seen from the following table, where the amount of dry matter corresponding to the solution used is stated :¹

¹ Of course, hygroscopic water of the soil was taken into account for the calculation of volume taken in the case of a).

PHOSPHORIC ACID.

	Dry matter used. gram.	Mg ₂ P ₂ O ₇ found. gram.	P ₂ O ₅ found. gram.	P ₂ O ₅ %	In average. %
a.	6.4691	0.0147	0.0094	0.145	0.1445
b.	13.5204	0.0305	0.0195	0.144	

SULPHURIC ACID.

	Dry matter used. gram.	Ba S O ₄ found. gram.	S O ₃ found. gram.	S O ₃ %	In average. %
a.	6.4691	0.0372	0.0128	0.198	0.200
b.	6.4691	0.0378	0.0130	0.201	

II. The ignited soil.

50 grams of air-dry soil were dried and ignited in a platinum dish, all precaution being taken to avoid loss, then the heat was raised gradually to a faint redness, stirring the soil from time to time with a platinum wire, until all organic matter was destroyed.

The product thus obtained, possessed a reddish colour somewhat similar to that of oxide of iron. After keeping for a few days in the room, this soil was treated with 100 c.c. of cold hydrochloric acid and digested for twenty four hours at the ordinary temperature etc, as just described in the case of I. b. Here, the treatment was quite the same in both cases, viz., a) and b).

The following table shows the analytical data, where the quantity of dry matter stated corresponds to the solution used :

PHOSPHORIC ACID.

	Dry matter used, gram.	Mg ₂ P ₂ O ₇ found, gram.	P ₂ O ₅ found, gram.	P ₂ O ₅ %	In average, %
a.	13.5204	0.0692	0.0441	0.326	0.327
b.	6.7602	0.0348	0.0222	0.328	

SULPHURIC ACID.

	Dry matter used, gram.	Ba S O ₄ found, gram.	S O ₃ found, gram.	S O ₃ %	In average, %
a.	13.5204	0.1278	0.0439	0.325	0.325
b.	13.5204	0.1280	0.0440	0.325	

III. The soil steamed in Koch's steam apparatus.

50 grams of air-dry soil were thoroughly mixed with 100 c.c. of water in a beaker and steamed in Koch's steam apparatus for three hours, thereupon the steamed soil was removed to a porcelain dish, and dried on the water bath. This sample after being exposed to the air for a few days was treated as those before, with the following result:

PHOSPHORIC ACID.

	Dry matter used, gram.	Mg ₂ P ₂ O ₇ found, gram.	P ₂ O ₅ found, gram.	P ₂ O ₅ %	In average, %
a.	13.5204	0.0316	0.0201	0.148	0.147
b.	13.5204	0.0310	0.0198	0.146	

SULPHURIC ACID.

	Dry matter used, gram.	Ba S O ₄ found, gram.	S O ₃ found, gram.	S O ₃ %	In average, %
a.	13.5204	0.0888	0.0305	0.226	0.2255
b.	13.5204	0.0886	0.0304	0.225	

IV. The soil steamed in an autoclave.

50 grams of airdry soil were put in a tall beaker, mixed with 100 cc. of water and placed in an autoclave containing some water, then heated until the pressure increased to three atmospheres and at that pressure the steaming was continued for three hours. The steamed soil was transferred into a porcelain basin, dried, and also left like the other samples exposed to the air for a few days. Phosphoric and sulphuric acid were determined by extraction with cold hydrochloric acid of sp. gr. 1.15 at 15°C as described before. The result obtained here was as follows:

PHOSPHORIC ACID.

	Dry matter used, gram.	Mg ₂ P ₂ O ₇ found, gram.	P ₂ O ₅ found, gram.	P ₂ O ₅ %	In average, %
a.	13.5204	0.0748	0.0477	0.353	0.351
b.	13.5204	0.0370	0.0236	0.349	

SULPHURIC ACID.

	Dry matter used, gram.	Ba S O ₄ found, gram.	S O ₃ found, gram.	S O ₃ %	In average, %
a.	6.7602	0.0480	0.0165	0.244	0.245
b.	6.7602	0.0485	0.0166	0.246	

The results here obtained are seen at a glance in the following table :

Treatment.	The raw soil.	The ignited soil.	The soil steamed in Koch's steam apparatus.	The soil steamed in an autoclave.
Percentage of Phosphoric acid.	0.145	0.327	0.147	0.351
Percentage of Sulphuric acid.	0.200	0.325	0.226	0.245

The amounts of phosphoric acid and of sulphuric acid obtained by extraction of the *original* soil with cold hydrochloric acid corresponds to those pre-existing as phosphates and sulphates respectively, while the differences between the percentages of those and of the phosphoric and sulphuric acid found in the *ignited* soil, might be derived from certain organic compounds. The soil steamed in Koch's apparatus did not liberate any considerable quantity of sulphates and phosphates, but the autoclave treatment had a better effect. These results are in favor of *Schmöger's* inference above mentioned. In the following table, a comparison of *Schmöger's* soil and ours is given :

	Raw Soil.		Steamed Soil. (in an autoclave).	
	P ₂ O ₅	S O ₃	P ₂ O ₅	S O ₃
Sedliner ¹ moor.	0.123	0.134	0.246	0.244
Soil from Komaba.	0.145	0.200	0.351	0.245
	In cold hydrochloric extract.		In hot hydrochloric extract.	
Soil from Komaba. ²	0.19	0.11	0.34	0.20

¹ Landw. Jahrb. Bd. XXV, 1896. This soil was analysed by Schmöger.

² Landw. Versuchst. Bd. XXX. This soil was analysed by Kellner. On boiling with hydrochloric acid, nuclein in the soil might be decomposed.

*Schmöger*¹ inferred that lecithin was not present in his soils. I made some experiments about this point with the soil of our field. Several hundred grams of the soil, after well-drying, were extracted first with ether and then with alcohol and the two liquids were united and evaporated on the water bath. A part of the evaporation-residue was heated with a mixture of sodium carbonate and some potassium nitrate, and the ash dissolved in nitric acid and tested for the presence of phosphoric acid therein with ammonium molybdate and the characteristic yellow colour was produced.² The presence of lecithin in the soil became thus very probable, hence I made a quantitative determination according to the method recommended by *E. Schulze*.³ The result is shown in the following :

Dry matter used, gram.	Ethereal and alcoholic extract, gram.	Mg ₂ P ₂ O ₇ found, gram.	P ₂ O ₅ found, gram.	Lecithin found, gram.	P ₂ O ₅ %
40.561	0.0616	0.0025	0.0016	0.0182	0.004
40.561	0.0535	0.0030	0.0019	0.0218	0.005
In average	0.05755	0.00275	0.00175	0.0200	0.0045

In 100 parts of dry soil, 0.0493 parts of lecithin were found, so that 100 parts of humus contained 0.452 parts of it. The ethereal extract of this soil was dark greenish brown. The coloring matter might be due to a partial decomposition product of the chlorophyll. The quantity of lecithin is but small so that it can not be taken into serious consideration.

To see how much phosphoric acid may be rendered available by destroying organic matters in this soil, I determined the phosphoric acid soluble in 1% citric acid⁴ in the following way :

¹ Landw. Jahrb. Bd. XXV. 1896.

² Besides, I made a qualitative test by decomposing the lecithin with baryta and preparing the double chloride of cholin and platinum.

³ Chemiker Zeitg, 1897.

⁴ Dyer : Jour. Chem. Soc. Vol. LXV. 1894.

a) 81.855 grams of dried soil were digested with 200 c.c. of 1% citric acid for forty eight hours at the ordinary temperature, shaking it from time to time. The filtrate and wash water was evaporated, and after separating silica, phosphoric acid was determined by the molybdic method in the usual way.

b) 81.855 grams of dried soil were ignited in a platinum dish and the extraction with 1% citric acid and after-treatment was quite the same as in the case of a). The result will be seen in the following :

	Dry matter used. gram.	Mg ₂ P ₂ O ₇ found. gram.	P ₂ O ₅ found. gram.	P ₂ O ₅ %
Ignited soil.	40.927	0.0071	0.0045	0.011
Raw soil.	40.927	0.0035	0.0022	0.005

It is clearly shown that the quantity of phosphoric acid soluble in 1% citric acid in the soil can be increased considerably by destroying organic matters.

Summary.

1. Phosphoric acid is present in humus soil in organic and anorganic forms.

2. The chief organic phosphoric compound is nuclein. Besides, a very small quantity of lecithin is present. Both compounds can be partially due to the bacterial flora of the soil, partially to the decaying plant roots.

3. The phosphoric acid in the organic compounds become available by burning the humus soil.

I determined also phosphoric acid contained in *Matiere noire* obtained from this soil : 0.249% P₂ O₅ was contained in *Matiere noire*, in the dry soil. Of course, this includes some P₂ O₅ of aluminium phosphate, because aluminium phosphate is somewhat soluble in ammonia. P₂ O₅ from raw soil (0.145%) + P₂ O₅ from *matiere noire* (0.249%) = 0.394%, while P₂ O₅ from the soil steamed under 3 atm. = 0.351%.

On the Behavior of the Rice Plant to Nitrates and Ammonium Salts.

BY

M. Nagaoka.

The question of the protein formation in plants has been the subject of numerous investigations during the last fifty years in Europe by various authors, as *Hartig*, *Borodin*, *Pfeffer*, *Kellner*, and especially *E. Schulze* and his students, and in our college *O. Loew*, *Y. Kinoshita* and *U. Suzuki* made also various observations in this line.

It was further known long since that the nitrogen of nitrates as well as of ammoniacal compounds can be utilized for the formation of asparagin and protein.

In the practical and economical point of view, the manurial effect of the nitrogen in both, nitrates and ammonium compounds, has also been compared by many agricultural chemists with different crops and also in different soils, but the results did not always agree.

In certain respects, even contradictory statements have been published by different observers, due to the different conditions of soil. *Boussingault* was the first to recognize the high manurial value of chili saltpetre. Since then *Barley*, *J. Bennet*, *Loewel*, *Kuhlmann*, *P. Wagner*, *Macreker* and others have carried out extensive experiments in regard to the efficacy of these salts in comparison with other nitrogenous manures and the valuable statements of these investigators have rendered the Chili-saltpetre very popular as a manure.

In 1896, however, *Pagnaul*¹ published the results of his experiments on the assimilability of nitric and ammoniacal nitrogen with several agricultural

¹ Ann. Agronomique 22 (1896).

plants and inferred that the ammonium sulphate was decidedly superior to the nitrates in all his experimental crops. *E. Klopfer*¹ observed also from his experiments that in presence of other necessary untriments the ammonium sulphate is more beneficial than the sodium nitrate and he expressed also his conviction that sulphate of ammonia is more economical than nitrate of soda, especially for application to cereals in the spring; but soon afterwards *P. Wagner*² inferred from his own experiments that *Klopfer's* observations were erroneous.

*H. F. Adams*³ has also made some observations regarding the relative assimilability of the various forms of nitrogen upon an acid soil limed and unlimed arrived at the following conclusions:

1. On a very acid soil, ammonium sulphate has worked like a poison instead of an effective fertilizer.
2. Where air slaked lime was applied with ammonium sulphate the nitrogen proved nearly as valuable as similar quantities in form of sodium nitrate.

In 1901, *H. Bachmann*⁴ compared the effect of sodium nitrate to ammonium sulphate upon potatoes, fodder beets, and kohlrabi; in which experiments the sulphate proved apparently more effective than the nitrate. The experiments, which were performed on barley, at the Japanese agriculture experiment stations proved the value of nitric and ammoniacal nitrogen to be varying according to the nature of the soils experimented on, while in the results obtained by *S. Machida*⁵ on the manuring experiments of *Polygonum tinctorium*, the value of both, nitric and ammoniacal nitrogen, was estimated as to be almost the same.

Although the relative effects of nitric and ammoniacal nitrogen have so often been made an object of practical investigations with different crops

¹ Jahresbericht f. Agri. Chemie 1899 s.s. 107-109 and Deutsch. Landw. Presse Bd. 25 (1898) No. 25 p.p. 271.

² Deutsch. Landw. Presse Bd. 25 (1898) No. 30 p.p. 327.

³ Rhode Island Station report 1897 p.p. 241.

⁴ Frühling's Landw. Ztg. 50 (1901) No 11 p.p. 386-387.

⁵ Report of the Imperial Japanese Experiment Station No 21.

and on different dry fields, yet their values are not yet certainly fixed for all kinds of soils, whilst, as to the nutritive effect of these two forms of nitrogen towards aqueous plants, especially to paddy rice, practical experiments, as far as I know, are entirely wanting.

However, as a whole, in all irrigated soils the so-called process of nitrification does not generally take place and ammonia is, in such soils, always the result of the putrefaction of some nitrogenous organic matters, hence rice plants growing in such conditions of soils might generally be supposed to be provided with ammoniacal nitrogen alone. Hence the investigation on the behavior to nitric nitrogen might seem to have no special importance. However when the richness, in nitric nitrogen, of some manures which are often used in the cultivation of rice plants are considered and also the occurrence of a slight nitrification in the common paddy fields during their exposure to the air in the late spring and in a nearly dry state, the question will certainly have some interest from the scientific as well as the practical point of view, especially for the cultivation of upland rice plants, which is generally cultivated in dry fields in which nitrification always largely takes place.

I have carried out several experiments, to study whether nitric nitrogen can be utilized as well as ammonium nitrogen by rice plants under different conditions.

Before entering upon the details. I must express my earnest thanks to the professors Loew, Kozai and Toyonaga, my colleagues in the college, who kindly overlooked and revised this and also my former papers; I must also pay the same thanks to J. Sudsuki and T. Koyasu, the assistants in our laboratory, who rendered me valuable assistance in my work.

I. Experiments on the effects of different nitrates as compared to ammonium sulphate, upon rice plants.

These experiments were carried out, in 1899, with 92 porcelain pots each measuring 25 centimetres in diameter and performed in the new glass house of our college. The principal questions, I had in mind, were:

1. How behave different nitrates and ammonium sulphate upon rice plants?

2. At what period of the plants growth, the nitrogen is most assimilated.
3. What doses of the nitric and ammoniacal nitrogen are profitable to the plants.

The soil was taken from one of our experimental fields which had been cultivated without manures for 5 years. After the field was well ploughed, it was irrigated and stirred to a condition of mud, and in order to get it free from all rootlets of the preceding crops, the mud was carefully sifted. It was then exposed to the air for three days.

Before the application of the thus prepared soil, all pots received about 4 kilograms of pebbles adjusting each pot to 10 kilograms in weight whereupon each received just 11 kilograms of the soil; the total weight of each pot then being 21 kilograms.

The nitrogen manures were the pure nitrate of sodium, potassium, calcium, barium, strontium and magnesium, besides the ammonium sulphate.

It was further proved by careful tests that all the nitrates were free from nitrite and perchlorate and the ammonium sulphate from sulphite and cyanide.

On July, 14 (1899) as the general manure, the phosphoric acid in the form of sodium phosphate and the potash as potassium carbonate were applied at the rate of 100 kilograms respectively per hectare. The various forms of the nitrogen were added on the 15th; the quantities of the nitrogen applied per hectare and per pot, are given in the following table:—

No of pots.	Kind of the nitrogenous manures.	Nitrogen applied per ha.	Nitrogen applied per pot.
		Kg.	Gr.
1, 32, — 2, 33, 63. 3, 34, 64.	Without nitrogen.	0 0 0	0 0 0
4, 35, 65. 5, 36, 66. 6, 37, 67. 7, 38, 68.	Ammonium sulphate.	25 50 50 50	0.1227 0.2454 0.2454 0.2454
8, 39, 69. 9, 40, 70. 10, 41, 71. 11, 42, 72.	Sodium nitrate.	25 50 50 50	0.1227 0.2454 0.2454 0.2454
12, 43, 73. 13, 44, 74. 14, 45, 75. 15, 46, 76.	Potassium nitrate.	25 50 50 50	0.1227 0.2454 0.2454 0.2454
16, 47, 77. 17, 48, 78. 18, 49, 79. 19, 50, 80.	Calcium nitrate.	25 50 50 50	0.1227 0.2454 0.2454 0.2454
20, 51, 81. 21, 52, 82. 22, 53, 83. 23, 54, 84.	Barium nitrate.	25 50 50 50	0.1227 0.2454 0.2454 0.2454
24, 55, 85. 25, 56, 86. 26, 57, 87. 27, 58, 88.	Strontium nitrate.	25 50 50 50	0.1227 0.2454 0.2454 0.2454
28, 59, 89. 29, 60, 90. 30, 61, 91. 31, 62, 92.	Magnesium nitrate.	25 50 50 50	0.1227 0.2454 0.2454 0.2454

On the 16th, after addition of 2000 c.c. of distilled water to each pot the specially nourished young rice plants (variety Satsuma) 65 days old were transplanted to all pots, each pot receiving 3 bundles of six healthy equally nourished individuals.

All pots were carefully weighed every day up to the middle of the blooming of the plants and the losses of weight owed to the water evaporation from the pots and to the perspiration of the plants were daily replaced by distilled water.

The chief difference observed was that the plants which received the ammonium sulphate exhibited a normal green colour and appeared quite healthy and further, they showed a vigorous tillering while the plants supplied with nitrates were yellowish and their tillering was very feeble but the seeds ripened about 10 days sooner than those of the former plants.

The pots of the small doses of nitrogen and the last series of the large doses of the respective experiments were left until the plants were in a state of full ripeness while the other two series of the large doses were harvested in two periods, the first cutting being taken on the 15th of August and the second on September 15th.

The following table contains the yields per pot, the averages of the three parallel trials and the contents of nitrogen given by the chemical analyses¹ of the crops.

¹ The determination of the nitrogen of these and the following experiments were performed after the common method of Kjeldahl, hence the results of the determinations do not contain the nitrogen of nitric acid in the harvested crops.

First crop.				Kind of nitrogenous manures.	Second crop.					
No of pots.	Yield per pot. gr.	Average gr.	N. in the total crop per pot. gr.		No of pots.	Yield per pot. gr.	Average gr.	N. in the total crop per pot. gr.		
1	6.12	6.14	0.093	Without nitrogen.	2	15.75	16.60	0.141		
32	6.16				33	16.04			63	18.01
5	10.25	10.01	0.159		Ammonium sulphate.	6			22.92	23.18
36	9.63			37		23.01	67	23.61		
66	10.16			9		6.36	Sodium nitrate.	10	16.74	
40	5.85	6.15	0.094	41	17.16					
70	6.25			71	16.65					
13	8.75	7.23	0.100	potassium nitrate.	14	18.19	17.17	0.146		
44	6.76				45	16.75			75	16.56
74	6.16				17	7.51			Calcium nitrate.	18
48	8.85	7.96	0.106	49	17.43					
78	7.52			79	18.11					
21	8.03	8.15	0.099	Barium nitrate.	22	18.06	18.06	0.154		
52	8.02				53	17.25			83	18.88
82	8.40				25	7.25			Strontium nitrate.	26
56	8.46	7.82	0.0997	57	18.44					
86	7.75			87	16.48					
29	8.31	7.01	0.102	Magnesium nitrate.	30	16.94	17.11	0.159		
60	6.77				61	17.51			91	16.88
90	5.95									
		7.39	0.1001	Average of the nitrates.			17.22	0.1475		

From the above two series of the results, it will be noticed that all nitrates had but little influence upon the organic matter production of the rice plants in both early periods of vegetation, while the ammonium sulphate caused a most prominent augmentation of the yield. Moreover the actual quantity of the absorbed nitrogen from the various nitrates was in all cases, equally trifling. There were no essential differences between the effects of the various nitrates.

When the actual surplus of the yield and also the surplus of the assimilated nitrogen of the ammonium sulphate over the pots not supplied with nitrogen, are assumed respectively, in both periods, to be 100, the ratio of the surplus yield and also the ratio of the surplus nitrogen assimilability of the nitrates, calculated from their average results, will be as follows:—

	Ratio of the surplus yield.		Ratio of the surplus nitrogen assimilability.	
	Ammonium sulphate.	Nitrates.	Ammonium sulphate.	Nitrates.
In the first period.	100	32.3	100	10.8
In the second period.	100	10.9	100	14.2

Thus it will be observed that the nitrogen of the nitrates was, in both periods, utilized by the plants in a considerable less degree than that of the ammonium sulphate and consequently the yields in all the pots supplied with the various nitrates did not reach the harvest yielded by ammonium sulphate.

If the average of the above two series of figures may represent the relative action between the sulphate and nitrates, the latter action will be, in the first period, 21.6 and in the second period 12.6 to the 100 of the former.

At last, I give, in the following two forms of table, the figures obtained from the harvests, which have been cut on November the 11th, from the pots supplied with two different doses of nitrogen that is to say 25 kilograms and 50 kilograms per hectare.

TABLE A.

Results obtained from the pots which were supplied with nitrogen at the rate of 25 Kg. per hectare.

No of. pots.	Kind of nitrogen manures.	Straw. gr.	Grains. gr.	Average per pot.			Nitrogen in the total crop per pot. gr.
				Straw. gr.	Grains. gr.	Total crop. gr.	
3	Without nitrogen.	16.53	16.33	15.80	17.02	32.82	0.2078
34		15.14	17.12				
64		15.73	17.61				
4	Ammonium sulphate.	20.13	19.86	21.39	19.74	41.13	0.3030
35		21.30	22.70				
65		22.74	16.67				
8	Sodium nitrate.	16.04	17.78	16.51	17.05	33.56	0.2082
39		16.62	16.78				
69		16.88	17.14				
12	Potassium nitrate.	16.91	16.44	16.29	16.41	32.70	0.2079
43		15.41	16.29				
73		16.51	16.50				
16	Calcium nitrate.	19.36	16.70	18.04	17.49	35.53	0.2089
47		16.78	17.51				
77		17.97	18.25				
20	Barium nitrate.	15.91	16.20	16.21	16.75	32.96	0.2086
51		15.92	16.93				
81		17.49	17.12				
24	Strontium nitrate.	15.89	16.65	16.11	15.92	32.03	0.2081
55		17.47	14.92				
85		14.98	16.20				
28	Magnesium nitrate.	17.24	17.30	16.77	16.19	32.66	0.2082
59		16.31	14.54				
89		15.86	16.72				

TABLE B.
Results obtained from the pots which were supplied with
nitrogen at the rate of 50 Kg. per hectare.

No of pots.	Kind of nitrogen manures.	Straw. gr.	Grains. gr.	Average per pot.			Nitrogen in the total crop per pot. gr.
				Straw. gr.	Grains. gr.	Total crop. gr.	
7	Ammonium sulphate.	22.41	22.21				0.3040
38		21.19	21.52	22.17	22.33	44.50	
68		22.91	23.27				
61	Sodium nitrate.	17.37	18.05				0.2081
42		15.60	16.62	16.57	17.11	33.43	
72		15.98	16.65				
15	Potassium nitrate.	18.63	16.75				0.2086
46		17.10	17.51	17.41	17.23	34.64	
76		16.49	17.43				
19	Calcium nitrate.	16.01	17.39				0.2085
50		16.63	17.41	16.75	17.71	34.46	
80		17.62	18.32				
23	Barium nitrate.	16.26	17.45				0.2084
54		16.02	17.10	16.70	16.70	33.47	
84		17.83	15.76				
27	Strontium nitrate.	16.40	18.27				0.2085
58		18.04	17.62	16.74	17.76	34.50	
88		15.77	17.39				
31	Magnesium nitrate.	16.50	17.21				0.2086
62		17.69	18.19	16.79	17.53	34.32	
92		16.18	17.18				

From the figures of the above two tables, we may learn that the small doses of the various nitrates (25 Kg. N. per hectare) had almost no influence

upon the rice production whilst that of the ammonium sulphate was more favorable.

On the other hand, from the larger doses (50 Kg. nitrogen per hectare) of the various nitrates a little more nitrogen was absorbed by the plants than in the cases of the smaller doses leading to an insignificant augmentation when compared to the yield of the pots not manured with any nitrogen; but as these increases varie within a very narrow limit, it is not worth attaching a practical importance. The larger dose of the ammonium sulphate had again a better action than that of the nitrates, yet when its increase in yield is compared to that of the smaller dose, its effect was not large, so that we can infer that the small dose of the sulphate was sufficient for the plants.

In 1896, *W. Schneiderwind*¹ has drawn the following conclusions from his experiments on the action of various nitrates upon the produce of oats:

1. Die aus den verschiedenen Nitraten aufgenommenen Stickstoffmengen waren vollkommen gleich.
2. Die Düngung mit Kaliumnitrat hat die grösten Strohmenngen erzeugt, im Körnerertrag jedoch das geringste Gewicht.
3. Die Düngung mit Magnesium nitrat hatte die grösste Körnermenge erzeugt.

With regard to the above statements, my present results agree only with his first conclusion and there were not, in fact, a difference noticeable between potassium nitrate and magnesium nitrate.

However, in the preceding tables there will be seen a peculiar phenomenon in regard to the proportion of the straw and grain, which have been produced in nearly equal quantities (the quotient of yield being about 100); in some cases the weight of grains even excuded that of the straw. This had never been observed in our former expriments with paddy rice plants and also in my later experiments, this is the a most exceptional occurrence this phenomenon cannot be due to a special influence of the manuring but to

¹ Jahresbericht f. Agric. Chemie. Bd. XX 1897, p.p. 228.

other conditions of which the exceptional late transplantation¹ of the young rice plants must specially be remembered.

As to the nitric nitrogen in all harvested plants, it was not quantitatively determined, but by the way of qualitative test with diphenylamin, it was shown that all plants which received the nitrates showed a distinct reaction for the nitric acid and the reaction was stronger in the earlier period than in the later.

II. Experiments with different doses and different application of sodium nitrate and ammonium sulphate to paddy rice plants.

These experiments were carried out in the field in the same year as the former in the glass house.

Fifty seven wooden frames, each representing an area of 0.826 square metre, were placed in a paddy field of our experimental farm. The soil was taken from the adjoining field which had not been manured for a long time; the general treatment of the soil in the frames was not essentially different from that described in my former papers. The potash was applied, July 29 as potassium carbonate and the phosphoric acid as double superphosphate, at the rate of 100 kilograms per hectare respectively.

The rate and actual quantity of the nitrogen, which was applied in the form of ammonium sulphate and sodium nitrate, per frame and also the mode of application are seen from following table.

¹ In my experiments, the transplantation of the young rice plants was about 20 days later than in common practice.

No of frames.	Kind of nitrogenous manures.	Nitrogen applied per ha. Kg.	Nitrogen applied per frame. gr.	Remarks on the nitrogen application.
1, 20, 39.	Without nitrogen.	0	0	0
2, 21, 40. 3, 22, 41. 4, 23, 42.	Ammonium sulphate.	25 50 75	2.083 4.166 6.249	The whole quantity was applied at the transplantation.
5, 24, 43. 6, 25, 44. 7, 26, 45.	"	25 50 75	2.083 4.166 6.249	$\frac{1}{2}$ was applied at the transplanting and $\frac{1}{2}$ after 30 days.
8, 27, 46. 9, 28, 47. 10, 29, 48.	"	25 50 75	2.083 4.166 6.249	$\frac{1}{4}$ was applied every 15 days.
11, 30, 49. 12, 31, 50. 13, 32, 51.	Sodium nitrate.	25 50 75	2.083 4.166 6.249	The whole quantity was applied at the time of transplanting.
14, 33, 52. 15, 34, 53. 16, 35, 54.	"	25 50 75	2.083 4.166 6.249	$\frac{1}{2}$ was applied at the plantation and $\frac{1}{2}$ after 30 days.
17, 36, 55. 18, 37, 56. 19, 38, 57.	"	25 50 75	2.083 4.166 6.249	$\frac{1}{4}$ was applied at every 15 days.

On the 30th of June, the young rice plants well nourished and 50 days old were transplanted as usually.

The crops were harvested on November 21 with the following results (the detailed figures of the yields per frame are seen from the last page). The last column of the table contains the figures of the total nitrogen contained in the total crop per frame, as found by chemical analysis.

No of frames.	Kind of nitrogenous manures.	N. applied per ha. Kg. / manner of application.	Straw. gr.	Full grains. gr.	Empty grains. gr.	Total crop. gr.	Nitrogen in the total crop per frame. gr.
1, 20, 39.	o	o	303.9	266.3	4.8	575.0	6.866
2, 21, 40.	Ammonium sulphate.	25. given	388.9	334.5	4.9	723.6	8.521
3, 22, 41.		50. in	441.5	379.5	6.0	827.0	9.825
4, 23, 42.		75. 1 dose.	480.6	401.3	5.4	887.3	10.802
5, 24, 43.	"	25. given	361.3	303.1	5.5	669.9	7.910
6, 25, 44.		50. in	408.5	359.1	5.7	773.3	8.966
7, 26, 45.		75. 2 doses.	463.1	406.6	6.4	876.1	9.739
8, 27, 46.	"	25. given	348.1	316.3	4.9	669.3	7.591
9, 28, 47.		50. in	398.9	347.6	5.3	751.8	8.763
10, 29, 48.		75. 4 doses.	406.3	358.9	5.9	771.1	9.094
11, 30, 49.	Sodium nitrate.	25. given	353.1	307.5	4.9	665.5	8.005
12, 31, 50.		50. in	349.5	312.7	5.1	667.3	8.152
13, 32, 51.		75. 1 dose.	368.1	328.4	6.4	702.9	8.360
14, 33, 52.	"	25. given	349.8	307.3	5.4	662.5	7.347
15, 34, 53.		50. in	347.2	309.8	5.6	662.6	7.616
16, 35, 54.		75. 2 doses.	338.0	302.7	5.4	646.1	7.484
17, 36, 55.	"	25. given	350.4	323.5	5.5	679.4	7.667
18, 37, 56.		50. in	323.2	287.6	5.1	615.9	6.807
19, 38, 57.		75. 4 doses.	339.5	303.5	5.4	648.4	7.458

These results demonstrate again an inferior action of the sodium nitrate upon the rice plants, as both the yield and assimilated nitrogen in all the frames with the nitrate in different doses varied but within a narrow limit; whilst the nitrogen of the ammonium sulphate was easily assimilated in the proportion to the quantities applied, as seen from the increase of the yields.

Since all paddy fields are irrigated from time to time during the whole

rice vegetation and since they are thoroughly covered with water, thus reducing processes (denitrification) being enhanced, losses of nitrates may occur in two ways.

Hence it might be deduced that the application of nitrates in several periods of the plants' growth would secure better results than the application in one dose ; but my results sufficiently show that no better effect can thus be gained.

In such paddy field as our's, the late and divided dressing with nitrate or ammonium sulphate to rice plants has no advantage whatever.

Since these experiments were performed more in accordance with the actual practice than the former experiments, it will be of some value to compare the manurial value of the sodium nitrate to that of the ammonium sulphate.

Thus when the surplus yield (2.52 grams) caused by the medium dose (applied at once) of the ammonium sulphate over the frames without nitrogen, is assumed to be 100, the relative increase caused by the same dose (applied also at once) of the sodium nitrate will be 36.6. Again, the actual percentage assimilability of the sulphate nitrogen is estimated as much as 71% while that of the nitrate is only 30.9%, hence when the former percentage number (71) to be assumed as 100 the relative assimilability of the nitrate will be 43.5. The average of the relative increase and assimilability constitutes its manurial value thus,

$$\frac{36.6+43.5}{2}=40.01$$

Hence it follows that the manurial value of the ammonium sulphate is 2.5 times greater than that of the sodium nitrate for paddy rice cultivation.

In order to determine the after-effects of the residual nitrogen of both, ammonium sulphate and sodium nitrate, in this second series, the soils of all the frames, having been untouched during the coming winter, were, in the next season (1900) cultivated again for the same species of rice plants and with fresh application of the general manures as in the preceeding year, but without further addition of ammonium sulphate or sodium nitrate. The transplantation of the young rice plants took place on the 28th of June, and the

crop were cut on the 11th of November. Here, the details in yields per frame being likewise left to the last pages, we shall in the following table only give the averages of the three equally treated frames.

No of frames.	Kind of nitrogenous manures.	N. applied per ha. Kg.	Straw. gr.	Full grains. gr.	Empty grains. gr.	Total crop. gr.
1, 20, 39.	Without N.	0	343.0	295.3	3.4	641.7
2, 21, 40.	Ammonium sulphate.	25.	371.2	326.6	4.9	702.7
3, 22, 41.		50.	370.3	330.3	3.6	704.2
4, 23, 42.		75.	411.3	359.0	4.4	774.7
5, 24, 43.	"	25.	370.7	339.3	3.6	713.6
6, 25, 44.		50.	361.1	312.7	4.4	678.2
7, 26, 45.		75.	400.0	356.3	6.2	762.5
8, 27, 46.	"	25.	338.8	315.7	3.2	657.7
9, 28, 47.		50.	377.7	317.7	3.4	698.8
10, 29, 48.		75.	403.7	336.3	4.2	744.2
11, 30, 49.	Sodium nitrate.	25.	348.0	295.0	4.6	647.6
12, 31, 50.		50.	355.7	322.3	3.6	681.6
13, 32, 51.		75.	367.5	357.0	3.6	708.1
14, 33, 52.	"	25.	366.0	322.0	3.3	691.3
15, 34, 53.		50.	374.7	316.3	3.2	694.2
16, 35, 54.		75.	347.0	295.7	4.4	647.7
17, 36, 55.	"	25.	360.8	307.0	4.9	672.7
18, 57, 56.		50.	364.0	336.3	4.3	704.6
19, 38, 57.		75.	379.0	337.0	3.9	719.9

By a superficial glance at the above figures, it might seem that the residual nitrogen displayed some special good influence upon the rice production. However, when we take into consideration the high proportion of the total crop also in the frames not supplied with any nitrogen, which frames

giving in average 66 grams more yield than in the first year, the general augmentation of the yields in all cases can not be due to the efficacy of the residual nitrogen but to other causes very probably to the highly favorable weather which prevailed in 1900.

As to the actual effects of the residual nitrogen, it is seen, as a whole, that, although the residues of the ammonium sulphate had again somewhat better influence than those of the sodium nitrate, yet in both cases the effects were far behind those when nitrogen compounds were freshly applied.

I give in the following table, the actual surplus yields obtained in the first and second year respectively.

No of frames.	Kind of nitrogenous manures,	N. applied in the 1st year per ha. Kg.	N. left for the second year per frame. gr.	Surplus yield. gr.	
				First year.	Second year.
2, 11, 40.	Ammonium sulphate.	25. given	0.428	148.6	61.0
3, 12, 41.		50. in	1.207	252.0	62.5
4, 13, 42.		75. one dose.	2.313	312.3	133.0
5, 14, 43.	"	25. given	0.439	94.9	71.9
6, 15, 44.		50. in	2.066	198.3	136.5
7, 16, 45.		75. 2 doses.	3.378	301.1	120.8
8, 17, 46.	"	25. given	1.358	94.3	16.0
9, 18, 47.		50. in	2.269	176.8	57.1
10, 19, 48.		75. 4 doses.	4.021	198.1	102.5
11, 20, 49.	Sodium nitrate.	25. given	0.944	90.5	6.1
12, 21, 50.		50. one	2.880	92.3	39.9
13, 22, 51.		75. dose.	4.755	127.9	66.4
14, 23, 52.	"	25. given	1.602	87.5	48.6
15, 24, 53.		50. in	3.416	87.6	52.5
16, 25, 54.		75. 2 doses.	5.631	71.1	6.0
17, 20, 55.	"	25. given	1.282	104.4	31.0
18, 27, 56.		50. in	—	40.9	62.0
19, 28, 57.		75. 4 doses.	5.657	73.4	78.2

Thus in the second year, the residual nitrogen of both, ammonium sulphate and sodium nitrate, had more or less action upon the rice plants yet, excepting but the residual nitrogen of the largest dose (last series of the nitrate) of the nitrate, there is no case in which their actions exceeded or attained an equal rate to that of the first year, although, as proved by the results of the chemical analysis, there were left still sufficient quantities of the nitrogen to produce crops similar to those of the first year.

As to the residues of the nitrogen after late application there was more left in the soil than in the case of early application, yet there was no better effect in the former case.

This may be due most probably to the fact that the nitrogen which was applied at the early period, was relatively more absorbed by the plants and a part of the assimilated nitrogen was reserved in the roots which by decomposition in the following year produced comparatively some good effect upon the second crops; on the other hand, the nitrogen which was applied later, having been less absorbed by the plants, was much washed away by irrigation in both first and second year, and consequently a certain quantity was lost.

Appendix.

YIELD PER FRAME (1st year).

No of frames,	Straw, gr.	Full grains, gr.	Empty grains, gr.	No of frames,	Straw, gr.	Full grains, gr.	Empty grains, gr.
1	314.0	268.5	4.6	3	433.8	383.5	5.5
20	301.3	252.5	5.1	22	413.8	345.0	6.4
29	296.5	278.0	4.7	41	476.8	410.0	6.2
2	397.2	342.0	4.2	4	491.3	395.0	7.0
21	393.3	345.0	4.9	23	483.3	407.0	6.0
10	362.2	316.5	5.5	42	467.3	402.0	3.1

No of frames.	Straw. gr.	Full grains. gr.	Empty grains. gr.	No of frames.	Straw. gr.	Full grains. gr.	Empty grains. gr.
5	342.5	274.0	5.2	13	351.8	298.5	7.0
24	328.3	289.0	5.5	32	341.3	302.5	7.1
43	413.0	346.0	5.7	51	411.3	384.2	5.0
6	351.3	294.2	5.2	14	311.3	263.0	4.5
25	434.8	374.5	6.5	33	368.8	315.0	5.4
44	439.3	408.7	5.5	52	369.3	344.0	6.3
7	495.8	418.2	6.5	15	297.3	258.0	6.2
26	456.3	413.0	5.4	34	346.0	308.5	5.3
45	437.3	388.5	7.3	53	398.3	363.0	5.4
8	391.0	355.0	5.3	16	277.3	253.5	4.5
27	334.0	309.5	4.3	35	341.5	301.5	5.8
46	319.3	284.5	5.2	54	395.3	353.0	5.8
9	371.5	317.0	6.8	17	323.8	300.5	5.3
28	443.3	375.2	4.8	36	308.5	272.0	5.4
47	381.8	350.5	4.4	55	418.8	398.0	5.9
10	390.8	351.2	5.0	18	328.3	278.5	6.0
29	408.8	366.2	5.6	37	257.5	206.0	4.0
48	419.2	359.4	7.0	56	383.8	378.3	5.2
11	331.0	279.7	2.9	19	339.3	309.0	4.0
30	323.3	277.7	6.0	38	339.5	301.0	5.2
49	405.0	365.0	5.9	57	339.8	300.5	6.2
12	346.8	310.0	4.3				
31	326.8	284.0	6.2				
50	375.0	344.2	4.9				

YIELDS PER FRAME (Second year).

No of frames.	Straw. gr.	Full grains. gr.	Empty grains. gr.	No of frames.	Straw. gr.	Full grains. gr.	Empty grains. gr.
1	353.0	282.0	3.9	9	396.0	311.0	3.2
20	335.0	294.0	3.5	28	383.0	346.0	3.0
39	336.0	310.0	2.7	47	354.0	296.0	4.4
2	362.9	308.0	5.7	10	374.0	313.0	4.2
21	420.8	380.0	4.2	29	413.0	366.0	3.0
40	330.0	291.8	4.9	48	424.0	330.0	5.5
3	368.0	325.0	4.2	11	358.0	278.0	5.1
22	388.0	351.0	3.7	30	332.0	295.0	3.2
41	355.0	315.0	3.0	49	354.0	312.0	5.5
4	457.0	379.0	5.0	12	331.0	303.0	4.2
23	392.0	349.0	3.7	31	379.0	339.0	2.7
42	385.0	349.0	4.5	50	357.0	325.0	4.0
5	370.0	328.0	3.9	13	372.0	331.0	3.2
24	411.0	372.5	3.9	32	373.0	359.0	3.9
43	331.0	317.5	3.0	51	357.5	321.0	3.7
6	371.0	277.5	6.0	14	383.0	357.0	3.0
25	364.5	316.5	3.5	33	350.0	286.0	3.2
44	348.0	344.0	3.7	52	365.0	323.0	3.7
7	439.5	381.5	6.2	15	388.0	302.0	4.0
26	368.5	346.5	5.0	34	374.0	334.0	2.5
45	392.0	341.0	7.4	53	362.0	313.0	3.2
8	315.5	271.5	3.5	16	358.0	276.0	5.2
27	327.0	312.5	3.0	15	331.0	297.0	2.4
46	374.0	363.0	3.2	54	352.0	314.0	5.5

No of frames.	Straw, gr.	Full grains, gr.	Empty grains, gr.	No of frames.	Straw, gr.	Full grains, gr.	Empty grains, gr.
17	349.0	276.0	6.5	19	384.0	348.0	3.0
36	365.5	342.5	3.0	38	383.0	347.0	3.2
55	368.0	302.5	5.2	57	370.0	316.0	5.5
18	341.0	302.0	4.5				
37	378.0	348.0	3.2				
56	373.0	359.0	5.2				

III. How do Sodium nitrate and Ammonium sulphate act on Upland rice, compared with Paddy rice?

As upland rice is always grown in ordinary dry fields and under quite different conditions as paddy rice it was of interest to compare also here the effects of nitric and ammonical nitrogen.

I paid attention to the following principal questions :

1. How and how much can nitric nitrogen be utilized by upland rice plants under ordinary conditions (that is to say without irrigation) ?
2. How is the behavior under conditions similar to those of paddy rice cultivation (with irrigation) ?
3. What difference can be observed between the upland rice plants and paddy rice plants when the latter are cultivated at the same time under just the same condition as the former plants ?

These experiments were commenced, May 1900, in porcelain pots which I have already described in the preceding pages.

The general plan of these experiments is given in the following table.

UPLAND RICE PLANTS.

No of pots.	Kind of nitrogenous manures.	N. applied	N. applied	Difference of Cultivation.
		per ha. Kg.	per pot. gr.	
1. 40, 97.	Sodium nitrate.	0	0	Without irrigation.
2. 50, 98.	" "	25	0.1227	
3. 51, 99.	" "	50	0.2454	
4. 52, 100.	" "	100	0.4908	
5. 53, 101.	Sodium nitrate.	0	0	With irrigation.
6. 54, 102.	" "	25	0.1227	
7. 55, 103.	" "	50	0.2454	
8. 56, 104.	" "	100	0.4908	
9. 57, 105.	Ammonium sulphate.	0	0	Without irrigation.
10. 58, 106.	" "	25	0.1227	
11. 59, 107.	" "	50	0.2454	
12. 60, 108.	" "	100	0.4908	
13. 61, 109.	Ammonium sulphate.	0	0	With irrigation.
14. 62, 110.	" "	25	0.1227	
15. 63, 111.	" "	50	0.2454	
16. 64, 112.	" "	100	0.4908	
17. 65, 113.	Sodium nitrate.	0	0	Without irrigation.
18. 66, 114.	" "	25	0.1227	
19. 67, 115.	" "	50	0.2454	
20. 68, 116.	" "	100	0.4908	
21. 69, 117.	Sodium nitrate.	0	0	With irrigation.
22. 70, 118.	" "	25	0.1227	
23. 71, 119.	" "	50	0.2454	
24. 72, 120.	" "	100	0.4908	

25, 73, 121.	Ammonium sulphate.	0	0	Without irrigation.
26, 74, 122.	„ „	25	0.1227	
27, 75, 123.	„ „	50	0.2454	
28, 76, 124.	„ „	100	0.4908	
29, 77, 125.	Ammonium sulphate.	0	0	With irrigation.
30, 78, 126.	„ „	25	0.1227	
31, 79, 127.	„ „	50	0.2454	
32, 80, 128.	„ „	100	0.4908	

In the beginning of May, all the pots received about 2 Kilograms of small pebbles besides 11 Kilograms of the air dry and well sifted soil which had been taken from one of our unmanured experimental dry fields.

For the experiments with the paddy rice plants, the soil of dry fields is certainly not favorable but for sake of comparison this had to be taken.

The general manures, at the rate of 100 Kilograms of potash as potassium sulphate and phosphoric acid as double superphosphate respectively per hectare, were mixed with the weighed soil which was then filled in the pots.

The sodium nitrate and ammonium sulphate were, in the form of a solution, given on the 14th of May. On the next day, one half of the pots which were to be cultivated without irrigation, received, 9 healthy seeds of the upland rice plants (the Variety Terishiradsu) and the other half paddy rice seeds (the Variety Satsuma).

On the same day, the remainders of the above two kinds of the rice seeds, were, as a preparation for other pots under irrigation, sown upon a seed bed specially prepared for the purpose and after the germination of the seeds, the young rice plants in the bed were nourished and treated in the usual manner.

When the young rice plants were 44 days old, they were transplanted in the designated pots, which had been previously and sufficiently irrigated and stirred. Each pot received 9 healthy equally sized plants in 3 bundles.

During the whole vegetation there were observed some differences of growth between the plants with and without irrigation; thus the former

plants in all cases gave out less tillers than the latter, and they had rather slender and somewhat narrow leaves while the plants without irrigation possessed, on the contrary, more tillers and their leaves were long and broad. The plants which received the nitric nitrogen exhibited a pale colour but their significance was not so eminent as in the cases of the preceding experiments, they bloomed about 2 days and ripened 5 or 6 days earlier than those plants which were manured with the ammonical nitrogen. The general differences will be seen in the plates of the last pages.

On the 2nd of November, all the plants were harvested at once and at that time, the plants without irrigation were just in the state of full ripeness while those with irrigation were in a condition of a little over-ripening.

The yields per pot and the averages of the 3 parallel experiments are given in the following 4 tables in which I give also the quantities of the total nitrogen contained in the whole crop per pot.

TABLE I.

Results of the upland rice plants without irrigation.

No of pots.	Kind of the manures.	N. applied per ha. Kg.	No of panicles.	Straw. gr.	Grains. gr.	Average per pot.			N. in the total crop per pot. gr.
						Straw. gr.	Grains. gr.	Total. gr.	
1	Without nitrogen.	0	15	14.9	12.5				
49		"	17	14.2	8.9				
97		"	15	15.1	11.0	14.95	10.55	25.50	0.160
9		"	16	14.2	12.5				
75		"	14	14.5	10.0				
105		"	14	16.8	8.4				
	Sodium nitrate.	25	20	21.5	8.7				
		"	20	23.1	9.0	21.67	8.90	30.57	0.191
		"	15	20.4	9.7				
	" "	50	17	25.4	14.9				
		"	15	23.4	14.1	24.70	14.37	39.07	0.228
		"	19	25.4	14.1				
	" "	100	16	25.4	12.5				
		"	22	33.4	12.6	30.63	11.77	42.40	0.265
		"	23	33.1	11.2				
	Ammonium sulphate.	25	17	21.8	13.4				
		"	17	21.4	11.7	22.33	11.37	33.70	0.223
		"	19	23.8	9.0				
	" "	50	21	27.4	14.2				
		"	16	26.6	14.5	26.00	14.30	41.20	0.248
		"	20	26.7	14.2				
	" "	100	22	35.1	15.7				
		"	24	44.0	15.5	39.57	15.57	55.14	0.285
		"	33	38.7	15.5				

TABLE II.

Results of the upland rice plants with irrigation.

No of pots.	Kind of the manures.	N. applied per ha. Kg.	No of panicles.	Straw. gr.	Grains. gr.	Average per pot.			N. in the total crop per pot. gr.
						Straw. gr.	Grains. gr.	Total. gr.	
5	Without nitrogen.	0	12	13.1	9.4	13.17	9.27	22.44	0.175
53		"	12	13.8	7.4				
101		"	12	13.1	9.2				
13		"	12	12.4	10.2				
61		"	14	13.6	10.2				
109		"	13	13.0	9.2				
6	Sodium nitrate.	25	12	16.7	9.0	16.50	9.83	26.83	0.223
54		"	15	16.8	9.9				
102		"	16	16.0	10.6				
7	" "	50	16	21.1	14.7	21.53	14.10	35.63	0.247
55		"	15	22.0	14.0				
103		"	13	21.5	13.6				
8	" "	100	21	33.1	22.2	34.33	21.30	55.53	0.379
56		"	25	36.6	22.5				
104		"	21	33.3	19.2				
14	Ammonium sulphate.	25	15	17.5	12.0	17.17	11.37	28.54	0.247
62		"	15	16.9	10.7				
110		"	13	17.1	11.4				
15	" "	50	19	24.4	15.5	23.87	14.70	38.57	0.260
63		"	16	23.7	15.1				
111		"	18	23.5	13.5				
16	" "	100	22	40.3	24.0	41.93	23.10	65.03	0.384
64		"	25	41.3	22.4				
112		"	23	44.2	22.9				

TABLE III.

Results of the paddy rice plants without irrigation.

No of pots.	Kind of the manures.	N. applied per ha. Kg.	No of panicles.	Straw. gr.	Grains. gr.	Average per pot.			N. in the total crop per pot. gr.
						Straw. gr.	Grains. gr.	Total. gr.	
17	Without nitrogen.	0	14	20.2	11.8				
65		"	14	23.1	13.9				
113		"	10	17.5	12.7	19.85	12.80	32.65	0.1995
25		"	15	19.1	11.7				
73		"	15	19.1	11.9				
121		"	14	20.1	14.8				
18	Sodium nitrate.	25	15	19.7	11.7				
66		"	14	21.9	12.7	21.23	12.13	33.36	0.219
114		"	13	22.1	12.0				
19	" "	50	19	30.2	13.4				
67		"	18	28.0	14.4	30.10	14.80	44.90	0.250
115		"	19	32.1	17.0				
20	" "	100	28	38.8	18.7				
68		"	25	38.1	17.0	38.97	18.20	57.17	0.348
116		"	26	40.1	18.9				
26	Ammonium sulphate.	25	20	26.8	15.7				
74		"	14	25.4	15.9	26.50	15.37	41.87	0.269
122		"	16	27.3	15.5				
27	" "	50	20	36.6	17.2				
75		"	20	32.3	15.7	33.13	16.70	49.83	0.307
123		"	20	34.5	17.2				
28	" "	100	30	43.3	17.2				
76		"	31	45.9	18.5	46.47	17.57	64.04	0.378
124		"	29	50.2	17.0				

TABLE IV.

Results of the paddy rice plants with irrigation.

No of pots.	Kind of the manures.	N. applied per ha. Kg.	No of panicles.	Straw. gr.	Grains. gr.	Average per pot.			N. in the total crop per pot. gr.
						Straw. gr.	Grains. gr.	Total. gr.	
21	Without nitrogen.	0	13	16.0	10.7				0.2345
68		"	12	18.0	12.0				
117		"	12	16.5	10.2	19.33	12.30	31.63	
29		"	12	21.7	13.7				
77		"	15	22.0	13.2				
125		"	12	21.7	14.0				
22	Sodium nitrate.	25	13	22.2	13.1				0.267
70		"	14	22.4	12.9	22.10	13.00	35.10	
118		"	15	21.7	13.0				
23	" "	50	17	30.2	12.9				0.287
71		"	18	28.4	13.0	29.23	12.87	42.10	
119		"	13	29.1	12.7				
24	" "	100	25	47.3	17.0				0.403
72		"	25	46.0	17.2	46.57	15.67	62.24	
120		"	24	46.4	12.8				
30	Ammonium sulphate.	25	17	25.5	13.7				0.317
78		"	14	26.5	15.2	26.73	15.47	42.20	
126		"	18	28.2	17.5				
31	" "	50	17	30.2	16.6				0.346
79		"	21	31.4	17.0	30.27	16.53	46.80	
127		"	17	29.2	16.0				
32	" "	100	22	44.7	17.5				0.443
80		"	23	45.1	18.2	45.87	18.07	63.94	
128		"	22	47.8	18.5				

From the numbers of the above 4 tables, some facts can be learned viz., *the upland* rice can also be cultivated like the paddy rice, with irrigation, whilst the paddy rice plant is also fitted for the cultivation without irrigation, although, both species of rice plants, when grown under their normal condition produce better results.

As to the effect of the sodium nitrate and ammonium sulphate, it will be generally seen that the latter salt acted better than the former; but before entering into a detailed discussion in regard to their efficacy, I shall calculate from the above results, the actual surplus yield over the pot not supplied with any nitrogen and also the actual extra of the assimilated nitrogen.

A. Upland rice plants.

WITHOUT IRRIGATION.

No of pots.	Kind of the manures.	N. applied per ha. Kg.	Surplus of the yield. gr.	Surplus of the assimilated N. gr.
2, 50, 98.	Sodium nitrate.	25	10.07	0.031
3, 51, 99.	" "	50	13.57	0.068
4, 52, 100.	" "	100	16.90	0.105
10, 58, 106.	Ammonium sulphate.	25	8.20	0.063
11, 59, 107.	" "	50	15.70	0.088
12, 60, 108.	" "	100	29.90	0.125

WITH IRRIGATION.

6, 54, 102.	Sodium nitrate.	25	4.39	0.048
7, 55, 103.	" "	50	13.19	0.072
8, 56, 104.	" "	100	33.09	0.204
14, 62, 110.	Ammonium sulphate.	25	6.10	0.072
15, 63, 111.	" "	50	16.13	0.085
16, 64, 112.	" "	100	42.50	0.209

B. Paddy rice plant.

WITHOUT IRRIGATION.

No. of pots.	Kind of the manures.	N. applied per ha. Kg.	Surplus of the yield. gr.	Surplus of the assimilated N. gr.
18, 66, 114.	Sodium nitrate.	25	0.71	0.0195
19, 67, 115.	" "	50	12.25	0.0505
20, 68, 116.	" "	100	24.52	0.1485
26, 74, 122.	Ammonium sulphate.	25	9.22	0.0695
27, 75, 123.	" "	50	17.18	0.1075
28, 76, 124.	" "	100	31.39	0.1785

WITH IRRIGATION.

22, 70, 118.	Sodium nitrate.	25	3.47	0.0325
23, 71, 119.	" "	50	10.47	0.0525
24, 72, 120.	" "	100	30.61	0.1685
30, 78, 126.	Ammonium sulphate.	25	10.57	0.0825
31, 79, 127.	" "	50	15.17	0.1115
32, 80, 128.	" "	100	32.31	0.2085

Thus it is generally seen from the above calculations that the ammonium sulphate acted upon both upland and paddy rice plants better than the nitrate, the latter being always less absorbed than the former; nevertheless it is also observed that the nitrate was more utilized by the upland rice plants than by the paddy rice.

Although these special properties of the upland rice which enables it to utilize nitrate better than paddy rice does have not yet been ascertained by any physiologist, yet it may be deduced from some observations of Loew¹ and U. Sudzuki,² that a higher concentration of sugar is attained in the

¹ Loew, The energy of living protoplasm.

² Bulletin, College of Agriculture, Imperial University. Vol. III. No. 5.

leaves of the upland rice plants, whereby the nitrates can be more easily transformed into asparagin and protein.

For the sake of better comparison I have calculated here, assuming respectively the surplus yield and also the surplus of the assimilated nitrogen caused by the medium dose (50 kg. per ha.) of the ammonium sulphate, to be 100, the percentages of the yield and assimilability for the nitrate applied in the same quantity (50 kg.) as follows:—

UPLAND RICE PLANT.

	Without irrigation.	With irrigation.
Surplus yield.	86.4	81.9
„ of the assimilated N.	77.3	82.4

PADDY RICE PLANT.

Surplus yield.	71.3	69.0
„ of the assimilated N.	47.0	47.0

Accordingly it is quite clear that in all cases the ammonical nitrogen has displayed a better action than the nitric nitrogen, while it also becomes obvious that the upland rice plants have utilized 30% more nitrogen in the case without irrigation and 35% more with irrigation, from equal dose of the nitrate than the paddy rice plants.

Although the paddy rice plants in these experiments have utilized the nitric nitrogen less than the upland rice plants, yet when these results are compared to those of the Series I and II, it will be seen that the paddy plants took up a somewhat higher amount of the nitric nitrogen than in the former cases. This difference of the nitric nitrogen assimilation will most probably be due to the difference of the soil used, since in these experiments dry field soil has been used instead of paddy field soil.

Concerning the chemical composition and physical properties of our dry and paddy field soils, there is no great difference, as we have frequently

described in our former bulletins,¹ yet the contents in humus, protoxide of iron and in alumina are evidently greater in the paddy soil than in the dry field soil.

Hence it might be inferred that when a nitrate is applied to the soil rich in humus and protoxide, a part of the salt is lost by denitrification.

IV. Experiments on the influence of lime compounds upon the effects of sodium nitrate, ammonium sulphate and fish manure with paddy rice plants.

It is well known that lime and its compounds have some beneficial effect upon the absorption of nitrate nitrogen by plant roots; but such observations have been made on the ordinary dry fields. It seemed to me of some interest to observe also the behavior in the paddy fields.

My experiments were carried out, in the year 1901, just in the same manner, as in the first series, with 120 porcelain pots in the glass house; the soil was also taken from the same paddy field as in the first experiments.

The general design of these experiments is shown in the following table:—

¹ The Bulletin, College of Agriculture, Imperial University. Vol I, No. 9 and 11.

WITHOUT NITROGEN.

No. of pots.	Nitrogen applied per ha. Kg.	Nitrogen applied per ha. gr.	Kind of lime compounds.	CaO applied per pot. gr.
1, 41, 81.	0	0	0	0
2, 42, 82.	0	0	Caustic lime	19.632
3, 43, 83.	0	0	Calcium carbonate	19.632
4, 44, 84.	0	0	Calcium sulphate	19.632

WITH SODIUM NITRATE.

5, 45, 85.	50	0.2454	0	0
6, 46, 86.	100	0.4908	0	0
7, 47, 87.	150	0.7362	0	0
8, 48, 88.	50	0.2454	Caustic lime	19.632
9, 49, 89.	100	0.4908	"	"
10, 50, 90.	150	0.7362	"	"
11, 51, 91.	50	0.2454	Calcium carbonate	"
12, 52, 92.	100	0.4908	"	"
13, 53, 93.	150	0.7362	"	"
14, 54, 94.	50	0.2454	Calcium sulphate	"
15, 55, 95.	100	0.4908	"	"
16, 56, 96.	150	0.7362	"	"

WITH AMMONIUM SULPHATE.

17, 57, 97.	50	0.2454	0	0
18, 58, 98.	100	0.4908	0	0
19, 59, 99.	150	0.7362	0	0
20, 60, 100.	50	0.2454	Caustic lime	19.632
21, 61, 101.	100	0.4908	"	"
22, 62, 102.	150	0.7362	"	"
23, 63, 103.	50	0.2454	Calcium sulphate	"
24, 64, 104.	100	0.4908	"	"

No. of pots.	Nitrogen applied per ha. Kg.	Nitrogen applied per ha. gr.	Kind of lime compounds.	CaO applied per pot. gr.
25, 65, 105.	150	0.7362	Calcium sulphate	19.632
26, 66, 106.	50	0.2454	"	"
27, 67, 107.	100	0.4908	"	"
28, 68, 108.	150	0.7362	"	"

WITH FISH MANURE.

29, 69, 109.	50	0.2454	0	0
30, 70, 110.	100	0.4908	0	0
31, 71, 111.	150	0.7362	0	0
32, 72, 112.	50	0.2454	Caustic lime	19.632
33, 73, 113.	100	0.4908	"	"
34, 74, 114.	150	0.7362	"	"
35, 75, 115.	50	0.2454	Calcium carbonate	"
36, 76, 116.	100	0.4908	"	"
37, 77, 117.	150	0.7362	"	"
38, 78, 118.	50	0.2454	Calcium sulphate	"
39, 79, 119.	100	0.4908	"	"
40, 80, 120.	150	0.7362	"	"

On the 21st of June, all the lime compounds were added to the soil at the rate of 4000 Kilograms of CaO per ha which makes 19.632 gram per pot, and as the general manure, phosphoric acid in the form of the sodium phosphate and at the rate of 200 Kilograms per hectare and the potassium sulphate at the rate of 150 Kilograms of K_2O were applied on the 23rd while the nitrogenous manures¹ were applied on the 24th of June.

The transplantation of the young rice plants took place on the 25th and each pot received 9 equally nourished plants in 3 bundles.

¹ The fish manure was prepared from sardine and contained 8.901% of nitrogen in the air dry state.

A fortnight after the transplantation, all the plants displayed a quite distinct and different development according to the nature of the nitrogenous manures; thus the plants which had received the ammonium sulphate grew the best and those with the fish manure came next while the plants with sodium nitrate remained pale and small in size. The general difference of the growth will be seen from the plates of the appendix.

All the crops were harvested on the 26th of November with the following results;¹ the numbers of the last column are the quantities of the total nitrogen in the whole crop per pot.

¹ The detailed figures per pot are given in the appendix of the last page.

WITHOUT NITROGEN.

No of pots.	N. applied per ha. Kg.	Compound of lime.	Straw. gr.	Grains. gr.	Total crop. gr.	Assimilated nitrogen per pot. gr.
1, 41, 81.	0	0	16.67	12.97	29.64	0.2045
2, 42, 82.	0	CaO	17.50	15.87	33.37	0.2536
3, 43, 83.	0	CaCO ₃	16.50	14.17	30.67	0.2202
4, 44, 84.	0	CaSO ₄	15.17	12.67	27.84	0.1836

WITH SODIUM NITRATE.

5, 45, 85.	50	0	16.33	13.23	29.56	0.2055
6, 46, 86.	100	0	17.33	14.23	31.56	0.2231
7, 47, 87.	150	0	17.33	13.43	30.76	0.2027
8, 48, 88.	50	CaO	22.33	19.47	41.90	0.3089
9, 49, 89.	100	,,	22.67	19.33	42.00	0.3205
10, 50, 90.	150	,,	21.00	17.57	38.57	0.2575
11, 51, 91.	50	CaCO ₃	11.67	15.10	32.77	0.2256
12, 52, 92.	100	,,	18.33	15.30	33.63	0.2256
13, 53, 93.	150	,,	18.33	15.30	33.63	0.2263

No of pots.	N. applied per ha. Kg.	Compound of lime.	Straw. gr.	Grains. gr.	Total crop. gr.	Assimilated nitrogen per pot. gr.
14, 54, 94.	50	CaSO ₄	17.00	13.43	30.43	0.2006
15, 55, 95.	100	"	17.33	13.37	30.70	0.2015
16, 56, 96.	150	"	17.17	13.33	30.50	0.1892

WITH AMMONIUM SULPHATE.

17, 57, 97.	50	o	30.50	22.77	53.27	0.3537
18, 58, 98.	100	o	42.67	31.67	74.34	0.5062
19, 59, 99.	150	o	49.67	40.43	90.10	0.6763
20, 60, 100.	50	CaO	32.00	26.07	58.07	0.3856
21, 61, 101.	100	"	35.33	35.13	70.46	0.5982
22, 62, 102.	150	"	39.33	39.23	78.56	0.6797
23, 63, 103.	50	CaCO ₃	29.50	24.87	54.37	0.3812
24, 64, 104.	100	"	37.83	30.83	68.66	0.4834
25, 65, 105.	150	"	44.33	37.83	82.16	0.6272
26, 66, 106.	50	CaSO ₄	28.67	21.33	50.00	0.3281
27, 67, 107.	100	"	41.17	33.10	74.27	0.5587
28, 68, 108.	150	"	47.67	39.17	86.84	0.7078

WITH FISH MANURE.

29, 69, 109.	50	o	27.33	20.50	47.84	0.3154
30, 70, 110.	100	o	33.67	29.40	63.07	0.4224
31, 71, 111.	150	o	47.50	37.43	74.93	0.4835
32, 72, 112.	50	CaO	28.67	25.13	53.80	0.3865
33, 73, 113.	100	"	38.50	31.87	70.37	0.4902
34, 74, 114.	150	"	42.33	38.83	81.16	0.6438

35, 75, 115.	50	Ca CO ₃	23.67	20.23	43.90	0.3111
36, 76, 116.	100	„	36.67	30.67	67.34	0.4624
37, 77, 117.	150	„	44.67	36.90	81.57	0.6439
38, 78, 118.	50	Ca SO ₄	24.33	18.17	42.50	0.2767
39, 79, 119.	100	„	32.67	25.40	58.07	0.3795
40, 80, 120.	150	„	41.00	34.27	75.27	0.5235

In considering these results, it will be observed that the ammonium sulphate had again a considerable good influence upon the production and next to it also the fish manure.

Before entering into a farther discussion of these results, I have calculated in the following table the extra yield caused by the 3 nitrogenous manures over the no nitrogen pot and also the extra of the assimilated nitrogen.

WITH SÓDIUM NITRATE.

No. of pots.	Nitrogen applied per ba. kg.	Compound of lime.	Extra.	
			Yield. gr.	Assimilated nitrogen. gr.
5, 45, 85.	50	o	(-) 0.08	0.0010
6, 46, 86.	100	o	1.92	0.0186
7, 47, 87.	150	o	1.12	(-) 0.0018
8, 48, 88.	50	CaO	8.53	0.0553
9, 49, 89.	100	„	8.63	0.0669
10, 50, 90.	150	„	5.20	0.0039
11, 51, 91.	50	CaCO ₃	2.10	0.0054
12, 52, 92.	100	„	2.96	0.0054
13, 53, 93.	150	„	2.96	0.0061
14, 54, 94.	50	CaSO ₄	2.59	0.0170
15, 55, 95.	100	„	2.86	0.0179
16, 56, 96.	150	„	2.66	0.0056

WITH AMMONIUM SULPHATE.

No. of pots.	Nitrogen applied per ha. kg.	Compound of lime.	Extra.	
			Yield, gr.	Assimilated nitrogen. gr.
17, 57, 97.	50	o	24.23	0.1512
18, 58, 98.	100	o	44.70	0.3017
19, 59, 99.	150	o	60.49	0.4718
20, 60, 100.	50	CaO	24.63	0.1320
21, 61, 101.	100	„	37.09	0.3446
22, 62, 102.	150	„	45.19	0.4261
23, 63, 103.	50	CaCO ₃	23.70	0.1610
24, 64, 104.	100	„	37.99	0.2632
25, 65, 105.	150	„	51.49	0.4070
26, 66, 106.	50	CaSO ₄	22.16	0.1445
27, 67, 107.	100	„	46.43	0.3751
28, 68, 108.	150	„	59.00	0.5242

WITH FISH MANURE.

29, 69, 109.	50	o	18.20	0.1109
30, 70, 110.	100	o	38.43	0.2179
31, 71, 111.	150	o	50.29	0.2790
32, 72, 112.	50	CaO	20.43	0.1029
33, 73, 113.	100	„	37.00	0.2366
34, 74, 114.	150	„	47.79	0.3902
35, 75, 115.	50	CaCO ₃	13.23	0.0909
36, 76, 116.	100	„	36.67	0.2422
37, 77, 117.	150	„	50.90	0.4237
38, 78, 118.	50	CaSO ₄	14.66	0.0931
39, 79, 119.	100	„	30.23	0.1959
40, 80, 120.	150	„	47.43	0.3399

These calculations show that the sodium nitrate, in spite of its large dose, had no great effect while the other two manures (ammonium sulphate and fish manure) gave a very satisfactory crop in proportion to their doses.

These results, in general, coincide well with those of the series I and II and hence it may be justified to conclude that paddy rice plants when cultivated in a genuine paddy soil like our's, have no sufficient capacity to assimilate the nitric nitrogen. Hence the nitrogenous manures to paddy fields should be applied either in the form of ammonium salts or of organic manures.

In regard to the beneficial influence of the lime and its compounds upon the absorption of the nitrate, it becomes clear from the above figures, that all the lime compounds have accelerated the assimilation of the nitric nitrogen to a certain extent. The caustic lime was most efficient whilst the calcium carbonate and sulphate remained but little behind.

Though the action of the caustic lime upon the nitric nitrogen assimilation was quite evident, the total increase of the organic matter was not sufficient enough to recommend the sodium nitrate as a profitable manure for paddy rice.

As to the ammonium sulphate, the lime compounds had no favorable but, on the contrary, an unfavorable effect on the assimilation of the nitrogen; only in one case (medium dose (100 kg.) of the ammonium sulphate with calcium sulphate) was an exception. The considerable diminution of the yield caused by the caustic lime in the pots with the ammonium sulphate, is doubtless due to the high alkalinity of the lime by which the ammonia of the sulphate was set free, causing a loss of active nitrogen.

As to fish manure the caustic lime seems to have acted somewhat beneficially but it was not so noticeable as in the case of the sodium nitrate. This action exerted by the caustic lime upon the fish manure is undoubtedly due to the influence upon the decomposition of organic compounds.

YIELDS PER POT.

No. of pots.	Straw. gr.	Grains. gr.	No. of pots.	Straw. gr.	Grains. gr.
1	16.0	11.5	9	23.0	19.0
41	17.0	12.7	49	22.5	19.5
81	17.0	12.7	89	22.5	19.5
2	17.0	15.8	10	17.0	13.0
42	17.0	15.8	50	23.0	19.7
82	18.5	16.0	90	23.0	20.7
3	17.0	15.0	11	18.0	14.3
43	16.5	14.0	51	18.0	16.5
83	16.0	13.5	91	17.0	14.5
4	15.0	12.3	12	19.0	15.3
44	15.0	12.0	52	18.5	15.5
84	15.5	13.7	92	17.5	15.1
5	16.0	13.7	13	17.0	13.2
45	16.0	13.5	53	19.0	16.2
85	17.0	12.5	93	19.0	16.5
6	17.0	14.7	14	17.0	14.0
46	17.0	13.5	54	17.0	13.8
86	18.0	14.5	94	17.0	12.5
7	17.0	13.5	15	17.5	13.3
47	18.0	14.0	55	17.0	13.8
17	17.0	12.8	95	17.5	13.0
8	22.0	20.6	16	17.5	13.5
48	22.0	18.5	56	17.0	13.0
88	23.0	19.3	96	17.0	13.5

YIELDS PER POT.

No. of pots.	Straw. gr.	Grains. gr.	No. of pots.	Straw. gr.	Grains. gr.
17	31.5	23.1	25	44.0	37.5
57	30.0	21.6	65	44.0	38.2
97	30.0	23.6	105	45.0	37.8
18	43.0	30.0	26	28.0	21.5
58	42.0	33.5	66	28.5	21.5
98	43.0	31.5	106	29.5	21.0
19	50.0	39.3	27	42.5	35.0
59	50.0	41.0	67	41.0	31.3
99	49.0	41.0	107	40.0	33.0
20	32.0	26.5	28	47.0	38.5
60	32.0	26.5	68	46.0	41.0
100	32.0	25.2	108	50.0	38.0
21	35.0	32.8	29	26.0	20.5
61	41.0	40.3	69	29.0	20.8
101	30.0	32.3	109	27.0	20.2
22	39.0	38.8	30	32.0	29.8
62	34.0	36.3	70	37.0	30.8
102	45.0	42.6	110	32.0	27.6
23	27.5	22.0	31	49.0	38.3
63	28.0	22.3	71	47.0	37.5
103	33.0	30.3	111	46.5	36.5
24	38.0	31.0	32	28.0	25.7
64	38.0	30.5	72	29.0	24.2
104	37.5	31.0	112	29.0	25.5

YIELDS PER POT.

No. of pots.	Straw, gr.	Grains, gr.	No. of pots.	Straw, gr.	Grains, gr.
33	38.5	32.5	37	45.0	36.7
73	38.5	33.0	77	44.0	37.0
113	38.5	30.1	117	45.0	37.0
34	45.0	38.8	38	24.0	18.0
74	38.0	36.5	78	25.0	18.5
114	44.0	41.2	118	24.0	18.0
35	27.0	22.2	39	33.0	24.2
75	19.0	16.5	79	33.0	25.5
115	28.0	22.0	119	32.0	26.5
36	36.0	30.8	40	40.0	33.5
76	38.0	30.0	80	41.0	35.8
116	36.0	31.2	120	42.0	33.5

V. Experiments on the action of a sodium nitrate and ammonium sulphate upon other aquatic agricultural plants.

Since there are, in Japan, several agricultural plants which are generally cultivated under the same conditions as paddy rice plants, it will not be uninteresting to investigate also the effects of sodium nitrate and ammonium sulphate upon some of such plants: and as the experiments of this sort have not yet been tried, as far as I know, among our agricultural chemists, the results of these investigation will be of some value in a theoretical and practical view.

A. Experiments with Fucus effusus L.

These experiments were tried, in 1900, and in conjunction with the series III. The soil also came from the same dry land. The treatment

of the Juncus were also similar to those of the rice plants in the 3rd series of experiments.

The rate and actual quantity of the nitrogen applied to each pot are as follows.

No. of pots.	Kind of nitrogenous manure.	Nitrogen applies per ha. kg.	Nitrogen applied per pot. gr.
33, 81, 129.	0	0	0
34, 82, 130.	Am. sulphate.	25	0.1227
35, 83, 131.	" "	50	0.2454
36, 84, 132.	" "	100	0.4908
37, 85, 133.	0	0	0
38, 86, 134.	Sodium nitrate.	25	0.1227
39, 87, 135.	" "	50	0.2454
40, 88, 136.	" "	100	0.4908

The crops were harvested on the 2nd of November, with the following results.

WITHOUT NITROGEN.

No. of pots.	Nitrogen applied per ha. kg.	Yield per pot. gr.	Average gr.	Assimilated nitrogen per pot. gr.	Surplus.	
					Yield. gr.	Assimilated nitrogen. gr.
33	0	19.7	20.50	0.1740		
81	"	19.7				
129	"	19.0				
37	"	21.5				
85	"	21.5				
133	"	21.6				

WITH SODIUM NITRATE.

No. of pots.	Nitrogen applied per ha. kg.	Yield per pot. gr.	Average. gr.	Assimilated nitrogen per pot. gr.	Surplus.	
					Yield. gr.	Assimilated nitrogen. gr.
34	25	19.4				
82	"	22.1	22.37	0.2082	1.87	0.0342
130	"	25.6				
35	50	24.7				
83	"	25.2	23.83	0.2225	3.33	0.0485
131	"	24.6†				
36	100	31.3				
84	"	28.5	29.03	0.2531	8.53	0.0791
132	"	27.3				

WITH AMMONIUM SULPHATE.

38	25	24.7				
86	"	24.9	24.87	0.2334	4.37	0.0594
134	"	25.0				
39	50	25.2				
87	"	26.2	26.20	0.2480	5.70	0.0740
135	"	27.2				
40	100	34.1				
88	"	33.8	33.40	0.3169	12.90	0.1429
136	"	32.3				

Thus it will be seen that the *Funcus effusus* utilised, like the paddy rice plants, more nitrogen from the ammonium sulphate than from the sodium nitrate, that hence the ammonium sulphate should also here be preferred as a manure to the nitrate.

As to the effect of the different doses of the nitrogen, it is seen that the larger the quantity of both salts was applied the larger was also the yield.

Since these experiments were, in contrary to the common practice, carried out with a dry field soil, I took up again, in 1901, a second series with ordinary paddy field soil. The soil was same as that of the IV series of experiments; the methods also were just the same as in the preceding experiments.

The crops were harvested on the 26th of November with the following results.

WITHOUT NITROGEN.

No. of pots.	Nitrogen applied per ha. kg.	Yield per pot. gr.	Average. gr.	Assimilated nitrogen per pot. gr.	Surplus.	
					Yield. gr.	Assimilated nitrogen. gr.
1	0	16.0	14.50	0.1720		
21	„	15.0				
41	„	13.0				
6	„	14.5				
24	„	14.0				
46	„	14.5				

WITH SODIUM NITRATE.

2	25	21.0	18.67	0.1874	4.17	0.0154
22	„	16.0				
42	„	19.0				
3	50	19.0	17.83	0.2047	3.33	0.0327
23	„	19.0				
43	„	15.5				
4	100	17.5	17.50	0.1795	3.00	0.0075
24	„	19.0				
44	„	16.0				

No. of pots.	Nitrogen applied per ha. kg.	Yield per pot. gr.	Average. gr.	Assimilated nitrogen per pot. gr.	Surplus.	
					Yield. gr.	Assimilated nitrogen. gr.
5	150	15.5				
25	"	15.0	15.33	0.1610	0.83	(-) 0.0110
45	"	15.5				

WITH AMMONIUM SULPHATE.

7	25	22.5				
27	"	22.5	22.17	0.2379	7.67	0.0659
47	"	21.5				
8	50	22.5				
28	"	22.0	22.83	0.2669	8.33	0.0949
48	"	24.0				
9	100	31.0				
29	"	29.0	30.00	0.3924	15.50	0.2204
49	"	30.0				
10	150	32.0				
30	"	31.0	31.67	0.5231	17.17	0.3511
50	"	32.0				

Accordingly the ammonium sulphate had again a better manuring action than the nitrate and the difference of the influence of both salts are, in general, still wider than in the former experiments.

Besides, the peculiar fact is observed in the above results that the yields of the nitrate pots gradually decreased with the increase of the nitrate in the soil whilst in the pots of ammonium sulphate the yield was gradually increased proportional to the quantity of the nitrogen applied. Denitrification or formation of the poisonous nitrites from nitrate may account for this.

At last, when the actual surplus of the yield and of the assimilated

nitrogen, caused by the 50 kilograms of the ammonium sulphate are assumed respectively to be 100, the ratio of the increase caused by the same dose of the sodium nitrate will be 40 for the plus yield and 34.6 for the surplus of the assimilated nitrogen, therefore the relative value of the nitrate for the *Juncus* plants will be $\frac{40+36.0}{2}=37.3$ for 100 of the ammonium sulphate.

B. Experiments with Arrow-heads.

(*Sagittaria sagittifolia* L.)

These experiments were performed under the same conditions and treatment as the preceding experiments A. (1901). The pots were 30 in number. Each pot received one healthy tuber of arrow-head. On the 9th of September, the roots and upper parts of the plants were harvested separately. At this time, the new tubers in the roots were very small but some of the leaves begun already to die.

The crops, after having been completely air dried, were weighed and analysed with the following results.

WITHOUT NITROGEN.

No. of pots.	Nitrogen applied per ha. kg.	Crop per pot.		Average per pot.			N. in the total crop per pot. gr.	Surplus.	
		Upper part. gr.	Roots. gr.	Upper part. gr.	Roots. gr.	Total.		Yield. gr.	Assimilated nitrogen. gr.
11	0	18.8	19.0						
31	„	18.0	21.6						
51	„	19.6	20.5	18.22	19.50	38.12	0.3966		
16	„	17.3	17.0						
36	„	20.0	20.5						
56	„	15.6	20.8						

WITH SODIUM NITRATE.

No. of pots.	Nitrogen applied per ha. kg.	Crop per pot.		Average per pot.			N. in the total crop per pot. gr.	Surplus.	
		Upper part. gr.	Roots. gr.	Upper part. gr.	Roots. gr.	Total.		Yield. gr.	Assimilated nitrogen. gr.
12	25	22.7	22.5						
32	"	17.2	24.0	20.40	23.03	43.43	0.4349	5.31	0.0443
52	"	21.3	22.6						
13	50	21.4	22.0						
33	"	21.9	23.4	23.43	22.33	45.76	0.4619	7.64	0.0713
53	"	27.0	21.6						
14	100	24.3	24.6						
34	"	35.8	23.2	30.20	21.93	52.13	0.5624	14.01	0.1718
54	"	30.5	18.0						
15	150	19.4	23.7						
35	"	26.4	25.2	22.47	22.87	45.34	0.4586	7.22	0.0680
55	"	21.6	19.7						

WITH AMMONIUM SULPHATE.

17	25	27.6	25.2						
37	"	23.5	30.0	26.03	26.23	52.26	0.5025	14.14	0.1119
57	"	27.0	23.5						
18	50	36.8	25.8						
38	"	28.0	27.7	32.93	27.00	59.93	0.6067	21.81	0.2161
58	"	34.0	27.5						
19	100	48.8	28.5						
39	"	46.6	23.9	47.70	26.20	73.80	0.7690	35.68	0.3784
59	"	Died.	Died.						
20	150	60.2	26.7						
40	"	57.8	31.8	56.23	32.73	88.90	0.9959	50.78	0.6053
60	"	50.7	39.7						

The arrow-heads thus took up a considerably larger quantity of the nitrogen from the ammonium sulphate and their yields were also uncomparatively greater than when supplied with the sodium nitrate.

With the largest dose (150 kg. per ha.) of the sodium nitrate a decrease of the crop was also observed, hence it may be assumed here that denitrification had, as in the case of *Juncus*, taken place.

By qualitative tests, the presence of nitric acid, was, as in the case of the rice plants, observed, in both, *Juncus* and Arrow-head supplied with large quantity of the sodium nitrate, wherefore we may be permitted to believe that these plants have like the paddy rice plants, not sufficient capacity for the transformation of nitric nitrogen into proteids. Now calculating on the same base as in the calculation of *Juncus*, the relative value is obtained for the sodium nitrate for arrow-head as $\frac{34.1 + 32.0}{2} = 33.2$ for 100 of the ammonium sulphate.

SUMMARY.

It was sufficiently proved in all of the preceding trials, that paddy plants cannot utilize nitric nitrogen as well as ammoniacal nitrogen. The causes of this phenomenon may be :

1. Paddy plants do not accumulate a sufficient quantity of sugar in the leaves to convert all of the nitric acid, absorbed into protein.

The pale yellowish colour of the rice plants supplied with nitrate is probably due to the physiological influence of accumulated nitrate.

2. In paddy soils, denitrification and also formation of poisonous nitrites may take place.

Indeed, the pots of I and IV of the series of experiments with heavy doses of the nitrate gave a slight Griess reaction for nitrite. In order to test my suppositions as to the denitrification a quantity of paddy soil with some sodium nitrate was kept in flasks well filled. Some nitrogen was indeed gradually developed. Further detailed studies will be made later on.

As to the relative value of the nitric and ammoniacal nitrogen upon the

paddy rice plant, Juncus and Arrow-head, it is seen that for 100 of the ammoniacal nitrogen, the nitric nitrogen had the following value :

With Paddy rice	40	(The result of the 2nd series of experiments.)
With Juncus	37	
With Arrow-head	33	

If the relative value for the paddy rice plant (40) is assumed to be 100, the value of the nitric nitrogen will be 90 for the *Juncus* and 80 for the Arrow-head.

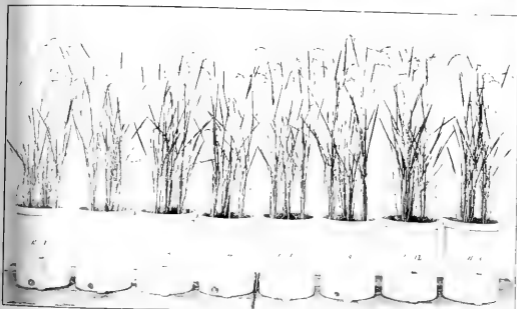




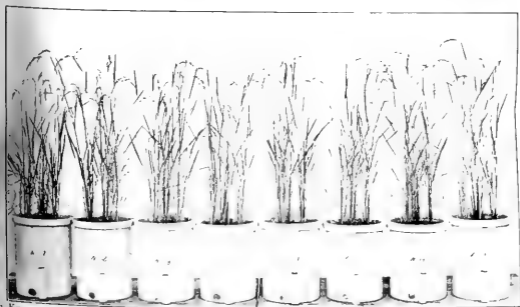




N per ha, Kg.	0	0	0	1	50	5	50
Lime Compound, ()		CaO	CaCO_3	CaSO_4	0	100	CaSO_4



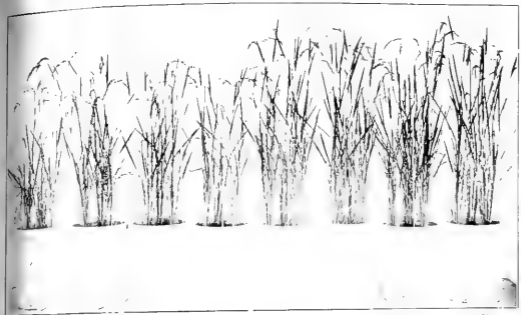
N per ha, Kg.	0	0	0	0	100	100	100
Lime Compound, ()		CaO	CaCO_3	CaSO_4	0	100	CaSO_4



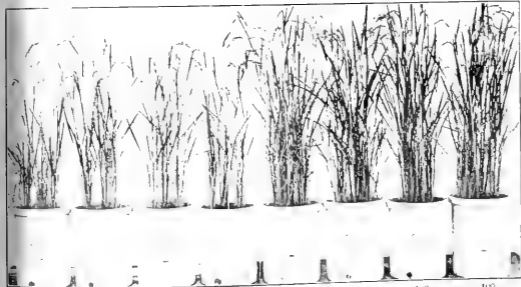
N per ha, Kg.	0	0	0	0	150	15	150
Lime Compound, ()		CaO	CaCO_3	CaSO_4	0	100	CaSO_4



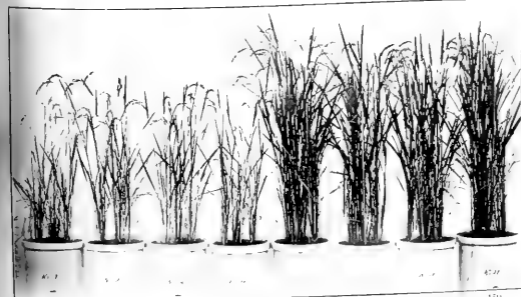
With Ammonium Sulphate.



N per ha, Kg.	0	0	0	0	50	50	50	50
Lime Compound,	0	CaO	CaCO_3	CaSO_4	0	CaO	CaCO_3	CaSO_4



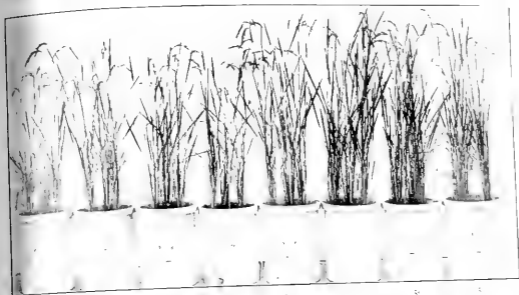
N per ha, Kg.	0	0	0	0	100	100	100	100
Lime Compound,	0	CaO	CaCO_3	CaSO_4	0	CaO	CaCO_3	CaSO_4



N per ha, Kg.	0	0	0	0	150	150	150	150
Lime Compound,	0	CaO	CaCO_3	CaSO_4	0	CaO	CaCO_3	CaSO_4



With Fish-murex.



N per ha, Kg. 0 150 300 450 600 750 900
 Lime Compound 0 0 0 0 0 0 0



N per ha, Kg. 0 150 300 450 600 750 900
 Lime Compound 0 0 0 0 0 0 0



N per ha, Kg. 0 150 300 450 600 750 900
 Lime Compound 0 0 0 0 0 0 0



N per ha, Kg. 0 25 50 100 200 300 400 500 600 700 800 900 1000
With Sodium Nitrate

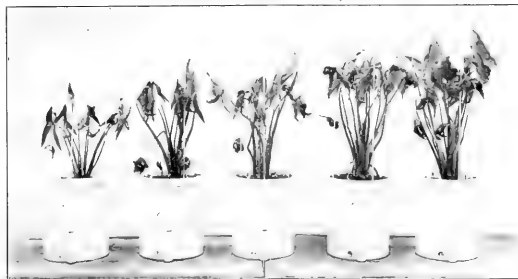
Arrow-head with Sodium Nitrate

1111 A.V. 1



0 25 50 100 200 400

Arrow-head with Ammonium Sulphate



N per ha, Kg. 0 25 50 100 200 400

On Different Degrees of Availability of Plant Nutrients.

BY

O. Loew and K. Aso.

The degree of availability of plant nutrients in the soil depends upon the size of the particles, the degree of solubility in water, the readiness of being dissolved by the humic acids of the soil or by the acidity of the rootlets and the extent of the root system.

The question of availability has recently been treated especially by *Fraps*¹, who distinguishes four factors of availability :

Chemically available plant food is that present in forms that can be taken up directly by plants.

Physical availability refers to enclosure in soil particles or protection of chemically available food.

Physiological availability refers to the difference in power of plants to assimilate food.

Weathering availability refers to the conversion of plant food into chemically available forms during the growing season of the plant.

We treat here only of some effects of different degrees of *chemical availability*.

Effects of different availability of lime and magnesia.

It has been pointed out by one of us some years ago that the most favorable ratio for plant growth of lime to magnesia in the soil is altered when the availability of these bases is not equal. The most favorable ratio, or the lime factor, $\frac{\text{CaO}}{\text{MgO}}$ was determined by us² for the condition that *the*

¹ Factors of Availability of Plant Food; Amer. Chem. Journ., vol. 32, No. 1. [1904].

² Cf. the contributions of *Aso, Furuta, Katayama* and *Daiuhara* in these Bulletins.

availability is equal. Then the best ratio was found for cereals = 1 or only a little higher while for crops with relatively more abundant foliage = 2 or 3; for tobacco it is = 4, as was recently ascertained by *Daikuhara*.

An equal availability can e.g. be assumed to exist in the soil if both these bases are present as carbonates or as hydrous silicates or as humates. In cases, however, in which the lime is present as carbonate while the magnesia as hydrous silicate or humate, perhaps a very rare case, the availability is not equal any longer, the ratio of CaO : MgO which enters into the plant body may differ somewhat from the former case, and the physiological effect of the different ratio in the plant will finally be recognized by the difference in yield—other things being equal.

It is a well known fact, further, that liming with slaked lime is more efficacious than with pulverized limestone, which is due to the most part to the finer condition of the particles of the resulting carbonate, when dissolved lime is exposed to the air.¹ It is impossible to pulverize limestone to such a degree of fineness. Hence also the degree of *availability* of both these products differs. Still more differs that of burnt magnesia and that of the commercial basic magnesium carbonate from that of pulverized magnesite.

A smaller amount of the more easily available form will not only suffice to produce the beneficial effect of the less available form, but also by an undue increase sooner reach the limit beyond which harmful effects will set in. Burnt magnesia is more injurious in overdoses than magnesite in the equivalent amount. Since exact quantitative data as to the respective physiological effects of the different forms are not yet known, we have recommended pulverized limestone and magnesite for the correction of very unfavorable ratios of CaO : MgO in soils, since in this way the natural conditions of soils was much better preserved than in the application of burnt lime or burnt magnesia.

But it is clear that sometimes very large quantities of those carbonates would be required what renders it desirable to apply smaller quantities of

¹ It occurs, however, that by insufficient attention the slaked lime left in heaps on the fields turns into a hard rock of carbonate.

the more efficacious compounds.¹ Quantitative studies, however, have to be made on an extensive scale in order to determine how much slaked lime, or artificially precipitated carbonate of lime will produce the same result as a given quantity of finely pulverized limestone and, in the case of a deficiency of magnesia, how much burnt magnesia, or artificial magnesium carbonate (basic), or magnesium sulphate would lead to the same increase in harvest as a given quantity of pulverized magnesite. We propose to call the figures obtained in comparison with 100 parts of the finest pulverized natural carbonates the *agronomical equivalents*. These figures depend only to a small extent on the chemical equivalent, they are determined mainly by the size of the particles,² solubility in water and acids of the rootlets and humus, and by the nature of the soil. A stiff clay soil will yield different figures from a loose sandy soil, especially when the presence of a considerable amount of hydrous silicates (calcium-zeolithes) in the former can change the nature of the compound applied, as, e.g., of magnesium sulphate. Recent experiments at the Imperial Japanese Central Experiment Station at *Nishigahara*, near *Tokyo*, which will be continued, have shown that when lime is present as *carbonate* and magnesia is applied as *sulphate* the best ratio of CaO : MgO which is = 1 with cereals (or nearly so) in case of *equal* salts changes into 30 : 1 with rice in sandculture, while with a soil rich in calcium zeolithe and relatively poor in magnesia it changed to 7 : 1.³ Thus we have for cereals the following best ratios under different conditions :

1 Also the price must be considered by the practical farmer. Burnt magnesia costs double as much as the same amount of magnesia in the sulphate and about 13 time as much as in the artificial (basic) carbonate. 100 Kilo crystallized magnesium sulphate cost about 7 yen (≈ 3,50).

2 Thus, the artificially prepared (basic) magnesium carbonate ($4 \text{ MgCO}_3 + \text{Mg}(\text{OH})_2 + 4 \text{ H}_2\text{O}$) being of a high degree of fineness of the particles is of much greater efficacy than the pulverized magnesite, while between artificial and natural calcium carbonate the difference in agronomical effects will be much smaller.

3 These experiments of *Kōzai*, *Daikuhara* and *Nakamura* will be published in the Bulletins of *Nishigahara*.

$\frac{\text{CaO}}{\text{MgO}} = \frac{1}{1}$	$\frac{\text{CaO as CaCO}_3}{\text{MgO as MgSO}_4} = \frac{30}{1}$	$\frac{\text{CaO as Ca-Zeolith}}{\text{MgO as sulphate}} = \frac{7}{1}$
when both bases are equally available,	with sandculture with MgO in highly available form.	with a clay soil from Kumamoto.

A recent author has declared that "magnesia as sulphate has only a very insignificant effect compared with the carbonate or citrate of magnesia" but this is an erroneous conclusion since he had compared *equal quantities* of these compounds and thus reached the point at which magnesia in the highly available form of the sulphate could exert already an injurious effect. If that author would have applied *much less* of the sulphate than of the carbonate he would soon have observed that the magnesia in that form can produce the same beneficial effect. The leading principle in the application of different magnesia manure must be to procure in equal times equal quantities of magnesia for the plant body and this end can be reached with much smaller quantities of the sulphate than of the carbonate. Beyond the most favorable ratio of CaO : MgO entering into the plant body, soon the line will be reached where an excess of magnesia will depress the yield again, and this line is sooner reached with the sulphate than with the artificial carbonate and sooner with this again than with pulverized magnesite, as was already pointed out above.

The great efficacy of magnesia in the form of sulphate had been also long ago recognized by *Nobbe* and by *Hellriegel*. *Nobbe* applied for 1 Liter of sand in his experiments 1.2 g. tertiary calcium phosphate and only 0.1 g. magnesium sulphate, and *Hellriegel* for

4 Kilo of sand

4 g. calcium carbonate and

0.18 g. magnesium sulphate

for the growth of barley in sandculture.

Magnesium sulphate at the rate of 112 Kilo per ha was frequently applied also with good results at *Rothamsted*; in this case the dose of the simultaneously applied superphosphate was such as furnished as much soluble lime, as corresponded about to the dose of the magnesia applied as sulphate.¹

¹ Cf. also Tarbaletrier, Ann. Agr. 1896.

The application of magnesia as magnesium chlorid cannot be recommended, (except in small doses in special cases) since this compound easily dissociates into base and acid and thus acts very injuriously. In the application of magnesium sulphate it must not be lost sight of that a slow and gradual action on the lime salts of the soil may take place whereby gypsum results. Our experiments showed that on addition of 1 g. calcium carbonate to 500 cc. of a 1% solution of magnesium sulphate 0.015 g. calcium sulphate was formed after two weeks standing with frequent shaking. When tricalcium phosphate was treated in the same manner 0.045 g. calcium sulphate was formed within two weeks.

Availability of lime in the form of gypsum.

It is an old observation that although calcium sulphate exerts in special cases a very favorable effect, it cannot replace slaked lime or calcium carbonate. On soils exceedingly poor in sulphates—and such soils occur more frequently than often assumed—it is the cheapest source of sulphur that can be applied. Also soils very poor in lime, especially loamy soils (but not dry sandy soils) are benefitted by gypsum to which also an indirect action, the liberation of potassa from certain compounds is ascribed. It has also been observed that many leguminous plants and potatoes are more benefitted by gypsum (200-500 Kilo per ha) than cereals are. Often it is recommended to apply gypsum together with woodash. But here it is not the gypsum which comes into action in the soil, since woodash contains potassium carbonate which transforms the gypsum into *calcium carbonate*.

The reason why lime in the form of the sulphate cannot replace the lime in the form of the carbonate¹ is due chiefly to the different degree of availability. It is true, gypsum is more soluble in water² than calcium carbonate,—since 100 p. of water dissolve at 12° C.=0.233 p. gypsum and at 60°=0.251 p., while the carbonate only very sparingly in absence of free carbonic acid,—but the reverse takes place in regard to dilute acids. An aqueous solution of carbonic acid will not dissolve more gypsum than plain

1 Also slaked lime passes of course into this form in the soil.

2 Certain salts, as ammonium sulphate or sodium chlorid increase the solubility a little.

water does (*Darcy*). Strong mineral acids augment the solubility of gypsum somewhat, but dilute organic acids exert only a very insignificant effect. Dilute acetic acid of 1 % will dissolve only traces of gypsum more than plain water. 10 g. powdered crystallized calcium sulphate was left in 100 cc. of a 1 % acetic acid for 2 days at 18-20° C. shaking the mixture repeatedly. The solution was found to contain 0.247 % calcium sulphate, while in the control flask with distilled water = 0.244 %. Hence it may be inferred that also the acidity of the roots does not enhance the absorption of lime in this form, nor has the acidity of the humus any effect on the availability of gypsum. Hence gypsum can in moderate doses neither exert the same beneficial effect as the carbonate nor in excessive doses the injurious effects in that degree as the carbonate or slaked lime.

From these facts it could be deduced that the lime content of the leaves would not increase even after addition of a great excess of gypsum to the soil. We have grown barley on a soil to which we added the enormous doses of 5% and 20% gypsum respectively. The check pots contained the original loamy humus soil and a mixture of this soil with the high dose of 5% calcium carbonate. As general manure served per pot of 8 Kilo soil:

.4 g. double superphosphate
5 g. potassium sulphate
5 g. ammonium nitrate.

The barley grains were sown (20 per pot) on March 9 and the young plants reduced to 9 per pot, leaving only such as were of equal size, on March 31. On April 17 the average height was:

Check	34.3 cm.
CaCO ₃ 5%	25.6 "
CaSO ₄ 5%	33.7 "
CaSO ₄ 20%	32.7 "

The plants were cut before the development of the ears and examined as to their lime content, with the following result:

IN 100 p. OF DRY MATTER :

	Total ash.	CaO.
Check plants	14.19	1.035
CaCO ₃ 5%.....	16.69	1.827
CaSO ₄ 5%.....	14.27	1.231
CaSO ₄ 20%.....	14.11	1.279

It will be seen that even with the enormous dose of 20% gypsum added to the soil the lime content of the leaves was not so much increased as at the dose of 5% of the carbonate. It remains to be mentioned that the gypsum plants showed a darker green than the others.

One exceptional case where gypsum has to be applied in place of the carbonate in order to avoid a depression of the harvest exists, when as the only phosphatic manure bone phosphate is at hand for manuring a soil very deficient in lime. Since calcium carbonate depresses usually the availability of phosphoric acid in bone dust, the sulphate has to take here its place, which has no such effect as was proved by *Katayama* and could be foreseen from theoretical reasons.

Sometimes the state of fertility of sandy soils rather poor in lime but producing moderate harvests, is much injured by liming with slaked lime or carbonate, perhaps by the phosphoric acid in the soil being rendered less available. In this case also the application of gypsum would be in order. But in such cases the available amounts of lime and magnesia ought to be also determined, in order to decide whether the liming was injurious on account of creating an unfavorable ratio of CaO : MgO. Such soils might be much benefitted not by increasing the lime alone, but only by a *simultaneous increase of lime and magnesia*. On this point see also the remarks farther on (p. 344).

*On the action of lime and magnesia on the soil compared
with the effects in the plant.*

Thus far the actions of lime on the soil have received much more attention from the agricultural chemists than those in the plant body. The

mechanical condition and the water holding capacity of certain soils can thus be improved, potassa can be made more easily available from certain compounds in the soil, acid humus can be rendered neutral, nitrification can be enhanced, etc. In the last mentioned two instances magnesia can exert probably the same effects as lime.¹ Since such a soil improvement leads to an increase in harvest also, it may be asked how can it be decided, whether an increase in harvest by liming is due to a physiological influence or merely to a soil improvement. In some cases this can easily be decided, in others it will be difficult; again in others the result will be a summation of both effects, since the liming in order to produce a proper ratio CaO : MgO in a soil may at the same time cause a substantial improvement of the mechanical condition.

Let us at first consider the case that a soil contains equal and sufficient quantities of lime and magnesia in equally available form. In this case no further liming would be required for physiological purposes for the growth of barley. If nevertheless the addition of lime or magnesia produces an increase in harvest, it must be inferred that a beneficial action on the soil was produced.

Some information may be gained in other cases by comparing the plus yield by liming without manure to the plus yield by liming in presence of a complete manure; sometimes the unlocking of potassa by liming an unmanured soil already too rich in lime may nevertheless produce an increase, that is, when the lime was present in a form in which it was incapable to act like caustic lime or carbonate. This may have been the cause for an increase in an experiment of *Dojarenko*.² He limed five soils, all richer in lime than magnesia, and observed in two of these cases with oats an increase of 50% in absence of manure. Also the effects of a given quantity on the soils differ—like the physiological effects—with the forms in which the lime or magnesia are applied. Slaked lime has a more powerful effect on the mechanical condition of a clay soil than carbonate, and magnesium sulphate will

¹ According to *Schl. sing.* also the mechanical condition of stiff clay soils can be improved by magnesia.

² Journ. f. exp. Landw. 1903, p. 183.

more easily unlock potassa from hydrous silicates than pulverized magnesite will do. *Meyer* added to a soil equal doses of magnesite, artificial magnesium carbonate and burnt magnesia respectively and observed plus yields in the ratio of 13 : 66 : 88. In this case an action on the soil itself has to be assumed, provided the available amounts of lime and magnesia had been determined after a reliable method. This author did not distinguish between actions on the soil and action of a certain ratio CaO : MgO in the plant, and hence was led to erroneous conclusions in regard to the limefactor.¹ The most recent experiments carried out at the Central Agricultural Experiment Station in Nishigahara again confirm our previous observations. *Kozai* overlimed intentionally a soil and regenerated the original fertility by adding the calculated amount of magnesia. *Daikuhara* increased the harvest for 100% by procuring the proper limefactor for barley on a soil from *Omagori* with 0.64% CaO and 1.91% MgO and *Nakamura* obtained an increase of 69% by increasing the amount of magnesia in a soil from *Kumamoto*, with a ratio of 1.76% CaO and 0.11% MgO. Here the absolute amount of magnesia would have sufficed for a normal crop but the ratio to the lime was so unfavorable, that an increase of magnesia was necessary for that reason alone.

Availability and Assimilability of phosphoric acid.

The availability relates to the condition in the *soil* while the assimilability to that in the *plant body*, to the transformation of the absorbed phosphates into lecithin and nucleoproteids, the latter forming the essential constituent of the nuclei and therefore being most important for the multiplication of cells, for the growth of the plants. This assimilation from the anorganic to the organic state proceeds, as one of us has pointed out, most probably from magnesium phosphate since this is much more easily hydrolyzed than the other phosphates occurring in the plant body. The amount of magnesia, however, should not exceed unduly that of lime in the cells, since under this condition a poisonous effect of the magnesium salts on the nuclei and chlorophyllbodies would result.² On the other hand a certain excess in the

¹ Cf. the criticism of O. L. in the *Landw. Jahrb.* 1905.

² As to the theory of the functions of calcium and magnesium salts cf. *Bul. No. 45* of the Bureau of Plant Industry, Washington, and these *Bulletins*, vol. IV., p. 381.

cells, of lime over the magnesia would decrease the assimilation of phosphoric acid by forming calcium phosphate and diminishing the formation of magnesium phosphate. It becomes clear therefore that—other things being equal—a certain ratio $\text{CaO} : \text{MgO}$ *within the plantcells* will be most favorable for the development. This inference was demonstrated as correct in various water, sand, and soil cultures for oats, barley, rice, bean, pea, onion, buckwheat and cabbage, and such experiments will here be continued.¹

As to the *availability* of phosphoric acid when offered in different forms, many investigations have been made of which however only a few of special relation to our questions can here come into consideration.

While the availability of the phosphoric acid in *bone phosphate* is increased by ammonium sulphate (much less by sodium nitrate), as *Seelhorst*, *Söderbaum* and *Prijanishnikow* have observed, it is on the contrary decreased by calcium carbonate, as *Kellner* and *Böttcher*, *B. Schulze*, *Nagaoka* and *Söderbaum* have found—at least under the usual conditions and in certain quantities.² In this particular instance therefore exists an analogy between the effect of a *lime excess in the cells* with that of a *lime excess in the soil*. In both cases the utilisation of phosphoric acid is depressed. But it would *not be justified* to ascribe a depression of the harvest by liming *always to a depression of the availability of phosphoric acid in the soil*, since, e.g., this is not depressed by carbonate, when the phosphoric acid is applied as secondary calcium phosphate (*Söderbaum*). Nevertheless a depression of the harvest by liming can here occur also, this is, when *the soil is already richer in lime than magnesia*, and cereals are to be grown.

The ratio of $\text{CaO} : \text{MgO} : \text{P}_2\text{O}_5$ plays a very important part in the cells, but no attention was thus far paid to this fact by agricultural chemists, the magnesia content of soils being considered generally as a negligible quantity. There may exist cases in which phosphoric acid *is easily available*

¹ In this regard it is also of interest, that *Joseph Seissl*, in his extensive investigations on the ash of potato leaves, has observed nearly the constant ratio $\text{CaO} : \text{MgO} = 2.6$ to $2.0 : 1$ notwithstanding certain variations in manuring.

² This is probably due to the neutralising of the acidity of the rootless and in certain cases to the neutralization of soil acidity.

but *not easily* assimilable, e.g., when superphosphate is added to a soil rich in calcium carbonate but relatively poor in magnesia.

Söderbaum¹ observed with *pea* that liming *depressed* the yield when small doses of superphosphate—30 Kilo per ha—were applied, but that liming *increased* the yield when large doses of superphosphate—150 Kilo per ha—had served. With oats, however, no increase was obtained in the latter case. This agrees with the inference of which one of us has drawn some years ago: *With the increase of lime over magnesia in a soil also the phosphoric acid has to be increased and with the simultaneous increase of carbonate of lime and superphosphate also the content of magnesia in the soil must be increased, when it was far below the amount of lime.* The extent of this increase is determined partly by the degree of availability of the magnesium compound applied. The above mentioned different behavior of pea and oats is easily explained by the pea requiring more lime than oats.

Summary.

The most favorable ratio of CaO : MgO or the limefactor was formerly determined by us for the condition that both those bases are present in an *equal state of availability*. This ratio changes however with the difference in availability since of the more available form also more of the base will enter into the plant and thus the ratio offered to the roots and that which enters into the plant body will differ. Magnesia in the form of burnt magnesia is more available than in the form of pulverized magnesite and in the form of magnesium sulphate still more available. The amounts in which the easily available forms of lime or magnesia can produce the same result as 100 parts of the natural carbonates in the finest powder, is proposed to call the *agronomical equivalent*. This magnitude changes with the nature

¹ Centrallbl. f. Agricultur. Chem. 1903, p. 737. Söderbaum mentions that in presence of sufficient phosphoric acid the yield was not essentially altered by increasing the amount of calcium carbonate from 500 to 5000 Kilo (increase of 0.05% CaO, to a depth of 33 Cm.). Slaked lime would perhaps shown here some difference. It is to be regretted that also here the *magnesia* content of the soil was ignored.

of the soils, and the partial transformation of the applied compounds into other forms in the soil.

The action of lime and magnesia in *physiological* respect has to be distinguished from the actions these bases exert on *the soil*.

The cause why lime in the form of gypsum acts differently from lime in the form of carbonate or slaked lime is the *low degree of availability*, since dilute acids do not increase the solubility. Even heavy doses of gypsum in the soil do not augment essentially the lime content of the leaves, and an excess of gypsum is not so injurious as an excess of carbonate.

The decrease in harvest by liming certain soils is not always due to a diminution of the *availability* of phosphoric acid, but may in many cases be due to the production of a very unfavorable ratio of lime to magnesia. *The magnesia content* of soils has always to be taken in account when *liming* and *phosphatic* manures come into application.



On the Injurious Effect of an Excess of Lime Applied to the Soil.

BY

S. Suzuki.

Various authors, *O. Kellner* and *O. Böttcher*, further *Söderbaum* and recently *M. Nagaoka* of this college, have observed that the availability of phosphoric acid of bone dust is considerably depressed by liming the soil, but not that of the superphosphate. *Kellner* and *Böttcher* believe that the dissolving action of the soil on bone dust would be paralyzed. ("das Aufschliessungsvermögen lahm gelegt wird"). However, it might also be explained by the neutralization of the acidity of the roots. It is therefore very probable that lime in other forms than carbonate and slaked lime, as for instance as calciumnitrate and sulphate, would not have this injurious action on the availability of the phosphoric acid of bone dust. It might now be supposed that whenever excessive liming depresses the yield it would always be due to the depression of the availability of the phosphoric acid of soil and manure. This is not however always the cause for the following reasons.

In the first place the experiments in *water cultures* made with culture solutions in which every nutrient with exception of ferric phosphate was present in the soluble condition, and lime in considerable excess of phosphoric acid, militates against that view¹, and in the second place the experiments with soil in which the phosphoric acid was administered as secondary sodium phosphate—certainly an available form—an excess of calcium carbonate also depressed the yield. It can hardly be assumed that

¹ In this case the depression of yield can impossibly be due to a diminished *availability* of phosphoric acid, since the latter is present in the soluble form.

the di-sodiumphosphate was transformed into the di-calciumphosphate by the calcium carbonate *in the soil*.¹ In all these cases the phosphoric acid was perfectly well available for the roots, but the transformation of the absorbed phosphate into nucleoprotein and lecithin *within the plant body* itself, or the *assimilation proper* in the cells might have been impaired by the excess of lime salt absorbed by the plant. We must distinguish between *availability* which relates to the condition in the *soil* and the *assimilability* which relates to the proper transformation of the absorbed phosphate in the *living cells*. The theory in regard to the function of lime and magnesia in the plant furnishes an explanation why an excess of lime as well of magnesia can interfere with the normal development of the cells.² In order to furnish further data for illustration of the different causes for the depression of availability and assimilability of the phosphoric acid the following experiments were made.³ The soil⁴ was taken from an unmanured field and each pot holding 8.18 kilo soil was manured,

with 10 grams potassium sulphate
15 „ sodium nitrate.

One pot received 3 grams bone dust (<0.25 m.m.) and 12 grams precipitated CaCO_3 ,⁵ and the second pot received 3 grams bone dust, and CaSO_4 equivalent to the 12 grams CaCO_3 . In the third pot the equivalent quantity of $\text{Ca}(\text{NO}_3)_2$ was applied, and in the fourth the phosphoric acid as secondary sodium phosphate, equivalent to the 3 grams bone dust (containing 22% P_2O_5), and 12 grams CaCO_3 . The fifth and sixth received only 3 grams bone dust and 1.33 grams Na_2HPO_4 respectively, while in the seventh no lime nor phosphatic compound was applied. Further, I

¹ Besides it has been shown by *Söderbaum* that di-calciumphosphate represents an easily available form of phosphoric acid.

² Comp. these Bull. IV. No. 5 p. 383.

³ The observation of *Dafert* on soils containing a considerable amount of CaCO_3 without causing the depression of the availability of phosphoric acid of bone phosphate shows that there are various influences in the soil which must come in consideration, one of these might be for instance the presence of very much humus, a source of carbonic acid.

⁴ This soil contained 92.34% of fine earth (<0.25 m.m.), and 0.55% CaO and 0.45% MgO.

⁵ This is nearly the ratio applied by *Kellner*.

intended to observe the action of soluble phosphate upon the yield in presence of a large amount of lime or magnesia in the soil. Thus, in pot No. VIII 116.86 grams precipitated CaCO_3 and in No. IX 158.93 grams burnt gypsum was applied to change the ratio of lime to magnesia in the original soil into 3 : 1, but in contrary in No. X into 1 : 3, applying 205.68 grams powdered magnesite. The quantities of general and special manures will be seen in the following table :—

No. of Pots.	General manure. g.	Bone dust. g.	Precipitated CaCO_3 . g.	Burnt gypsum. g.	$\text{Ca}(\text{NO}_3)_2$ (anhyd.) g.	Na_2HPO_4 (anhyd.) g.	Powdered magnesite. g.	Ratio of Lime to magnesia in the soil. g.
I.	{ 10 K_2SO_4 15 NaNO_3	3	12	—	—	—	—	$\frac{1.42}{1}$
II.	"	3	—	16.32	—	—	—	$\frac{1.42}{1}$
III.	"	3	—	—	19.68	—	—	$\frac{1.42}{1}$
IV.	"	—	12	—	—	1.33	—	$\frac{1.4}{1}$
V.	"	3	—	—	—	—	—	$\frac{1.24}{1}$
VI.	"	—	—	—	—	1.33	—	$\frac{1.2}{1}$
VII.	"	—	—	—	—	—	—	$\frac{1.2}{1}$
VIII.	"	—	116.86	—	—	1.33	—	$\frac{3}{1}$
IX.	"	—	—	158.93	—	1.33	—	$\frac{3}{1}$
X.	"	—	—	—	—	1.33	205.68	$\frac{1}{3}$

On April 23, seeds of upland rice were sown, 25 in each pot, and the young shoots reduced to 11 on June 6. At the middle of June a great difference in the development of plants became noticeable. Length measurement was made on July 12 with the following result :—

¹ Lime was here added in the form of gypsum to produce this ratio 3 : 1.

No. of Pots.	CaO : MgO.	Average length of stalks, c.m.	No. of Pots.	CaO : MgO.	Average length of stalks, c.m.
I.	1.42 : 1	92.7	VI.	1.2 : 1	84.0
II.	1.42 : 1	87.2	VII.	1.2 : 1	75.3
III.	1.42 : 1	87.0	VIII.	0.3 : 1	61.0
IV.	1.40 : 1	101.5	IX.	0.3 : 1	97.6
V.	1.24 : 1	97.0	X.	0.3 : 1	48.0

On July 20, ears appeared at first in pot IV. and then followed pots II., III., V., VI. and IX. on July 22. Irrigation was stopped on September 15, as at this time the plants became yellow-ripe. The plants were cut on October 10 and left to dry in each pot. The weight of the air-dry harvest was as follows :—

Addition of phosphates and magnesia or lime salts,	Total weight	Weight of seeds.	Number of stalks, bearing ears.	Average length of stalks, c.m.	Relative harvest, taking the yield in the Check pot (VII.) as 100.	
	g.	g.			Total harvest.	Seeds.
I. (Bone dust + CaCO ₃).	93.7	39.7	17	74.5	184.8	193.7
II. (Bone dust + CaSO ₄).	83.2	36.2	14	80.6	164.1	176.6
III. (Bone dust + Ca(NO ₃) ₂).	102.0	39.4	23	71.3	201.2	192.2
IV. (Na ₂ HPO ₄ + CaCO ₃).	109.4	51.0	22	67.3	215.8	248.8
V. (Bone dust).	95.0	40.5	18	73.7	187.4	197.6
VI. (Na ₂ HPO ₄).	84.0	39.5	17	75.6	165.7	192.7
VII. (o)	50.7	20.5	11	82.8	100.0	100.0
VIII. (Na ₂ HPO ₄) + Large amount of CaCO ₃ .	43.0	17.4	10	65.5	84.8	84.9
IX. (Na ₂ HPO ₄ + Large amount of CaSO ₄).	102.0	68.5	20	73.8	201.2	334.1
X. (Powdered magnesite + Na ₂ HPO ₄).	29.2	8.0	15	49.8	57.6	39.0

From this table we learn :

1. That an excessive liming with CaCO₃ depresses the yield very much, notwithstanding the phosphoric acid being present in the easily available form of secondary sodium phosphate (See Pot VIII.).

2. On the other hand, the application of CaSO_4 in equivalent quantities, under otherwise, the same conditions, led in contrary to the greatest production of seeds. This shows that in VIII not the depression of *availability* of the phosphoric acid was caused by the liming, but a depression of the *assimilability* of the phosphoric acid in the cells themselves. The great difference of the action between the calcium carbonate and calcium sulphate is very easily explained by the fact that the CaSO_4 is only absorbed from the soil in the measure as it is soluble in water, which is but little, while the absorption of lime in form of CaCO_3 depends chiefly on the *acidity* of the rootlets, and hence the amount of lime which is taken in this form into the plant body is much larger than the amount of CaSO_4 .¹
3. That the powdered magnesite added in such a ratio that the amount of magnesia became three times as high than that of lime led to a very great depression in the yield.
4. Comparing VI. with V., the seed production was almost equal, showing that the action of phosphoric acid in the form of bone dust and of di-sodium phosphate was nearly the same.
5. The application of a *moderate* amount of lime together with the bone dust has not noticeably diminished the yield (compare I with V) which may be explained by the fact that the soil applied contained 11% of humus, and since the humus has more or less an acid character it would be explained why the CaCO_3 did here in the small quantities (applied 12 grams per pot) not depress the availability of bone dust phosphoric acid, while the increase from 12 to 116.8 grams. depressed the yield more than 50% in seeds.

¹ Only small quantities of gypsum could have been transformed into phosphate, since the amount of sodium phosphate relatively to gypsum was but small.



Is the Availability of Phosphoric Acid in Bone Dust Modified by the Presence of Gypsum ?

BY

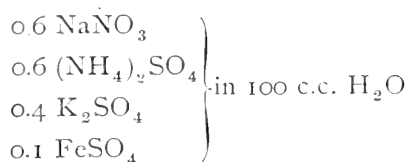
T. Katayama.

The investigations of *Kellner* and *Böttcher* of *Nagaoka*, *B. Schulze* and *Söderbaum* have shown that the availability of phosphoric acid in bone dust is very much depressed by the presence of calcium carbonate while that availability in the secondary and primary calcium phosphate is not essentially altered.

The question can now arise, how has a soil, very poor in lime, to be provided with lime, if there is no other phosphatic manure at hand but bone dust? The idea suggested itself that here the application of gypsum in place of carbonate of lime or slaked lime would be in order. This led me to compare the CaCO_3 and the calcium sulphate in this regard. The sand serving for this culture was treated first with conc. hydrochloric acid and washed with distilled water until every trace of HCl was removed. Each pot contained 2.5 kilo of this sand and was manured with 1.5 gram bone dust (0.303 grams P_2O_5). The pots received further the following ratios of powdered limestone, powdered magnesite and gypsum,

- I. $\frac{\text{CaCO}_3 = 3.6 \text{ grams.}}{\text{MgCO}_3 = 4.18 \text{ ,,}}$
- II. $\frac{\text{CaSO}_4 2\text{aq} = 6.19 \text{ grams.}}{\text{MgCO}_3 = 4.18 \text{ ,,}}$
- III. $\frac{\text{CaCO}_3 = 3.6 \text{ grams.}}{\text{MgCO}_3 = 1.04 \text{ ,,}}$
- IV. $\frac{\text{CaSO}_4 2\text{aq} = 6.19 \text{ grams.}}{\text{MgCO}_3 = 1.04 \text{ ,,}}$

For further manure a solution of



was prepared and of this solution 50 c.c. were first applied at the starting, on June 3, while another dose of 33 c.c. was applied, on July 21.¹

Upland rice served for this experiment of which 7 young shoots (2-2.5 c.m. high) were transplanted into each pot from the seed bed. The irrigation was carried out upon the principle that 65% of absolute water capacity was almost continuously present.

As early, as on the 18th of June, very remarkable differences were observed, the gypsum plants being much more luxuriantly developed than the carbonate plants and showing further a nice dark green color, while the carbonate plants had a yellowish appearance.

The average height was as follows :

	July 1.	July 21.
I.	15 c.m.	30 c.m.
II.	30 "	55 "
III.	14 "	33 "
IV.	32 "	57 "

While furthermore the ears with the gypsum plants No. II and IV had developed on August 16, there was no ear developed even 4 weeks later with the carbonate plants I and II. The plants were cut Sept. 15, and weighed in the air dry state with the following results :

¹ *Seelhorst* had observed that bone dust can be better utilized when nitrogen is applied as ammonium sulphate than when applied as sodium nitrate, but in our case the calcium carbonate interfered.

	Average height, c.m.	Weight of grains, grams.	Total weight, grams.
I.	30.0	0.00	3.2
II.	67.0	6.50	21.5
III.	31.0	0.00	4.8
IV.	69.8	7.94	28.0

This result shows 1) that the availability of P_2O_5 in bone dust was not prevented by gypsum what could already foreseen by the theory, 2) that an increase of magnesia as magnesite did not act favorably.

Since the availability of gypsum depends not upon the acidity of the rootlets¹ but simply upon the rather low solubility in water, an excess of gypsum will under most conditions not act so unfavorably as an undue corresponding excess of carbonate of lime.

One more experiment was made in which phosphoric acid was applied as secondary calcium phosphate,² all other conditions were the same as in II and IV mentioned in the above experiment. Also the time of starting and watering of the upland rice were the same. The final result was as follows :

	Average height, c.m.	Weight of grains, grams.	Total weight, grams.
II.	69.0	7.15	27.0
IV.	71.0	7.63	28.0

Also from this result it will be seen that a certain excess of lime in the form of gypsum over the magnesia as carbonate in a soil does not depress essentially the harvest of rice.

Finally it may be pointed out that while in my experiments in which humus was absent the depression of the availability of bone dust by calcium

¹ Compare the article of *Loew and Aso* in this Bulletin.

² This was prepared by adding so long dilute milk of lime to double superphosphate until the acid reaction had just disappeared.

carbonate¹ was again clearly demonstrated, the effect was very different with a soil containing 11% humus, as shown by the foregoing experiments of S. Suzuki. No depression was noticed in that case by moderate liming.

¹ This could, in my sand culture experiment, be due only to the neutralization of the acids of the roots.

Ueber den Kalkgehalt verschiedener tierischer Organe, IV.

VON

M. Toyonaga.

In meinen früheren drei Mitteilungen¹ habe ich nicht nur den Kalk- und Magnesia-Gehalt in verschiedenen Organen nach meinen Untersuchungen mitgeteilt, sondern auch darauf hingewiesen, dass der absolute Kalkgehalt der Drüsen bedeutend grösser ist als derjenige der Muskeln und der weissen Hirnsubstanz. Nur bei den Hoden hat sich eine weit geringere Kalkmenge ergeben als bei anderen Drüsen. In dieser Mitteilung handelt es sich um den Kalkgehalt der Leber von Pferd, Rind und Schwein, ferner um den der Schilddrüse des Pferdes. Für die Schilddrüse existieren noch keine derartigen Bestimmungen, während für die Leber vom Menschen und Hund solche bereits bekannt sind. Für die Leber hat Oidtmann in 1000 Teilen frischer Substanz 0,2842 Teile Ca und 0,0125 Teile Mg gefunden, während Aloy² in 1000 Teilen frischer Hundeleber 0,175 bis 0,259 Ca und 0,048 bis 0,066 Teile Mg fand.

Meine Bestimmungen wurden für die Schilddrüse der Pferdes ebenso ausgeführt wie in meinen früheren Mitteilungen erwähnt, mit folgendem Resultat:

SCHILDDRÜSE DES PFERDES.

Frische Substanz.	Wasser.	Trocken- substanz.	Asche.	CaO, (in 1000 Teilen frischer Substanz.)	MgO, (in 1000 Teilen frischer Substanz.)	Ca, Mg.
67.508 g	39.0586 g	28.4494 g	0.4818 g	0.3517 %	0.116 %	1.85
	57.858 %	42.142 %	7.137 %			

¹ Diese Bulletin, Bd. V u. VI.

² Jahr. Bericht f. Tierchemie Bd. 32. S. 700.

Was die Lebern anbetrifft so wurde hier das Wasser-extrakt separat untersucht, während das unlöslich Gebliedene mit Essigsäure von 1% extrahiert und hierin auch Kalk und Magnesia bestimmt wurde. Das unlösliche wurde zuletzt mit Alkohol (93%) extrahiert und auch in diesem Auszug Kalk und Magnesia bestimmt, Schlieslich wurde der Rückstand ebenfalls auf Kalk und Magnesia untersucht. Das Resultat ist aus folgender Tabelle zu erschen :

PFERDELEBER.

	Trocken- substanz. grams.	Asche. grams.	CaO. grams.	MgO. grams.	Ca. Mg.
Wasser-Extrakt	17.300	0.925	0.0103	0.0248	0.5
Essigsäure-Extrakt.....	12.800	0.4901	0.0261	0.0264	1.2
Alkohol-Extrakt	6.700	0.2076	0.0050	spur	
Rückstand	15.975	0.1968	spur	0.0028	
Summe	52.775	1.8195	0.0414	0.05417	0.9
In 1000 Teilen frischer Substanz.....	263.875%	6.0975%	0.207% = 0.1478%Ca	0.27085% = 0.1681%Mg	

RINDSLEBER.

	Trocken- substanz. grams.	Asche. grams.	CaO. grams.	MgO. grams.	Ca. Mg.
Wasser-Extrakt	21.070	1.0568	0.0202	0.0336	0.7
Essigsäure-Extrakt.....	8.200	0.7028	0.0242	0.0260	1.1
Alkohol-Extrakt	5.350	0.1140	0.0048	0.0024	2.4
Rückstand	12.0056	0.0538	0.0045	0.0035	1.5
Summe	42.6256	1.9274	0.0537	0.0655	1.0
In 1000 Teilen frischer Substanz	233.128%	9.637%	0.2685% = 0.1918%Ca	0.3275% = 0.1977%Mg	

SCHWEINSLEBER.

	Trocken- substanz, grams.	Asche, grams.	CaO, grams.	MgO, grams.	Ca. Mg.
Wasser-Extrakt	26.560	2.3544	0.022	0.0356	0.7
Essigsäure-Extrakt.....	12.280	0.6018	0.0209	0.0223	1.1
Alkohol-Extrakt	4.750	0.083	0.0043	spur	
Rückstand	9.863	0.0446	0.0026	0.0035	0.9
Summe.....	53.543	3.0348	0.0498	0.0614	1.0
In 1000 Teilen frischer Substanz	267.715%	15.441%	0.249% = 0.1779% Ca	0.397% = 0.1853% Mg	

Während im Wasser extrakt die Magnesia über den Kalk überwiegt, sind im Essigsäure extrakt keine wesentlichen Unterschiede zu bemerken Ob die geringe Menge Ca und Mg im Alkohol-extrakt vielleicht auf Spuren von Ca-und Mg-Seifen zurückzuführen ist, wäre wohl einer weiteren Prüfung wert.

Vergleichen wir den Calciumgehalt dieser drei Lebern mit demjenigen der Hunde- und Menschenleber, so finden wir eine ziemlich gute Uebereinstimmung, nämlich für 1000 Teilen frischer Substanz :

	Ca.	Mg.
Hundeleber	0.175-0.259	0.048-0.066
Menschenleber	0.2842	0.0125
Pferdeleber	0.1479	0.1681
Rindsleber	0.1918	0.1977
Schweinsleber	0.1779	0.1853

Jedoch ergibt sich für den Magnesiumgehalt eine sehr bedeutende Schwankung; während nämlich *Oidtmann* in 1000 Teilen Menschenleber nur 0.0125 Teile Magnesium fand, beobachtete *Aloy* in der Hundeleber das vier-bis fünffache, nämlich 0.048-0.066 Teile Magnesium in 1000 Teilen.

Hier bei unseren drei vegetabilisch lebenden Tieren¹ ist der Magnesia-gehalt bedeutend grösser.

Schliesslich mag noch darauf hingewiesen werden, dass Aloy in anderen Organen den folgenden Calcium- und Magnesiumgehalt beobachtet hat :

IN 1000 TEILEN FRISCHER SUBSTANZ.

Hund.	Ca.	Mg.	Mittel Ca/Mg.
Gehirn.....	0.014-0.028	0.072-0.084	0.26
Muskeln	0.147-0.196	0.270-0.332	0.57
Herz	0.280-0.357	0.440-0.498	0.78
Leber	0.175-0.259	0.048-0.066	3.7
Niere	0.238-0.350	0.126-0.192	1.8
Milz.....	0.392-0.448	0.054-0.072	6.8

Aus diesen Zahlen erhellt, dass der Herzmuskel bedeutend reicher an Calcium und Magnesium ist, als andere Muskeln, was jedenfalls von speciell-lem Interesse ist, weil gerade jener Muskel weit mehr Arbeit leistet als andere. Ferner ergibt sich aus Aloy's Bestimmungen, dass der Magnesium-gehalt der Niere (beim Hund) weit grösser ist, als derjenige der Milz und Leber, er ist in der Hundeniere etwa so gross als ich ihn bei der Leber von Rind, Schwein und Pferd fand.

¹ In Japan werden die Schweine ausschliesslich vegetabilisch ernährt.

Ueber das Verhalten von Fluornatrium zum Blut.

VON

M. Toyonaga.

Das Verhalten von Fluornatrium zum Blut und Muskel erregt unser specielles Interesse. Man sollte vermuten, dass es wegen seiner kalkfällenden Wirkung ganz analog den neutralen, löslichen oxalsauren Salzen wirken müsste. Die vorhandenen Angaben jedoch sind nicht in Uebereinstimmung mit dieser Folgerung. Nach Arthus¹ verhindert 0.3% NaF die Koagulation des Blutes nicht, während 0.5% einen Niederschlag giebt. Da es nur sehr auffallend schien, dass NaF so ganz verschieden auf das Blut wirken sollte als das Kaliumoxalat, stellte ich einige weitere Versuche an. In Cylindergefäße von 25–50 c.c. Capacität wurde Blut und die berechnete Menge NaF in 4% iger Lösung gegeben, während das Kontrollblut ebenso viel Wasser bekam als das Volumen dieser Lösung betrug. Ein Teil der Cylinder wurde sofort nach dem Durchschütteln in den Thermostaten gebracht, während die anderen bei gewöhnlicher Temperatur stehen blieben. Das Resultat war überraschend:

	0.3%.....	Ueber 3 Tage flüssig.
	0.5 „.....	„ „ „
Na F.	1.0 „.....	„ „ „
	2.0 „.....	„ „ „
	4.0 „.....	Nach 24 Stunden Serumschicht dickflüssig.
Controll.....		Coaguliert.
Kaliumoxalat 0.3 ...		Flüssig.

¹ Jahres-Bericht f. Tierchemie 1901–1902.

Im zweiten Versuche verwendete ich fein zerriebenes NaF anstatt der wässerigen Lösung und liess die Mischung gut durchgeschüttelt bei Zimmertemperatur stehen. Das Blut zeigte nach 10 Minuten im Kontrollgefäss beginnende Coagulation. In den Proben mit 0.3 u. 1% NaF dagegen senkten sich die Blutkörperchen ohne dass Coagulation zu bemerken war. Die Färbung des Blutes war jedoch bei der Blutprobe mit 1% NaF heller und in der Blutprobe mit 4% NaF ganz auffallend hellrot. Bei Neigung der Cylinder liess sich sogar nach mehreren Stunden beobachten, dass die untere Schicht von Blutkörperchen sich noch bewegte wie die obere Serum-schichte. Bei der Blutprobe mit 4% NaF hatte die Trennung aber noch nicht begonnen, selbst nach einer Stunde noch nicht, die Mischung war aber flüssig geblieben. Die Serumschichte bei 1% NaF wurde bedeutend trüber als die mit 0.3%, aber letztere war noch trüber als das Serum im Kontrollgefäss. Erst nach zwei Stunden begann bei dem Blut mit 4% NaF die Senkung der Blutkörperchen. Nach 24 Stunden war bei 0.3% NaF das Serum- und die Blutkörperchenschichte noch immer flüssig geblieben, letztere dickflüssig und in der Serumschichte selbst hatte sich ein geringer Niederschlag abgesondert. Bei 1% NaF war dieser weisse Niederschlag weit bedeutender, und es hatte sich im Serum selbst eine gallertartige Masse abgesondert, während bei 0.3% absolut nichts Derartiges zu sehen war. Der Blutkörperchenbodensatz war durchaus nicht koaguliert, sondern nur etwas dickflüssig. Bei 4% NaF zeigte sich nach 24 Stunden das Serum endlich getrennt von den Blutkörperchen, aber es war rot gefärbt, ein Beweis von Hämolyse. Ferner war auch hier Serum sowohl wie Bodensatz flüssig geblieben.

Es wirkt also das kalkfällende NaF ebenso Koagulation verhlindernd als neutrale Oxalate. Die Beobachtung aber, dass bei 4% NaF bei 28°C. und bei 1% NaF bei Zimmertemperatur das Serum dickflüssig, fast gallertartig geworden war, aber nicht bei 0.3% NaF, scheint mir anzudeuten, dass das Fluornatrium durch Verbindung mit dem Bluteiweiss¹ diesen Zustand herbeiführte, ebenso wie den erwähnten geringen weissen Niederschlag in

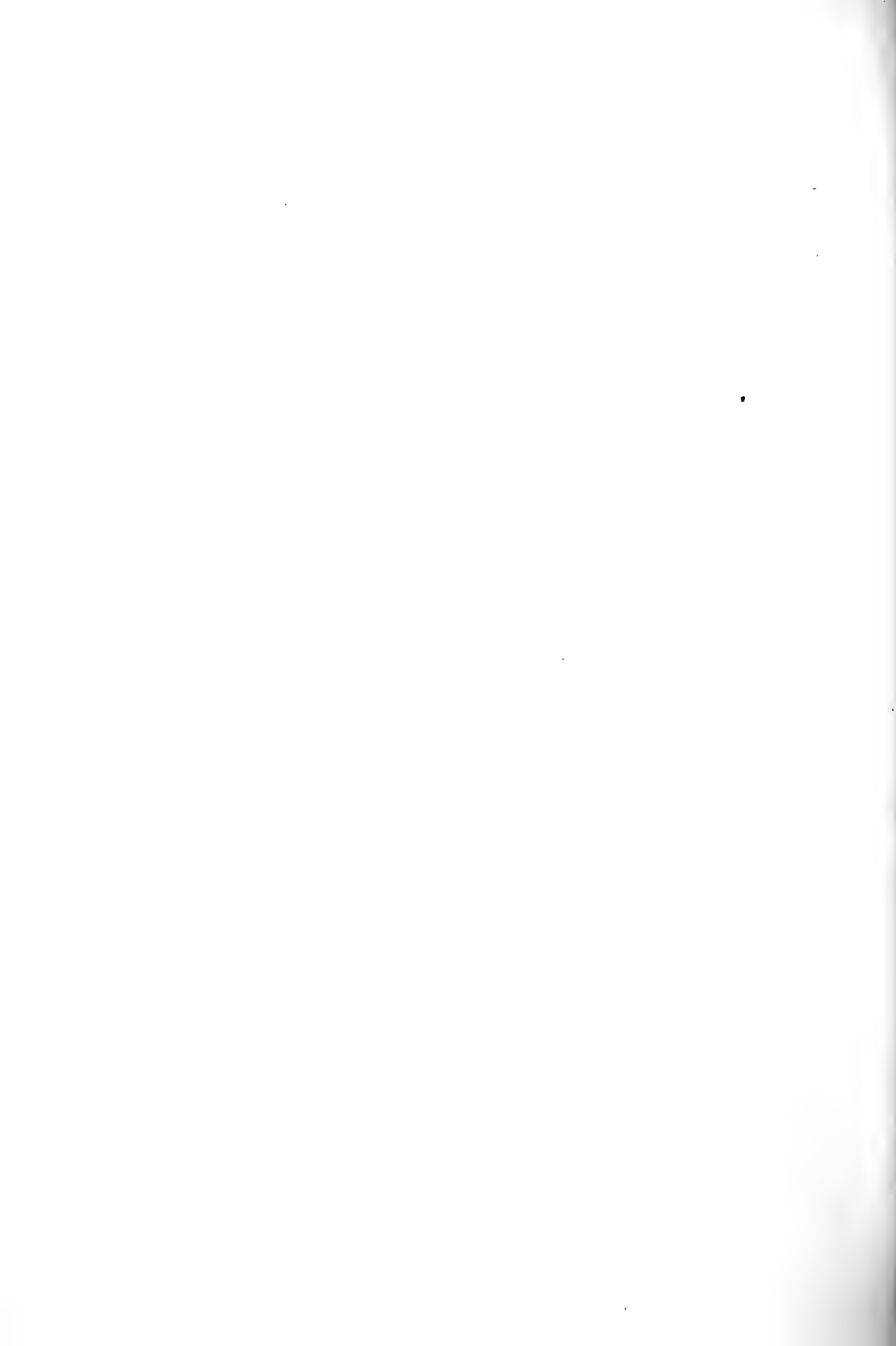
¹ Fluornatrium kann sich mit manchen Eiweisskörpern verbinden, es gibt z. B. eine Fällung mit Hefepresssaft; NaF und HF geben ferner Doppelverbindungen, welche Chloride nicht geben.

der Serumschichte, und dass diese Erscheinung keine eigentliche Koagulation ist. Auf diese Weise wäre wohl am einfachsten auch eine Beobachtung an Muskel von *Otto v. Fürth*¹ zu erklären; derselbe beobachtete: „Eine 3%-ige Lösung von NaF ruft bei Injektion in die Schenkelarterie eines frisch getöteten Kaninchens augenblicklich eine hochgradige Muskelstarre hervor, während eine 5%-ige Natriumchloridlösung selbst nach einer Stunde und später die Muskeln noch weich und beweglich lässt.“

Dass diese Erscheinung nichts mit dem Kalkgehalt der Gewebe zu tun hat, geht auch daraus hervor, dass neutrales Kaliumoxalat diese Erscheinung nicht hervorbringt. Ich habe mich selbst überzeugt, dass bei Injektion von 30 c.c. einer 10%-igen Lösung dieses Oxalats in die Schenkelarterie eines eben getöteten Kaninchens die Muskel selbst nach 15 Minuten noch weich und beweglich blieben.

¹ Hofmeister's Beiträge III, S. 565 (1903).





On the Flowering of Bamboo.

BY

Oscar Loew.

To those agricultural plants, the flowering of which is considered as a very undesirable feature by the farmers, belongs the bamboo, since after flowering and fruiting the bamboo dies off like other Gramineæ. There may pass 20 to 60 years, however, before the flowering takes place and during these many years the bamboo groves forming so often an essential part of a Japanese farm, has produced annually innumerable shoots from the extensive system of rhizomes, thus increasing considerably the income of the farmers. These shoots are cut when 20-50 cm. high and sold in the market. They form a much esteemed, popular, dish and are prepared in various ways.

Of the varieties growing in Japan it is especially *Phyllostachys mitis* that yields the most palatable shoots. *David G. Fairchild*¹ writes on the bamboo as follows :

“ The bamboo groves of Japan are not only one of the most striking features of its landscapes but one of its most profitable plant cultures. No other nation has found so many artistic uses for the plant as the Japanese and in no other country, except it be China, is such a variety of form employed by the common people. The plant is a necessity to the Japanese peasant ; it forms one of the favorite themes of the Japanese artist and out of it are manufactured some of the most delicate works of Japanese art.

¹ Bul. No. 43 of the Bureau of Plant Industry, Washington. Mr. *B. Lathrop* has warmly recommended the introduction of the Japanese bamboos into the United States. Very valuable studies on bamboos have been published by Prof. *T. Makino*.

The bamboo is in fact one of the greatest cultivated plants of this plant-loving race."

It can easily be perceived that it is considered as a great calamity when an extended bamboo grove commences after many years of existence, to develop flower.¹ Climatical conditions, as very warm and dry summers, also the age of the rhizome and perhaps the gradual exhaustion of the soil when manuring is not properly attended to, may play a rôle.

The simplest way to prevent the evil would seem to cut away all the buds as soon as they appear. But with these tall plants densely crowded in groves this would involve too many difficulties, which have also been encountered with the flowering of the sugar cane, also a dreaded phenomenon. The possibility however cannot be denied that under certain condition of nutrition the leaf and shoot formation may be so much favored that all organic nutrients are again consumed in this process, not sufficient being left for development of flowerbuds. It is well known that on different soils and with different manuring the ratio of straw to grains varies between wide limits with the Gramineæ. Although some influences acting in this direction are known, a proceeding to prevent the flowering altogether in a well developing plant is not yet known. What may be gathered from the literature in this direction is about the following :

Gypsum acts more on leaves and stems than on grains.² *Blomeyer*³ observes: "Gips lässt die Vegetation, die er offenbar sehr begünstigt, bisweilen nicht zum Abschluss kommen. Die Bohnen hören nicht auf, an der Spitze zu grünen und zu blühen, sehr zum Schaden der Ansatzes und Ausbildung der Früchte am unteren Stengelteile."

Also a rich manuring with sodium nitrate seems to act more on leaves than on flowers, as *H. Müller*⁴ observed with potatoes and sugarbeets; the starch was rapidly transformed into protein, the leaves assumed a deep

1 Of other late flowering plants may here be mentioned *Agave America*, *Larix europæa* (20-30 years), *Pinus* and *Abies* (12-40 years, according to climate).

2 E. Wolff, *Praktische Düngerlehre*, Berlin, 1892.

3 *Die Kultur der landwirtschaftlichen Versuchspflanzen* I, p. 330.

4 *Centralbl. f. Agriculturchem.* 24, p. 454; also *Chem. Centralbl.* 95, II, p. 682.

green and contained 2.5 times as much chlorophyll as the check plants, but the *formation of flowers was retarded*.

*Schneiderwind*¹ reports that potassium nitrate acts with cereals more on the development of leaves than on that of the grains, while it is the opposite with magnesium nitrate. *Nagaoka*² however did not observe such an effect of magnesium nitrate on rice.

Wilfarth³ found that manuring with little potassa and much nitrogen induced the sugarbeet to develop a rich leafy vegetation, the roots however remained poor in sugar, while under the opposite condition the roots accumulate much sugar. When the amounts of phosphoric acid and nitrogen are reduced at the same time the plants will remain very small. It has further been repeatedly observed that rich phosphatic manure leads to an early, Chilisalpeter to a late ripening. Also different potassium salts seem to have an influence on the rapidity of flowering.

Several reasons led the writer to suppose that an excess of lime and nitrogen over the other mineral nutrients should favor the growth of leaves most, to the detriment of flower formation. An experiment made in this direction led however not to a quicker leaf-formation but to a very marked increase of the size of the leaves. A barley plant which had been grown in the following culture solution :

Calcium nitrate	0.2 %
Magnesium sulphate	0.1 ..
Monopotassium phosphate	0.1 ..
Potassium nitrate	0.2 ..
Ferric phosphate	0.01 ..

and had developed three large and two small stalks, was at a height of 29 c.m. transferred into the following solution (Febr. 15)

Calcium nitrate	0.5 %
Magnesium sulphate	0.01 ..
Ammonium sulphate	0.10 ..

¹ Journ. f. Landw. 1898.

² These Bulletins VI, No. 3.

³ Zeitschr. f. Zuckerindustrie, 51, p. 323 [1901].

Monopotassium phosphate	0.10%
Potassium nitrate	0.20 „
Ferric phosphate	0.01 „

The amount of magnesium sulphate was here exceedingly small in order to make the effect of the excess of lime more clearly appear. Since some more magnesia had very probably been absorbed from the first culture solution, than absolutely needed, further growth to a certain stage could be expected; indeed within the next five weeks the height increased to 48 cm. However, the root and the lower leaves had stopped growth, it were only the youngest leaves that had grown and their growth in length and width continued three weeks longer until they had reached April 15, a quite unusual size as will be seen from the photograph, reproduced on Plate XXVII. The measurements of the two largest leaves were as follows:

Length 30 and 31 cm.—Checkplant.....	20 and 22 cm.
Width 3.6 and 3.6 cm. „	1.5 and 2.1 cm.

Soon afterwards a gradual yellowing set in and the examination of the roots showed that they had partly died off, leading to an early death of the entire plant. The cause was very probably the prevention of assimilation of phosphoric acid and the precipitation of the soluble phosphates of the protoplasm as calcium phosphate.

Manuring experiments with bamboo with the intention to check the formation of flowerbuds would naturally require a long series of years. Observations, however, made on annual grasses may justify a heavy application of gypsum and chilesalpeter in conjunction with irrigation when groves are suspected to soon develop flowerbuds, which is to be feared especially in very warm and dry summers.

The question of retarding flowering and fruiting naturally suggests the further question whether the life of the leaves of the flowering *Gramineæ* might not be prolonged beyond the usual duration. The ripening of the grains depends here upon the death of the leaves which gradually turn yellow from the lowest to finally the uppermost. Since the seeds require relatively much dipotassium phosphate it is probably this salt, so necessary for the protoplasm of every cell, which is drawn chiefly from the leaves to

the forming seeds thus causing the death of the leaves.¹ *Heinrich* observed that even the root becomes finally exhausted. This process has its analogy in the migration of a certain amount of potassa, magnesia, phosphoric acid and nitrogen from the deciduous leaves in autumn into the bark of the branches and trunk for later use again. Hence the idea suggested itself whether a dose of dipotassium phosphate applied as top dressing just after the flowering period would act beneficially in prolonging the life of the leaves which thus could produce more starch. The result would be larger and heavier seeds. I have made in conjunction with Prof. *K. Aso* several experiments in this direction but the results were not decisive, although we have not only applied single salts (6-10 g. per pot of 8 Kilo soil) but also complete nourishing solutions as top dressing after the flowering period. In some cases the leaves did not die off as rapidly as in others and also the weight of 100 grains was a little larger than with the check-plants, but these differences were too insignificant to bear any weight.²

1 According to *Wolff* and to *Ritthausen* it is one of the offices of SiO_2 to hasten here this dying process in favor of the seed.

2 Deficiency of nitrogen enhanced the yellowing process, a certain excess of lime or of dipotassium phosphate retarded it. With KCl the green was a little longer preserved than with K_2SO_4 as a potassa manure. Also a certain excess of water retards the yellowing.



Growth of Leaf caused by an Excess of Lime. To page 308, of "Flowering of Bamboo."

Further Observations on Oxidases.

BY

K. Asō.

In order to furnish further proofs that the substance contained in plantjuices which produces the guaiac reaction is not identical with a substance contained in some plantjuices that liberates iodine from potassium iodid, I have made some further experiments to show that the latter is merely a nitrite. In the first place, the following observation was made in order to test the assertion of *Bach* and *Chodat*,¹ that the guaiac reaction upon peroxids is more sensitive than the liberation of iodine by peroxids.

The common paraldehyd of commerce has generally an acid reaction and yields with potassium iodid-starch very soon an intense blue reaction due to the liberation of iodine. I entertained the supposition that this reaction is not caused by the pure paraldehyd, but by an admixture of an organic peroxid, very probably by acetylhydroperoxid.² Similar peroxids have been observed as a result of autoxidation of other aldehydes and also the common ether forms after long contact with the air, an organic peroxid, which sometimes even causes explosions in distilling such an old ether to the last drop. I shook therefore about 20 c.c. of commercial paraldehyd with an equal volume of 10% sodium carbonate solution and after washing until the alkaline reaction disappeared, a portion of that paraldehyd was distilled off. There was now observed that the iodine reaction above-mentioned did not take place neither at once nor within fifteen minutes, but only

¹ Berichte der D. Chem. Ges. 1904, XXXVII. Heft 1.

² R. H. Page, (Amer. Pat.) mentioned in the Chemiker Zeitg. Benzaldehyde (commercial) produced also the iodine reaction in traces.

an exceedingly weak reaction slowly appeared later on, which however was not intensified by the addition of some acetic acid. The original paraldehyd, however, gave an intense reaction within a few minutes. This result was sufficient to prove that it is not the paraldehyd itself, which causes the iodine reaction, but some impurity, which can only have been the peroxid above-mentioned, to judge from analogy. Now it was interesting to observe that the original paraldehyd which produced such an intense iodine reaction *had no reaction whatever on tincture of guaiacum*, not even on addition of some hydrogen peroxid. These mixtures were still colorless even after half an hour. Therefore I can not agree with Bach and Chodat when they believe „dass die Guajakreaktion auf Peroxyde bei weitem empfindlicher ist als die Jodkalium-Stärke-Reaktion.”

Also in regard to nitrites, both reagents were compared with the result that the guaiac reaction is less delicate than the potassium-iodid-starch reaction.¹ Most of plant juices produce very strong guaiac reaction, but no potassium iodid reaction. Hence the substance which produces the guaiac reaction must be quite different from that which produces the potassium iodid starch reaction, that is, the former is caused by oxidase very frequently in plant juices, and the latter by nitrite which is present in certain plant juices, as I had positively proved in one case.

But if the iodine liberation by certain plant juices would be due always to traces of nitrite and not to enzymes, how is the fact to be explained that this property is lost in most cases on heating? The probable answer is here that plant juices are often slightly acid and contain at the same time small quantities of amido-compounds. Under this condition traces of nitrites must disappear on warming, while after addition of some alkali, the reaction will probably be maintained after boiling. 30 c.c. of 0.001% potassium nitrite solution were mixed with 30 c.c. of 1% asparagine solution and divided into three parts. To one, was added a drop of dilute acetic acid, to the other a drop of dilute caustic potash solution, while the third served as control.

¹ For instance, with the solution of 0.0005% potassium nitrite, a distinct iodine reaction appeared immediately, but no guaiac reaction at once and only a trace after half an hour.

These solutions were kept boiling for five minutes and tested with potassium iodid starch with the following result :

Control.	Alkaline solution.	Acid solution.
The reaction appeared, but slower and weaker than in the alkaline liquid.	Distinctly and immedialy.	No reaction at all after several hours.

This experiment was repeated several times with the same result. Hence I became convinced that amido-compounds decompose nitrite in a very faint acid solution and it is necessary to make the solution alkaline to preserve the nitrite. Thus I made analogous experiments with plant juices. 18 grams of the buds of *Sagittaria* were crushed, extracted with 100 c.c. water and divided into three equal parts. To one, a few drops of acetic acid, to the other a few drops of caustic potash were added while the third served as control. Each solution was heated to 95° C. for 10 minutes and filtered after acidification with acetic acid, which had produced some precipitate, and tested :

	Control.	Alkaline solution.	Acid solution.
Potassium-iodid starch reaction.	No reaction at first, but after 10 min., it appeared gradually.	Distinctly at once.	No reaction at first, but after 10 min., a reaction appeared although weaker than in the control case.
Griess reaction.	Distinctly.	Distinctly.	Distinctly.
Guaiac reaction.	No reaction at all.	No reaction at all.	No reaction at all.

In order to separate the substance which produces the guaiac reaction from that which yields the reaction of Griess, the following experiments were made: 35 buds of *Sagittaria* (about 33 grams) were crushed with 55 c.c. water. To 60 c.c. of the pressed juice which yielded a very strong reaction with potassium-iodid-starch, 200 c.c. of strong alcohol (90%) were added. The mixture was left for twenty four hours and filtered. The filtrate was evaporated on a waterbath and the residue was dissolved in

20 c.c. water and filtered. The filtered liquid gave a strong Griess reaction as well as the iodine reaction very decidedly, but not the guaiac reaction while the aqueous solution of the well-washed precipitate gave, in the contrary, not the Griess reaction nor the iodine reaction, but a strong guaiac reaction. *This result proved positively that the substance which gives the guaiac reaction is not the same that liberates iodine from potassium iodid.* Similar experiments were repeated with the bud and the skin of the bulb of *Sagittaria* and the same results were obtained in each case. Experiments with buds of potato were also made with similar results.¹

CONCLUSION.

1. The guaiac reaction for peroxids is not so sensitive as the potassium-iodid-starch reaction, and the guaiac reaction for nitrites is much weaker than the iodine reaction for nitrites.
2. The reason why certain plant juices which can liberate iodine, lose that property on heating is very probably due to the acidity of the juice and the presence of traces of amido-compounds, which are very favorable conditions for the decomposition of nitrites.
3. It was positively shown against the assumption of *Bach* and *Chodat*, that the substance which gives the guaiac reaction is not the same as that which liberates iodine.

¹ A more detailed report on this subject will appear in *Beihefte Bot. Centr.-Bl.*

On the Large Bacillus observed in Flacherie.

BY

S. Sawamura.

Since *Pasteur* had demonstrated the presence of a large bacillus in the intestinal canal of the silk-worm suffering from flacherie, it has been the subject of much discussion. *Macchiati*¹ gave the bacillus the name of *Bacillus bombycis*, while the writer² and Lo Monaco³ regarded it as *Bacillus megaterium* De Bary. It seems to be also the same as the bacillus, whose pathogenic activity was first observed by *Ishiwata*, and is called sometimes by the name of "Sudden Death Bacillus."

By further investigations on this subject the writer found that the large bacillus usually seen in a large number in flacherie has slightly different culture-characters from *Bac. megaterium* De Bary; that is:—the bacillus in question produces the indol reaction which is usually wanting in culture of *Bac. megaterium*; and the colony on agar seems to grow larger than the latter. The question, whether this bacillus may be regarded as a distinct species, or a variety of *Bac. megaterium*, must be decided by further investigations. But for the sake of convenience we shall call this bacillus, for the present, by the name of *Bacillus megaterium bombycis*.

Experiment I.

This experiment was performed to observe the pathogenic action of *Bac. megat. bombycis*. 1904, May 10. The following materials were fed

¹ Le Stazioni sperimentali Agarari Italiane Vol. XX, Part II.

² Bulletin of Agricultural College, Tokyo, V, No. 4.

³ Dall' Archivio di Farmacologia e Scienze affini. Year II, Vol. II, Part VI.-VII.

together with mulberry-leaves respectively to 20 silk-worms on the fourth day of the second stage.

1. Six days old agar-culture of *Bac. megat. bombycis* suspended in water.
2. The fluid I. to which a few drops of dilute acetic acid were added.
3. The fluid I. diluted with 10 times its volume of water (average 5 bacilli in an eye-field of 800 fold magnification).
4. Pepton-glucose culture of *Bac. megat. bombycis* (10-15 bacilli in an eye-field of 800 fold magnification).

The larvae fed with an agar culture of *Bac. megat. bombycis* lost appetite, vomited a yellow fluid and died. The dead body shrunk,¹ and many of the large bacilli multiplied in the digestive canal, but the appearance of the excreta was normal.

The number of the dead was as follows :-

Date of observation.	Control.	I.	II.	III.	IV.
May 11, 3 p.m.	0	0	3	0	0
„ 12, 8 a.m.	0	10	2	0	0
„ 13, 8 „	0	1	0	1	0
„ 14, 9 „	0	-	6	0	0
Total.	0	20	11	1	0

From these figures it will be seen :

1. That it is certain that *Bac. megat. bombycis* exerts a pathogenic action on silk-worm.
2. That when *Bac. megat. bombycis* had served in a small number, however, the pathogenic effect was greatly decreased.
3. That *Bac. megat. bombycis* cultured in fluid media showed no pathogenic action.

¹ With old larvae the dead bodies stretched.

Experiment II.

This experiment was performed to observe a second time whether Bac. megat. bombycis loses its pathogenity by being cultured in fluid media.

May 14. The following materials were fed respectively to 10 larvae in the second stage.

1. Agar-culture of Bac. megat. bombycis suspended in water.
2. Five days old bouillon-culture of Bac. megat. bombycis (5 bacilli in an eye-field of 800 fold magnification).

The number of the dead within 5 days was as follows:—

Control	0
I.	10
II.	0

The results of this experiment shows again that Bac. megat. bombycis loses nearly all its pathogenity by being cultured in fluid media. Why the bacillus cultured in fluid media is less pathogenic, only further investigations can decide.

Experiment III.

This experiment was performed to confirm again that Bac. megat. bombycis does not injure silk-worms when inoculated in a small number.

May 18. The following materials were fed respectively to 10 larvae on the third day of the third stage.

1. Agar-culture of Bac. megat. bombycis suspended in water.
2. The above fluid diluted with 10 times its volume of water (average 5 bacilli in an eye-field of 800 fold magnification).
3. The fluid I. diluted with 50 times its volume of water.
4. The fluid I. diluted with 100 times its volume of water.

Two series of the experiments were performed, and one series was kept at 25° C. and the other at 18.9 C. The number of the dead was as follows:—

Date of observation.	Temperature.	Control.	I.	II.	III.	IV.
May 19, 9 a.m.	25° C.	0	7	2	0	0
„ 20, „ „	„	0	3	7	5	0
„ 21, „ „	„	0	—	0	2	1
Total.		0	10	9	7	1
May 19, 9 a.m.	18.9° C.	0	4	1	0	0
„ 20, „ „	„	0	6	6	0	0
„ 21, „ „	„	0	—	2	2	0
Total.		0	10	9	2	0

We may conclude from these results.

1. That when *Bac. megat. bombycis* is inoculated in a small number the pathogenic effect is greatly decreased.
2. That higher temperature increases the pathogenicity of *Bac. megat. bombycis*.

These facts may explain why flacherie was regarded as terribly infectious by Lo Monaco,¹ while as quite harmless by Bolle,² the cause of the difference probably being the quantity of the bacillus fed and the temperature in which the trial larvæ were kept.

Experiment II.

This experiment was performed in order to observe the action of *Bac. megat. bombycis* on animals other than silk-worm.

May 12. *Dasychira lumulata* Butl. was fed with a large quantity of agar-culture of *Bac. megat. bombycis*. On the next day one of them was killed and the multiplication of the large bacilli was observed in the intestinal canal. The remainder were kept for 5 days without observing any symptoms of the disease.

¹ Dall' Archivio di Farmacologia Sperimentale e Scienze affini. Year II., Vol. II, Part VI-VII.

² Bericht über die Tätigkeit der K. K. Landw. chemischen Versuchsstation in Görz, 1902.

In another experiment 0.1 c.c. of a water suspension of *Bac. megat. bombycis* was injected into the intestines of *Dasychira lumulata* through the anus by a specially constructed syringe. They all died on the next day. The leave-fragments in their intestinal canals were brown and the alkalinity of the intestinal fluid was weaker, and many large bacilli were detected therein.

June 23. Ten larvae of *Gastropocha pini* L. were fed with an agar-culture of *Bac. megat. bombycis* from which two died; but the cause of death seemed to be another than the infection with the bacillus, because it was not detected in the intestinal canal of the diseased. The remainder were quite healthy. Five of the other larvae were inoculated subcutaneously by stabbing softly with a sharp needle holding *Bac. megat. bombycis* on the point. On the next day 3 of them died, in whose blood the large bacilli had multiplied. On the following day one more died, but the last formed a cocoon.

Two white mice were fed with a large quantity of agar-culture of *Bac. megat. bombycis*, and one was subcutaneously inoculated, both showing no symptom of disease.

From these facts it may be seen:

1. That *Bac. megat. bombycis* is pathogenic also for other insects, although they have stronger resistance-power than the silk-worm.
2. That *Bac. megat. bombycis* is not pathogenic for mammalia.

Experiment V.

This experiment was made to decide whether *Bac. megat. bombycis* produces any poisonous substance. The difficulty in this experiment lies on the fact, that as *Bac. megat. bombycis* adheres always to mulberry leaves, it multiplies very soon in the intestinal canal of the silk-worms, when their health is injured in any way, such as feeding a sterilized bacterial culture. To obviate, therefore, any errors in this direction mulberry-leaves sterilized with formalin served. The feeding of the filtrate of the bouillon culture passed through Chamberland's filter was without effect on silk-worms.

May 30. Twenty silk-worms on the first day of the fifth stage were fed with agar-culture of *Bac. megat. bombycis* sterilized with a few drops of formalin, and afterwards reared with sterilized leaves. Other 20 larvae were also fed with sterilized leaves as a control. On May 31 two and on June 1, one of the experimental larvae died, in whose intestinal canal no bacillus was detected, the leave-fragments being green, whilst no death took place in the control larvae. The average live weight of the larvae on June 1 was as follows:—

Experimental larvae	0.790	grams.
Control	1.340	„
Difference	0.550	„

After June 1 natural leaves served from which 4 of the experimental larvae died, in whose intestinal juice the large bacilli propagated. From these results it is very probable that *Bac. megat. bombycis* produces some substance strongly poisonous for silk-worms.

Experiment IV.

This experiment was performed to ascertain the distribution of *Bac. megat. bombycis*.

1. May 2. Silk-worms, which had just been hatched from the eggs and were not yet fed, were washed with strong alcohol, and the alcohol adhering was burnt in order to sterilize the larvae. They were then cut with sterilized scissors and put into bouillon. Among 3 tubes of bouillon 2 remained clear, but one became turbid, whence plate-cultures were prepared. From these plates a large bacillus was isolated, which was proved to be *Bac. megat. bombycis* by killing 5 out of 10 larvae when the bacillus was fed to them.
2. *Bac. megat. bombycis* was also isolated from the excreta of a young larva fed 16 times with mulberry-leaves, and then it was fed to 10 larvae, which died from this within five days.

3. This experiment was repeated with the same result when this bacillus from the fresh excreta of an older larva in the third stage was used.
4. Bac. megat. bombycis cultured on agar and derived from mulberry leaves directly, killed 8 out of 25 larvae within 4 days by feeding this culture.
5. *Dasychira lumulata* died within a few days when it was injected with pure water through the anus. In the intestinal canal large bacilli always were observed which were proved to be Bac. megat. bombycis by its pathogenity to silk-worm.

From these facts it may be inferred that Bac. megat. bombycis is one of very widely distributed bacteria. It exists always in the alimentary canal of healthy insects without causing any injury to them. But when it is cultured on solid media and fed to silk-worms, death soon follows the operation. The peculiar fact can not at present be explained. Further investigations on this point are necessary.

Experiment VII.

Various other bacteria were fed to silk-worms for comparison with Bac. megat. bombycis. The results were as follows:—

Name of Bacteria.	Date.	Age of larvae.	Number of larvae used.	Dead.
1. Bacillus isolated from cherry leaves.	May 10-14.	First day of the second stage.	10	0
2. Bacillus prodigiosus.	"	"	10	0
3. Bacillus coli from Nukamiso.	"	"	10	0
4. Bacillus coli from silk-worm.	"	"	10	0
5. Bacillus II. from mulberry.	"	"	10	0
6. Bacillus IX. " "	"	"	10	0
7. Micrococcus I. B. " "	"	"	10	0
8. Micrococcus II. B. " "	"	"	10	0
9. Micrococcus III. B. " "	"	"	10	0
10. Micrococcus VII. B. " "	"	"	10	0
11. Micrococcus XI. B. " "	"	"	10	0
12. Micrococcus VIII. B. " "	May 18-21.	Third day of the third stage.	10	0
13. Bacillus megaterium, ¹	June 4-6.	Fifth day of the fifth stage.	5	0
14. Bacillus coli from silk-worm.	"	"	5	1

Except Bac. coli there were no bacteria which caused the disease of silk-worm by feeding, as Bac. megat. bombycis did.

¹ Obtained from Germany.

Experiment VIII.

The following bacteria were used to observe their pathogenity for silk-worms by subcutaneous infection.

Name of bacteria.	Date.	Age.	Number of larvae used.	Dead.	Remarks.
1. Sudden death bacillus, ¹	May 30 -June 3.	First day of the fifth stage.	6	6	Numerous large bacilli in the blood; dead bodies soft and black.
2. Micrococcus II. B. from mulberry.	"	"	5	0	
3. Micrococcus VIII. B. from mulberry.	"	"	5	0	
4. Micrococcus VII. B. from mulberry.	"	"	5	0	
5. Micrococcus from healthy silk-worm.	"	"	5	0	
6. Bacillus typhi murium.	"	"	5	0	
7. Bacillus coli from silk-worm.	June 1-4.	Second day of the fifth stage.	5	5	Short bacilli in the blood; dead bodies shrunken.
8. Bacillus pyocyaneus.	"	"	5	4	Many bacilli in the blood; dead bodies with green tint.
9. Bacillus megaterium, ²	"	"	5	2	Large bacilli in the blood; dead bodies soft and black just as those killed by Bac. megat. bombycis.
10. Micrococcus II. A. from mulberry.	"	"	5	0	

¹ Provided by Mr. Hayashi. Nomura believes this identical with *B. alvei*.

² Obtained from Germany.

Name of bacteria.	Date.	Age.	Number of larvae used.	Dead.	Remarks.
11. <i>Bacillus megaterium</i> , ¹	June 4-6.	Fifth day of the fifth stage.	5	5	Same as 9.
12. <i>Bacillus coli</i> from silk-worm.	"	"	5	4	Same as 7.
13. <i>Bacillus coli</i> from Nukamiso.	"	"	5	4	Same as 7.
14. <i>Bacillus pyocyaneus</i> .	"	"	5	5	Same as 8.
15. <i>Bacillus acidilactici</i> Hueppe.	"	"	5	0	
16. <i>Bacillus subtilis</i> .	"	"	5	0	
17. <i>Micrococcus</i> II. A. from mulberry.	"	"	5	1	Large bacilli in the blood; dead body black.
18. <i>Micrococcus</i> I. from silk worm with black spots on the fore-part of the body.	June 6-8.	Seventh day of the fifth stage.	3	0	
19. <i>Micrococcus</i> II. from the same worm.	"	"	3	0	
20. Short bacillus from the same worm.	"	"	3	0	
21. <i>Micrococcus</i> I. from healthy silk-worm.	June 7-10.	Fifth day of the fifth stage.	5	0	

¹ Obtained from Germany.

Name of bacteria.	Date.	Age.	Number of larvae used.	Dead.	Remarks.
22. Micrococcus V. from healthy silk-worm.	June 7-10.	Fifth day of the fifth stage.	3	0	
23. Micrococcus VI. from healthy silk-worm.	"	"	3	0	
24. Micrococcus II. from sick worm with black spots.	"	"	5	0	
25. Micrococcus IV. from the same worm.	"	"	5	0	
26. Short bacillus from the same worm.	"	"	3	0	
27. Bacillus megaterium bombycis.	"	"	3	3	Same as 1.
28. Bacillus from healthy silk-worm.	June 9-13.	First day of the fifth stage.	5	0	
29. Micrococcus pyogenes aureus.	June 15-17.	Sixth day of the fifth stage.	5	3	Many micrococci in the blood; dead body of one hard but two soft; not blackened.
30. Bacillus prodigiosus.	"	"	3	0	
31. Sarcina lutea.	"	"	5	0	
32. Bacillus megaterium bombycis from mulberry.	June 9-14.	First day of the fifth stage.	5	5	Same as 1.
33. Bacillus from sick worm.	"	"	5	0	

From these results it follows that many bacteria, such as *Bac. megat.*, *coli*, *pyocyaneus*, *Micrococcus pyogenes aurcus*, &c., can multiply in the blood of silk-worms and kill them as *Bac. megat. bombycis*. As the action of *Bac. megat.* on silk-worm is the same as of *Bac. megat. bombycis*, it is very probable that the latter is a variety of the former.

GENERAL CONCLUSION.

In the former investigations the writer assumed that flacherie is caused not by any special bacterium but by several, which occur commonly on mulberry-leaves. This assumption is also confirmed by the results of these experiments.

The writer expresses his sincere thanks to Mr. Hayashi who provided him with a culture of "Sudden Death Bacillus" and to Mr. Yamasaki, Assistant of the College.

TEMPERATURE DURING THE EXPERIMENTS.

Date.	Temperature.	Date.	Temperature.	Date.	Temperature.	Date.	Temperature.
May 1	15.0° C.	May 13	20.0° C.	May 25	12.8° C.	June 6	23.2° C.
" 2	12.2	" 14	11.1	" 26	15.0	" 7	22.3
" 3	21.1	" 15	15.0	" 27	16.7	" 8	23.7
" 4	17.8	" 16	18.9	" 28	17.2	" 9	21.6
" 5	18.3	" 17	23.3	" 29	17.8	" 10	22.5
" 6	15.0	" 18	18.9	" 30	18.9	" 11	19.9
" 7	16.1	" 19	18.9	" 31	19.4	" 12	20.5
" 8	17.2	" 20	18.9	June 1	21.6	" 13	21.5
" 9	17.2	" 21	18.9	" 2	21.1	" 14	20.2
" 10	21.7	" 22	20.5	" 3	21.7	" 15	20.3
" 11	20.6	" 23	19.4	" 4	20.6	" 16	21.0
" 12	22.2	" 24	12.2	" 5	21.9		

Some New Varieties of Mycoderma Yeast.

BY

T. Takahashi.

The "Kahmhefe" well known by the rapid formation of a covering film on the surface of nutrient liquids occurs very abundantly in Sake, Sake-mash and Kōji i.e. boiled rice covered with a vegetation of *Aspergillus Oryzæ*. But the researches hitherto made on this subject are very scanty. Klöcker and Schioning¹ mentioned the presence of a variety of *S. Anomalus* in sake-kōji, Yabe² observed the presence of a mycoderma yeast on the rice straw used in the sake factories. Kozai cultivated from kōji two kinds of "kahmhefe" viz. a variety of *Saccharomyces anomalus* with hat shaped spores and an asparogenous yeast, both causing a feeble alcoholic fermentation and producing acetic ether in beer wort. Quite recently K. Saitō isolated from sake a kind of *S. Anomalus*, which produced acetic and butyric ethers in beer wort or in kōji extract and was capable of fermenting dextrose, levulose, saccharose, galactose and sparingly also maltose.

As this kind of yeast causes various changes in the constituents of the substrata, in which it grows, it is very important to investigate its character and also the changes which it produces in nutritive liquids.

From this point of view, I have isolated several varieties of Mycoderma yeast³ from sake, kōji and sake-mash and studied their morphological as well as physiological properties. They are briefly described in the following table.

1 Centralblatt f. Bakt. Parasitenkunde. Alth II. Bd. I 1895, p. 777.

2 Bulletin of Imp. Univ. College of Agr. Vol. III, No. 3, P. 223.

3 By mycoderma yeast I mean the kind of *Kahmhefe*, which is in capable of forming spores.

MYCODERMA A, FOUND IN KŌJI. (Plate XXVIII A).

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Elliptic and sausage shaped, 1.5-2 μ . in b. and 12-15 μ . l. Rich in vacuoles (4-5), which contain refractive and revolving bodies or granules.	On the wort (kept at 22° C.), a thin white mealy film is formed after 22 hours. The film changes to greyish brown and assumes a mesenteric structure. The colonies on the wort-gelatine are round chalky white with mesenteric surface. Streak culture on wort or kōji-extract gelatine have a coarse mesenteric appearance; the gelatine is very slowly liquefied. Giant colonies on wort-gelatine are greyish in color and folded especially on the margin; on sugar-bouillon-agar, it gives white mesenteric coatings.	Ferments glucose, saccharose, but not maltose. Assimilates glucose saccharose, maltose.	Optimum temperature; 19-25° C. Killed at a temperature of 50° C. in 10 minutes.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH_3OH .	Assimilation of nitrite, ³ glycerin and alcohol.
Alcohol (0.7-1 %) contained in Mayer's and Nægeli's solution devoid of sugar, is changed into acetic acid, which is gradually further oxidized into CO_2 and H_2O . This phenomenon is also observed in the kōji-extract culture. In sake containing 13.2% (16.33 vol. %) of alcohol, no growth takes place.	It formed 2.2% of alcohol after standing for 10 days in wort at 21-26° C. 3.35% alcohol, 0.199% of fixed ¹ and 0.201% of volatile acid were reformed in kōji's-extract after 10 days culture at 19-26° C., while after 15 days under the same condition were found only 0.024% fixed and 0.057% of volatile acid.	CH_3OH was found in kōji-extract culture.	Assimilate glycerin and alcohol.

1 The fixed acid was calculated as succinic acid, the volatile acid as acetic acid.

2 As inferred from the reaction of *Carcucuve* and *Colton* and by the formation of anilin-violet. This small amount of methyl alcohol is probably an oxidation product.

3 Nitrite was given in nutritive solution containing glycerin instead of sugar. Assimilation can not take place when the solution is made distinctly acid by acetic acid (1 per mille). Here evidently a poisonous action took place.

MYCODERMA B (Plate XXVIII B), WAS FOUND IN MOTO-MASH.

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to Sugar.	Optimum and killing temperature.
Oval or elongated but not filamental as in mycoderma A. 3-5 μ . long, 2-3 μ . broad. The revolving granules in vacuole are rare.	On wort or kōji-extract it forms a yellowish brown corrugated film often with a reddish tinge. Colonies on the wort-gelatine are rounded, chalky or a loose mass. Streak cultures on wort-gelatine are mealy but granular somewhat concave; on kōji-extract gelatine colonies waxy and finely striated. Liquefies gelatine very slowly on the margin. Giant colonies on wort-gelatine are elevated toward the centre and decorated with concentric rings, and radiating lines. The margin is almost smooth. On sugar-bouillon agar it forms chalky white mesenteric giant colonies.	Ferments glucose but not saccharose or maltose. Assimilates these three sugars.	Optimum temp. 26° C. 56° C. for 10 m. is not sufficient to destroy the vitality; it is killed at 60° C.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of $\text{C}_2\text{H}_5\text{OH}$.	Assimilation of nitrite, glycerine and alcohol.
Alcohol (0.7-1 %) contained in Mayer's and Nāgeli's solution, devoid of sugar, was assimilated but not converted into volatile acid. In sake with moderate contents of alcohol growth was observed. When the amount of alcohol reaches, however, 10.7-13.23%, no development takes place. Oxidation of alcohol took place in kōji-extract culture.	In wort or kōji-extract after 10 days 2.2% of alcohol, and in the latter media 0.028% of fixed and 0.0095% of volatile acid, while in 15 days 1.8% of alcohol, 0.120% of fixed and 0.0185% of volatile acids. After 15 days was found 1.8% of alcohol in the wort culture.	$\text{C}_2\text{H}_5\text{OH}$ was found in kōji-extract culture.	Assimilates nitrite glycerine and alcohol.

This mycoderma differs from mycoderma A, by having no filamental form, not fermenting saccharose, developing on sake and *assimilating nitrite*.

MYCODERMA C (Plate XXVIII C), ISOLATED FROM KŌJI.

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Oval, elongated sausage or clup-shaped and rich in vacuoles after containing revolving refractive granules. Oval $2.5 \times 3.5 \mu$, elongated $1.4 \times 4.9 \mu$, sausage and clup-shape $1.4 \times 1.2 \mu$.	On wort or kōji-extract kept at 22 or 26.5°C . no film was formed but on the latter kept at $28-30^\circ \text{C}$. there was soon a formation of a film. Therefore this mycoderma differs from two preceding species being only capable of forming a film at higher temperature on the above media. On <i>Hayduck's</i> solution it forms a film also at lower temperature ($15-22^\circ \text{C}$). On the wort-gelatine it forms mesenteric waxy colonies. Streak cultures on the wort-gelatine give coarse pasty colonies; on kōji-extract gelatine greyish, firmly corrugated colonies. The gelatine is very slowly liquefied. Giant colonies on wort-gelatine are white mesenteric, with irregular margin; on sugar-bouillon-agar white radiated and folded colonies.	Ferments only glucose. Assimilates glucose, saccharose but not maltose.	Optimum temperature $28-30^\circ \text{C}$. The cells are killed at 54.4°C . in 10 Minutes.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of $\text{CH}_3 \cdot \text{OIL}$.	Assimilation of nitrite, glycerin and alcohol.
Alcohol (1%) contained in Mayer's solution, devoid of sugar, was converted into acetic acid. In sake containing 13.3% of alcohol no growth takes place.	In kōji-extract 2.78% of alcohol and 0.155% fixed, 0.0095% volatile acid were formed at $19-26^\circ \text{C}$. after 10 days; while after 15 days there were found 2.6770% alcohol, 0.034% fixed and 0.032% of volatile acids, chiefly formic and butyric acids. In wort at $21-25^\circ \text{C}$. after 10 days, was found 2.5% of alcohol. Also the formation of <i>acetic acid from glycerin</i> , which is contained in <i>Hayduck's</i> solution, devoid of sugar.	$\text{CH}_3 \cdot \text{OH}$. was found in the culture of kōji-extract.	Assimilates alcohol, glycerine but not nitrite.

This yeast differs from the preceding species by the higher optimum temperature and the formation of acetic acid from glycerin and by varying shapes.

MYCODERMA YEAST D (Plate XXVIII D),
ISOLATED FROM KŌJI.

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Oval, elliptic, sometimes wedge-shaped, rich in vacuoles containing refractive granules of fatty nature. 2-3.5 μ . b., 3-7 μ . l.	On wort or kōji-extract forms a strongly developed, highly corrugated film, consisting chiefly of oval cells. The color of the film is in the beginning chalky white (on kōji-extract) or white (on wort), but afterwards changed into greyish yellow, often with a reddish tinge. On wort-gelatine it forms a round, chalky white colony. Streak cultures on wort or on kōji-extract gelatine have a mealy appearance. The gelatine is very slowly liquefied. Giant colonies on wort-gelatine are brownish yellow, remarkably elevated toward the centre and highly corrugated, on sugar-bouillon agar have a feather like appearance.	Ferments only glucose, assimilates glucose, saccharose, maltose.	Optimum temperature 25-30° C. Killing temperature 56° C. (15 min.) 100° C. (1 hr.)

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of $\text{C}_2\text{H}_5\text{OH}$.	Assimilation of nitrite, glycerine and alcohol.
Alcohol (1%) contained in Mayer's solution, devoid of sugar, is changed into acetic acid. The same phenomenon was found in kōji-extract culture. In sake containing 13.3% of alcohol kept at 25° C. film formation took place. After 13 days there was found only 2.28% of alcohol.	In kōji-extract kept at 19-26° C. was found after 10 days 2.39% and after 15 days 5.44% of alcohol and in the latter case 0.0911% of fixed, and 0.01422% of volatile acids were found. Among volatile acids, acetic and butyric acids were present. In wort kept at 21-26° C. after 10 days was found 3.23% of alcohol.	$\text{C}_2\text{H}_5\text{OH}$ was formed in kōji-extract culture.	Assimilates all three compounds.

Thus this yeast is distinguishable from the wedge-shaped cells, by the production of a higher percentage of alcohol (5.44%) and the growth on sake containing a higher percentage of alcohol (13.3%).

MYCODERMA YEAST E (Plate XXVIII E), FOUND IN KŌJI.

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugar.	Optimum and killing temperature.
Round, oval shaped or elongated, sausage, often wedge-shaped. The elongated cells are especially rich in vacuoles containing revolving refractive granules. 1.2-1.5 μ . b. 1.4-0.4 μ . l.	On kōji-extract forms slowly a thin film. On wort at first white and thickly fouled, altering in color to gold yellow afterwards. Colonies on the wort-gelatine or kōji-extract-gelatine it forms chalky white <i>mesenteric colonies</i> , of which the color changes gradually to greyish-yellow. Giant colonies on wort-gelatine are white with a yellow tinge and highly corrugated. Those on sugar-bouillon-agar are slightly brown and more or less granular in the centre, while toward the periphery radiating lines, run regularly. In addition concentric rings occur on the surface.	Ferments <i>glucose, saccharose</i> but not maltose. Assimilates the former two, but not maltose.	Optimum temperature 22-24° C. On heating 55.4° C. for 10 m. is sufficient to kill the cells.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH_3OH .	Assimilation of nitrite, alcohol and glycerine.
Alcohol (7-1%) contained in Mayer's and Nægeli's solutions, devoid of sugar, changed into <i>acetic acid</i> . The same change was observed in kōji-extract culture. In sake containing 13.3% of alcohol no growth was found.	In wort kept at 21-26° C. during 10 days was formed 4.62% of alcohol. In kōji-extract kept at 19-26° C. in 10 days 2.4% of alcohol and 0.0589% of fixed, 0.00% of volatile acid, while in 15 days 4.75% of alcohol, 0.0392% of fixed and 0.161% of volatile acids.	CH_3OH was formed in culture of kōji-extract.	Assimilates alcohol, glycerine, but not nitrite.

By these properties this yeast can be distinguished from the preceding varieties, except *Mycoderma A*, by fermenting saccharose. Further from *Mycoderma A*, it differs by not assimilating maltose and having various cell forms and also by forming larger quantities of alcohol.

MYCODERMA F (Plate XXVIII F), FOUND IN SAKE.

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Mostly elongated cells. 1.5-2 μ . b., 2-10 μ . l. Cells are rich in vacuoles containing revolving refractive granules.	On the wort or kōji-extract forms a thin white mesenteric film, which alters gradually to yellowish or brownish white with a rosy tinge (on wort) resembling to that of Mycoderma yeast B and I. On wort or kōji-extract-gelatine, it forms powder like colonies. The liquefaction of gelatine sets in slowly. Giant colonies on wort gelatine are conical in shape and yellowish white in color. Thin margins are irregularly defined, while the surface are ornamented with deep lines regularly running from the centre to the periphery. On sugar-bouillon agar it forms a coarse mesenteric giant colony.	Ferments glucose only. Assimilates glucose, saccharose, and maltose.	Optimum temperature 22-24° C. 56° C. for 10 m. will cause the death of the cells.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH_3OH .	Assimilation of nitrite, alcohol and glycerine.
Alcohol (7-1%) contained in Mayer's and Nāgeli's solutions, devoid of sugar, was changed into acetic acid. The same phenomenon was observed in kōji-extract culture. In sake containing 10.7% of alcohol, it grows very well forming a very thick mesenteric film. After 36 days there was found only 0.32% of alcohol. Higher percentage (13.3%) of alcohol in sake depressed its growth.	In wort kept at 21-26° C. after 10 days was found 2.72% of alcohol. In kōji-extract held at 19-26 C. in 10 days, there was found 2.11% of alcohol, 0.0627% of fixed and 0.0057% of volatile acids, the latter was found to consist chiefly of acetic acid with a trace of butyric acid. After 15 days alcohol increased to 3.41% and 0.021% of fixed and 0.0010% of volatile acid were found.	CH_3OH found in the kōji-extract culture.	Assimilates all three.

It will be seen that, this yeast differs from Mycoderma A, C, E by assimilating nitrite. From Mycoderma B, D by destroying the fixed acids and still from the former by having lower killing and optimum temperatures. Moreover from species D by not showing wedge shape. From Mycoderma C and E by growing on sake.

MYCODERMA YEAST I (Plate XXVIII I),
FOUND IN MOTO MASH.
GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Mostly oval or elliptic somewhat resembling <i>Sacch. exiguus</i> being 1.0-2.2 μ b. and 1.4-2.2 μ l. The cells have generally a homogenous protoplasm provided with vacuoles with 1 or 2 revolving granules.	On kōji-extract or wort this species forms a finely corrugated film. The color is chalky white in the beginning. It changes gradually into a faint yellow (on kōji-extract) or bright rosy brown (on wort). The film formation is complet on wort after 2.5 hours at 26.5° C. ¹ On kōji-extract-gelatine it formes a somewhat waxy colonies, but on wort-gelatine rather mealy. The gelatine is liquefied rapidly. Giantcolonies on wort-gelatine have conical form of slight yellowish color. The margin is well defined and the surface is furnished with fine radiating lines. On sugar-bouillon agar it forms rather flat and smooth giant colony.	Ferments only glucose. Assimilates glucose, saccharose, and maltose.	Optimum temperature 22 - 24° C. Ten minutes heating at 5.44 C. is not sufficient to kill the cells.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH ₃ .OH.	Assimilation of nitrite, alcohol and glycerine.
Alcohol contained in culture solution, devoid of sugar, was converted into acetic acid. In sake containing 10.77% of alcohol there was found a good growth. After 30 days the percentage of alcohol was reduced to 4.50%.	In kōji-extract kept at 19-26° C. after 10 days cultivation were formed 3.41% of alcohol, 0.126% of fixed and 0.0057% of volatile acids; after 15 days 5.09% of alcohol, 0.0514% of fixed and 0.0361% of volatile acids. In wort kept at 21-26° C., there were found 3.41% of alcohol after 10 days.	CH ₃ .OH was found in kōji-extract culture.	Assimilates all three.

This yeast differs from *Mycoderma* A, C, E by assimilating nitrite. From all the preceding species by its very small size. Further it differs from *Mycoderma* C and E by assimilating maltose, and from species A, E by not fermenting saccharose. Still further it differs from *Mycoderma* A, C, E by the growth on Sake, and from species B, D by the faculty of decomposing fixed acid. From species F it differs by the very quick formation of the film on wort at 26.5° C.

¹ This is one point which distinguishes this yeast from *Mycoderma* F, which forms the film after 4.3 hours under the same condition.

MYCODERMA YEAST M (Plate XXVIII M),
ISOLATED FROM KŌJI.

GENERAL CHARACTERISTICS.

Form and usual size	Growth.	Behavior to sugars.	Optimum and killing temperature.
Round, oval or long filamental form. The long cells have many vacuoles, in which revolving refracting granules are present. 2.0-2.5 μ . b., and 2.5-15 μ . l.	On wort or kōji-extract it forms a rather brownish and very thick film. On wort-gelatine the colonies are chalky white with central elevation. The margin part has very fine lineations. Streak culture on kōji-extract-gelatine gives light brown colonies. They are rather smooth but the margin part has fine lineations. It liquefies gelatine slowly. Giant colonies on wort-gelatine seems white mesenteric and somewhat moistened, while on sugar-bouillon-agar white with very coarse folds.	Ferments only glucose. Assimilates glucose, saccharose, and maltose.	Optimum temperature 27-30° C. 10 m. is not sufficient to kill the cells; it suffices at 50° C. for 10 m.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH_3OH .	Assimilation of nitrite, alcohol and glycerine.
Alcohol (7-1 %) contained in Mayer's and Nægeli's solution is changed into acetic acid. This fact is found in the kōji-extract culture. In sake containing 13.3% of alcohol no growth was observed.	In kōji-extract, there was found at 19-26° C., 2.39% of alcohol, 0.0374 % of fixed and 0.00761% of volatile acids in 10 days, while in 15 days was found 2.56% of alcohol, 0.0935 % of fixed and 0.0266% volatile acids. In wort held at 21-26° C. was found 2.11% of alcohol in 10 days. The volatile acid consisted chiefly of acetic and butyric acids.	Trace was found in kōji-extract culture.	Assimilates glycerine alcohol, but not nitrite.

Thus it is obvious that this yeast differs from Mycoderma B, D, F, I by not assimilating nitrite. From Mycoderma C and E by assimilating maltose, and from mycoderma A by not fermenting saccharose. Further it differs from all of the preceding species except C by having a higher optimum temperature for the growth.

MYCODERMA N (Plate XXVIII N), WAS FOUND IN KŌJI.

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Very long filamental form, rich in vacuoles which containing revolving granules and the size: 3 μ . b., 7-30 μ . l.	On kōji-extract or wort makes <i>very quick</i> development of the film, which is at first white mesenteric changing afterward yellowish (kōji-extract) or yellowish-brown (on wort). The colonies on wort-gelatine seems greywhite and mesenteric. Streak culture on wort or kōji-extract-gelatine gives beautiful greyey coarse mesenteric colonies. It liquefies gelatine rather quickly. Giant colonies on wort-gelatine are white and seem mesenteric without elevation; on sugar-bouillon-agar it forms a coarse, chalky white mesenteric colonies.	Ferments only glucose. Assimilates glucose, saccharose, and maltose.	Optimum temperature 22-30° C. The heating to 54° C. for 10 m. causes the death of cells.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH ₃ . OH.	Assimilation of nitrite, alcohol and glycerine.
Alcohol contained in culture solution changed to acetic acid. The same fact was observed in kōji-extract culture. In sake containing 10.77% of alcohol it forms a yellowish-brown mesenteric film, but the increase of the alcoholic content to 13.3% depressed the growth.	In kōji-extract kept at 19-26° C. in 10 days, there was found 2.94% of alcohol, 0.0864% of fixed and 0.047% of volatile acids, while after 15 days the alcohol decreased to 2.56%. In wort it forms 3% of alcohol at 21-26° C. during 10 days. The volatile acids consist of acetic and butyric acids.	Forms CH ₃ . OH in kōji-extract culture.	Assimilates glycerine, alcohol but not nitrite.

This yeast differs from mycoderma B, D, F and I by not assimilating nitrite, it differs from species C and E by assimilating maltose. From species A and E by the incapability of fermenting saccharose. Further from species M, E, C, A by the growth on sake.

MYCODERMA YEAST O (Plate XXVIII O),
 WAS FOUND IN KÖJL.
 GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Round, clup or long ellipse and the latter has vacuoles rich in revolving granules. 2.5-3 μ . b., 10-18 μ . l.	On kōji-extract or wort forms somewhat greyey film with thick foulds. The culture on wort-gelatine gives waxy and more or less mealy colonies carrying veins on the margin. Streak culture on wort-gelatine mesenteric with a slightly greyey color, on kōji-extract-gelatine it makes a chalky white growth. Giant colonies on wort-gelatine are pasty and half granular, but with no coloration, on sugar-bouillon-agar they are slightly brown with granular folds on central part, <i>differing from species E</i> . Also, on glycerine solutions it shows a most energetic growth.	Ferments only glucose, assimilates glucose, saccharose, maltose.	Optimum temperature 22-24° C. Exposure to 54.4° C. for 10 m. kills the cells.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH_3 , OII.	Assimilation of nitrite, alcohol and glycerine.
Alcohol (7-1%) contained in Mayer's or Nāgeli's solution, devoid of sugar, is converted into acetic acid. The same fact is also observed in kōji-extract culture. In sake containing 10.77% of alcohol it shows no growth.	In kōji-extract kept at 19-26° C. for 10 days, there was found 2.33% of alcohol, 0.014% of fixed and 0.000% of volatile acids, while after 15 days cultivation 3% of alcohol, 0.0178% of fixed and 0.0180% of volatile acids were found. In wort held at 21-26° C. was found 3.41% of alcohol.	Trace of CH_3 , OII was found in kōji-extract culture.	Assimilates glycerine, and alcohol.

This yeast is distinguishable from all preceding species by the vigorous growth on solutions containing glycerin (21-26° C.). Like species E the formation of volatile acid is slower than with the other species. From varieties A, E it differs by not fermenting saccharose; from C and E by the assimilation of maltose. The incapability of the growth on sake distinguishes this yeast from species B, D, F, I and N. The lower optimum temperature distinguishes from species M.

MYCODERMA YEAST Q AND R (Plate XXVIII),
WERE FOUND IN KŌJI.

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Round, ellipse or long filament. The revolving granules in vacuole occur very rare. 2-2.5 μ . b., 10-20 μ . l.	On kōji-extract or wort both varieties form a very thick mesenteric film of a yellowish-brown color. The colonies on wort-gelatine are chalky white and have two main folds on the surface. Streak culture on kōji-extract-gelatine give a very coarse beautiful greyey (sp. Q) or yellowish (sp. R) mesenteric coating. The former liquefies gelatine quicker than the latter species. The same culture on wort-gelatine gives moistened (sp. R) or dry (sp. Q) mesenteric coatings. Giant colonies on wort-gelatine are yellowish with an elevated mesenteric film. The margin is irregular. Those on sugar-bouillon-agar seem mesenteric and especially in the case of species Q are colored more or less yellowish.	Both species ferment glucose, saccharose but not maltose. Assimilate three sugars.	Optimum temp. 27-30° C. killing temp. 54.4° C. for 10 m.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH ₃ . OH.	Assimilation of nitrite, alcohol and glycerine.
Alcohol (7-1 %) contained in Mayer's and Nægeli's solution, devoid of sugar, is converted into acetic acid. In sake containing 10.77% of alcohol both species make very quick growth and after 36 days there was found 4.50% (sp. Q) and 5.56% (sp. R) of alcohol. If alcohol is increased to 13.3% both failed to develop.	The species Q forms in kōji-extract at 19-26° C. during 10 days 3.82% of alcohol, .0682% of fixed and .0199% of volatile acids, while after 15 days 4.5% of alcohol .0486% of fixed and .045% of volatile acid. The species R forms at the same condition during the first period 2.67% of alcohol, .0112% of fixed and .0285% of volatile acids. And after 15 days 3.06% of alcohol, .0561% of the former acid and .0380% of the latter acid. The volatile acid consist of acetic and formic acids (sp. R) or only acetic acid (sp. Q). This property and the destroying faculty of the species Q for fixed acid distinguishes from species R.	Both formed CH ₃ . OH in the culture of kōji-extract. Species R formed larger quantity.	Assimilate glycerine and alcohol.

By these properties we can distinguish the two species from all the varieties except A and E, by the fermenting faculty of saccharose, while the former two varieties do not develop on sake and by this properties differ from the species Q and R.

MYCODERMA YEAST S (Plate XXVIII S), FOUND IN
THE AIR OF A SAKE FACTORY.

GENERAL CHARACTERISTICS.

Form and usual size.	Growth.	Behavior to sugars.	Optimum and killing temperature.
Chiefly round and very rare ellipse. The cell contents are very bright 2.5-5 μ . b., 3-7 μ . l.	On kōji-extract or wort it forms a ring at common temperature, but on an artificial solution containing alcohol (Nägeli's solution), or glycerin instead of sugar (Hayduck's solution) or Hayduck's solution shows a very energetic growth. It forms on wort-gelatine a waxy conical colony with a ring-like canal near the top of the colony. Streak culture on wort or kōji-extract gelatine gives a white waxy coating. It liquefies gelatine very slowly. Giant colonies on wort-gelatine are a white, elevated mass of waxy nature. On the central part some petal-like figures radiate from centre very similar to that of <i>S. hyalosporus</i> . On sugar-bouillon-agar it gives rather flat and waxy colonies.	Ferments only glucose. Assimilates glucose, saccharose but not maltose.	Optimum temp. 27-30° C. Killing temperature lies at 54.4° C.

CHEMICAL BEHAVIOR.

Behavior to alcohol.	Formation of alcohol and acids.	Formation of CH_3 , OH.	Assimilation of nitrite, glycerin, and alcohol.
Alcohol contained in artificial nutrient solution is changed into acetic acid. In sake containing 10.77% of alcohol there was no growth.	In kōji-extract kept at 19-26° C. for 10 days, it formed 2.61% of alcohol, 0.028% fixed and 0.01212% of volatile acids, while after 15 days 3.24% of alcohol, 0.03% of fixed and 0.00237% of volatile acids, the latter acid consisting of acetic and butyric acids. In wort at 21-26° C. for 10 days 3.35% of alcohol was found.	Forms CH_3 , OH in kōji-extract culture.	Assimilates the latter two.

The round form of the cell is the essential character in which this yeast differs from the described varieties. The incapacity of assimilating maltose distinguishes it from the preceding varieties, except C and E, while species E ferments saccharose and hence differs from this yeast. The species C has a different cell form and may thus be distinguished from this yeast.

The properties which distinguish our mycoderma yeast varieties from certain well known mycoderma yeasts :

They differ from

Mycoderma Cerevisiae, " vini, Desm, " " I, II, Seifert, Endoblastoderma amycoïdes (I, II, III, IV), Endoblastoderma liquefaciens. (These varieties do not cause fermentation).	Endoblastoderma glu- comyces (I, II, III, IV). (Do not liquefy gela- tine, ferment <i>glu- cose</i>).	Endoblastoderma pul- verulentum. (Ferment <i>maltose</i> , saccharose).	Henneberg's Mycoder- ma a and b. (Ferments, besides glu- cose, levulose and <i>maltose</i> , but not sac- charose. Produces <i>acetic ether</i> . The opt. temp. 32-51° C.
Mycoderma A. by fermenting glucose.	By fermenting saccha- rose, and liquefying gelatine.	By not causing fer- mentation of mal- to-c.	By not fermenting mal- tose, and the lower optimum tempera- ture, and failure to form acetic ether.
Mycoderma B. ditto.	By liquefying gela- tine.	By not fermenting both, saccharose and maltose.	"
Mycoderma C. ditto.	"	"	"
Mycoderma D. ditto.	"	By incapacity of fer- menting saccharose and maltose.	By not fermenting mal- tose and lower op- timum temperature and not forming acetic ether.
Mycoderma E. ditto.	By the property of fermenting saccha- rose and liquefying gelatine	By not fermenting malto-c.	"
Mycoderma F. ditto.	By liquefying gela- tine.	"	"
Mycoderma I. ditto.	"	"	"
Mycoderma M. and N. ditto.	"	"	"
Mycoderma Q. and R. ditto.	By liquefying gelatine and fermenting sac- charose.	"	"
Mycoderma S. ditto.	By liquefying gela- tine.	"	"

Although a number of mycoderma yeast varieties has hitherto been isolated by various authors, it is very difficult to ascertain whether our Mycoderma species are identical with any of the organisms described in the literature; for not only the majorities of these organisms have not exactly been investigated as to their morphological and physiological properties, but also some of these properties are not quite constant. For this reason I shall point out, in the following, only those races which are especially noted by their characteristic properties.

Among my cultures, the varieties B. D. F. and I. are most interesting on account of their property of assimilating some *nitrogen from nitrite*, when at the same time glycerin is applied as the source of carbon. Such kinds of yeast have hitherto not been observed, *S. acetæthylicus* of Beijerinck being only capable of assimilating N from nitrates.

The varieties of Mycoderma yeast (D. F. I. Q. R. N.) are so far interesting as they can grow in sake containing 10.77 w % or 13.32 w % of alcohol.

Not less interesting is the fact that most varieties of Mycoderma yeast isolated by myself have the property of producing from kōji-extract besides ethyl alcohol a noticeable trace of *methyl alcohol*,¹ the latter is frequently, as I have proved, present in common sake.

Furthermore all kinds produce acetic acid from alcohol and some (species C.) also from glycerin; certain varieties (O. E. B. C.) form besides acetic acid, butyric acid and a few (E. C.) also formic acid in saccharine solution.

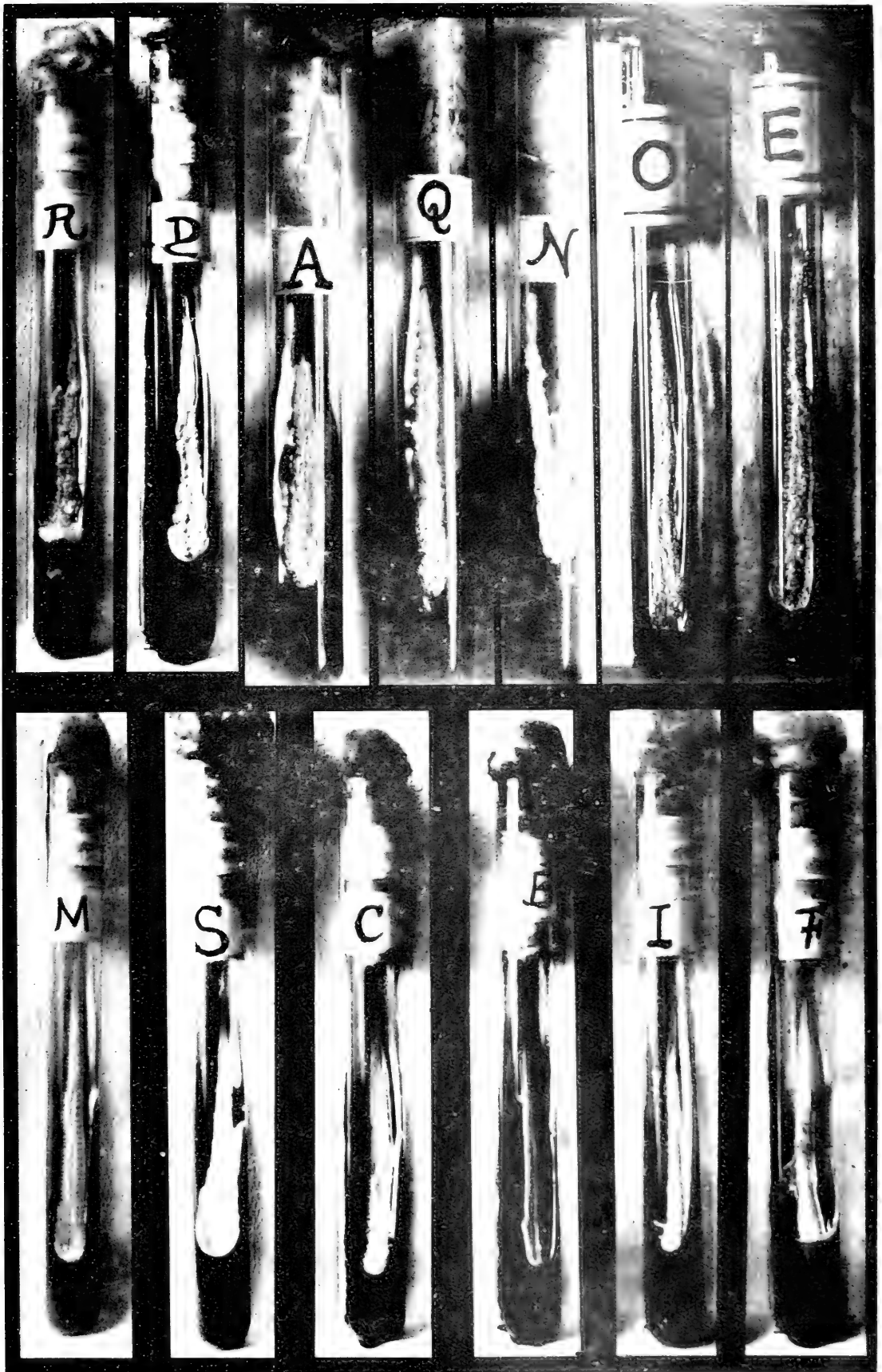
¹ This alcohol may be one of the oxidation products of ethyl alcohol.

EXPLANATION OF PLATE XXIX AND XXX.

All of these mycoderma yeast cells were taken from the plate culture of wort-gelatine. Magnified 750 times. f: Fatty matters. m: Revolving particles. L: Light refracting globules.

Fig. A.	Taken from after 10 days culture of Mycoderma yeast A.							
Fig. B.	The cells of Mycoderma yeast B, after 7 days cultivation.							
Fig. C.	„	„	„	„	C,	„	10 „	„
Fig. D.	„	„	„	„	D,	„	13 „	„
Fig. E.	„	„	„	„	E,	„	12 „	„
Fig. F.	„	„	„	„	F,	„	11 „	„
Fig. I.	„	„	„	„	I,	„	19 „	„
Fig. N.	„	„	„	„	N,	„	19 „	„
Fig. O.	„	„	„	„	O,	„	9 „	„
Fig. R.	„	„	„	„	R,	„	18 „	„
Fig. Q.	„	„	„	„	Q,	„	18 „	„
Fig. S.	„	„	„	„	S,	„	9 „	„





Streak cultures on Löfflerextract-gelatine 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000.



FIG. A.



FIG. D.



FIG. B.



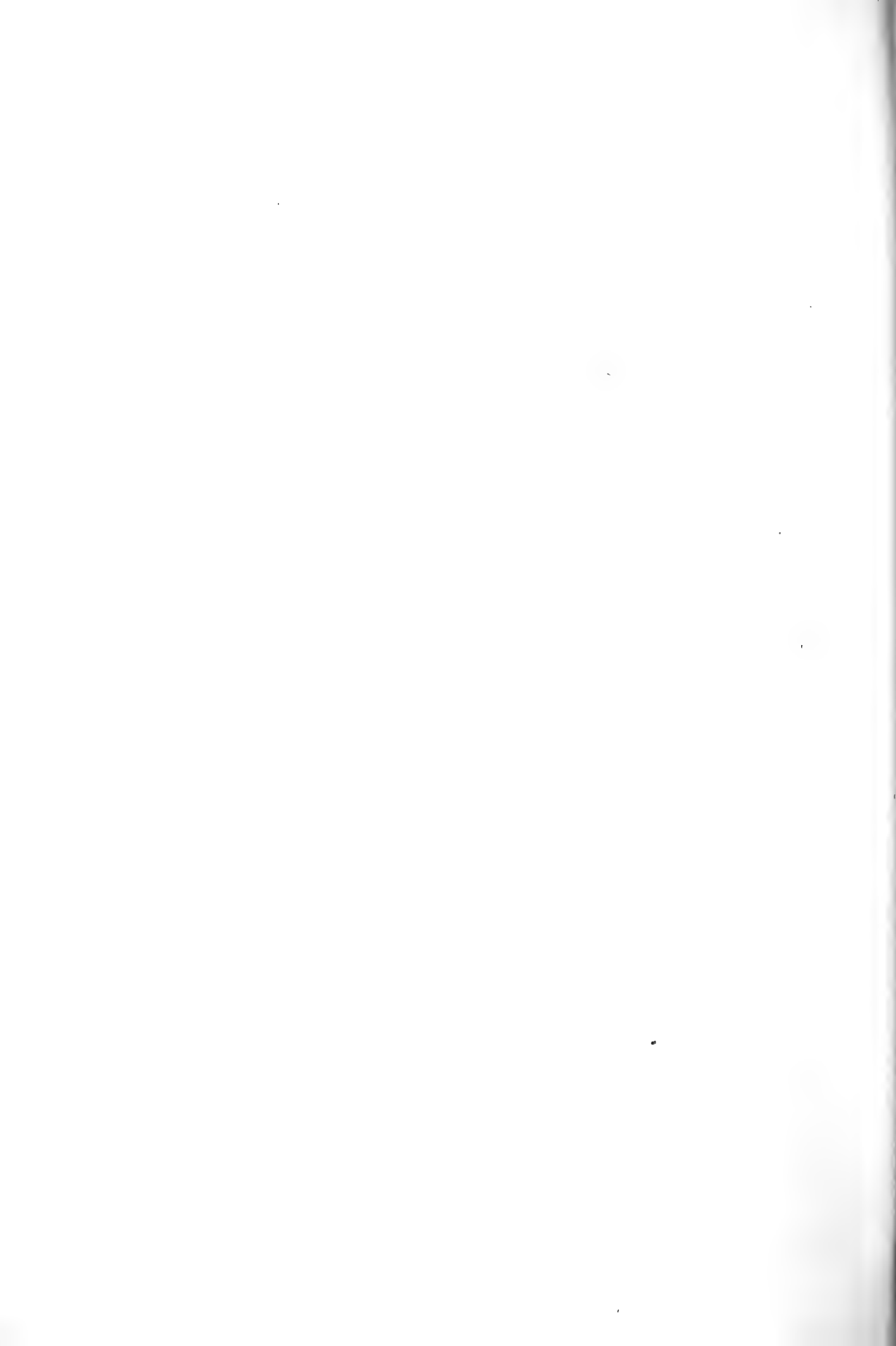
FIG. E.



FIG. C.



FIG. F.



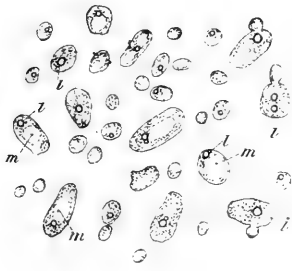


Fig. I.



Fig. N.

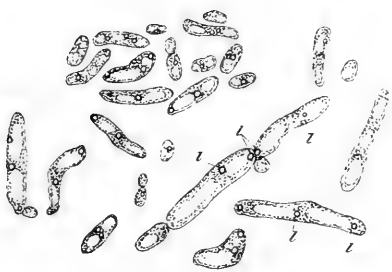


Fig. R.



Fig. O.

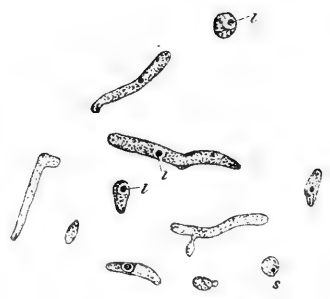


Fig. M.

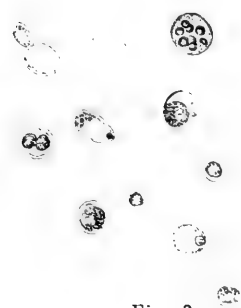
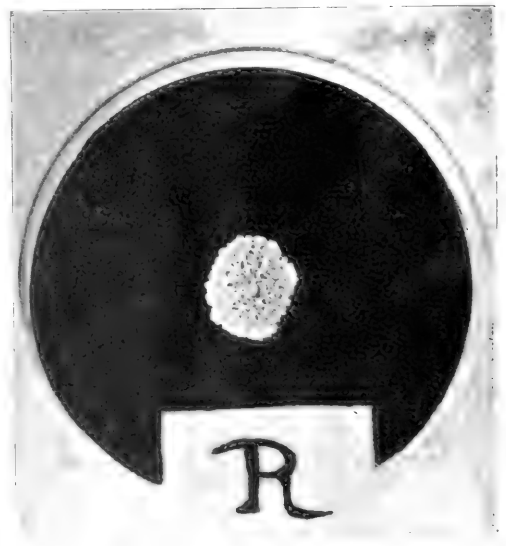
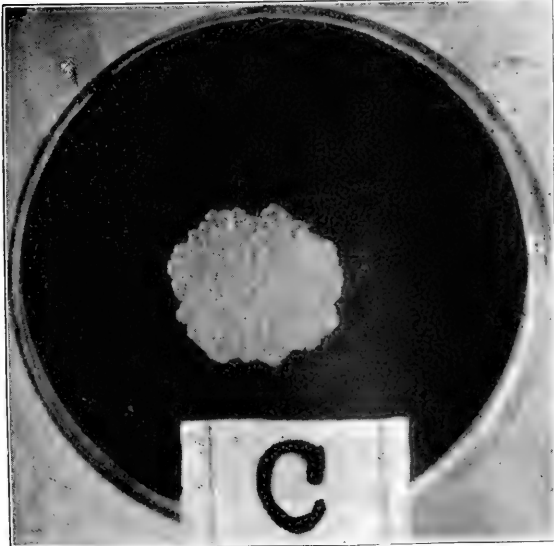
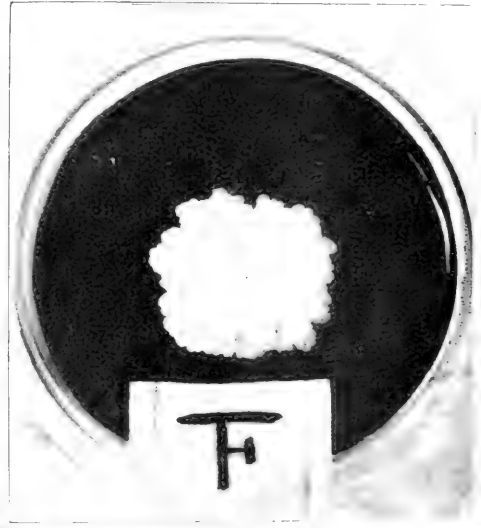
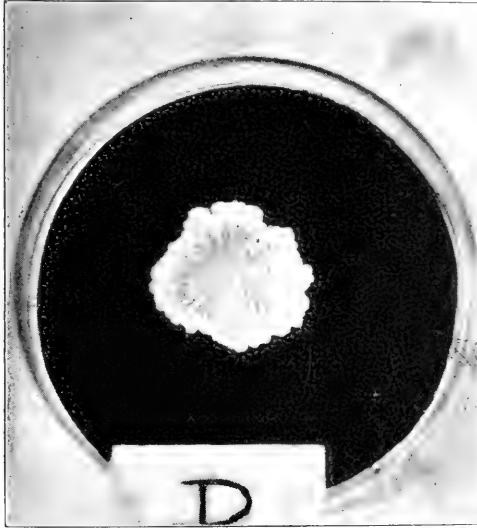
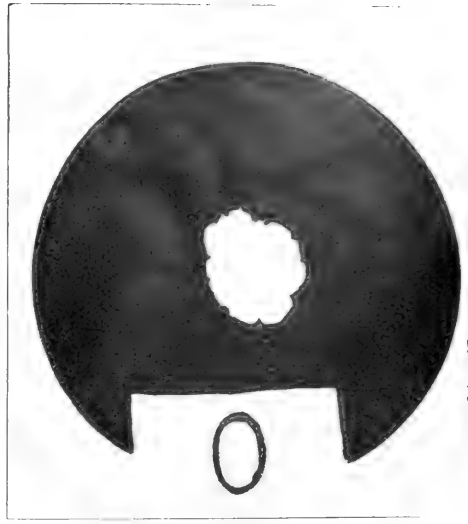
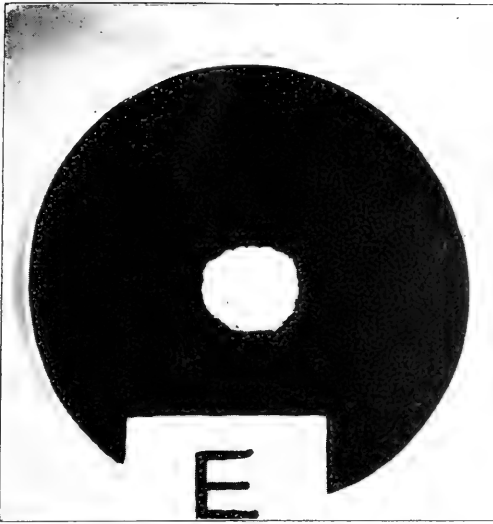


Fig. 2.



Fig. Q.





Giant colonies on sugar-bouillon-agar, after 5 days at 25° C.

Can Nitrite Provide Oxygen in Anaerobic Culture of Bacteria ?

BY

T. Takahashi.

Some time ago *Weissenberg* reported that *Bacillus pyocyaneus* can develop under anaerobic conditions when nitrite of sodium is present in the culture solution. It would seem therefore that this microbe could utilize the oxygen of the nitrite when atmospheric oxygen is withheld. Since, however, similar experiments made long ago by *Loew*¹ with potassium nitrate and aerobic microbes had failed to yield positive results, the result of *Weissenberg* seemed rather doubtful, for obligate aerobic microbes at least.

The writer has therefore made some experiments with nitrite. 0.25 grams of sodium nitrite was dissolved in bouillon and after sterilization infected with *Bac. pyocyaneus*. The test tubes were kept anaerobically (*Buchner's* contrivance) at 30-32° C. After 20 days a very thin scum on the surface was observed and the solution exhibited a weak opalescence. A growth worth mentioning had not taken place. Only very few gas bubbles and that only in the first few days had been observed. The control tubes had remained perfectly clear under the same conditions and it seems therefore that the nitrite enabled the growth of a mere trace of microbes.

Similar experiments, in all of which the bouillon was rendered weak alkaline, were made with *Bac. subtilis*, *Bac. mesentericus vulgatus*, *Bac. mesentericus fuscus*, *Bac. acidii lactici*, Hüppe, *Proteus mirabilis*, and *Bac. of typhoid of mice*. The result was here essentially the same as before, namely the *oxygen of nitrite cannot replace the molecular oxygen of the air in the life of microbes*, at least not with the varieties tested.

¹ Those culture solutions contained leucin and in some cases sodium acetate as organic nutrients, and were infected chiefly with *Bac. fluorescens liquefaciens*. Note of *O. Loew*.



On Manuring with Kainit.

BY

S. Suzuki.

It has attracted considerable attention that kainit which is not only a most effective and valuable manuring compound but also the cheapest source of potassa in commerce for the agricultural-plants, exerted in certain cases an injurious action, depressing the yield of agricultural crops. This action was ascribed by *Detmer* to the presence of chlorids in the kainit. The observation of *Schulz-Lupitz*, however, that the application of lime in conjunction with kainit can prevent the injurious action of kainit¹ militates against the view of *Detmer* and renders it very probable that it is the magnesia content of the crude kainit which causes the injurious action on certain soils.² *Ennenbach*³ who made recently some experiments on the action of kainit in water culture, ascribes, like *Detmer*, the injurious action he observed, to the presence of chlorids, especially of sodium chlorid. However, his experiment can not throw any light on the cause of the injurious action of kainit on certain soils; especially since he carried out his experiments in presence of a sufficient amount of lime which of course counteracted the injurious action of the magnesia of the kainit. Thus it can easily be understood why in his case no injurious action of the magnesium salts in the kainit was observed. *Schreiber* wants to avoid the crude kainit on heavy

¹ Many have therefore applied recently kainit in conjunction with lime, some with excellent results. It is to be regretted, however, that the authors ignored the original magnesia content of the soil.

² Perhaps only such soils come here into consideration which contain much more magnesia than lime.

³ *Landwirt, Jahrbücher*, XXX, Ergänzungsband III, 1902.

soils on account of its chlorid content, while the action on loose sandy soils is beneficial. He also calls attention to the facts that in some cases the magnesia content of the kainit can act injuriously, in other cases, however, very favourably also.¹ Such a case was published recently² in which 0.46% of lime in soil had an injurious effect on lupins, and that this was overcome by an application of kainit; in this case it was very probably chiefly the magnesia content of the kainit that counteracted the effect of the excess of lime. This original soil was very probably very poor in magnesia.

*Gerlach*³ ascribes the injurious action of kainit also to chlorids and believes this could be counteracted by lime. But it is quite impossible to conceive here any connection between the injurious action of chlorids and the supposed counteraction by lime. It was very probably the injurious action of the magnesia in the kainit that was counteracted by liming the soil in *Gerlach's* case. It is true that chlorids can exert in certain quantities an injurious effect on plants and numerous experiments have been made with sodium chlorid. The results, however, do not closely agree, the doses applied and the nature of the soil influencing the action,⁴ The depressing action of potassium or sodium chlorid on the starch content of potato⁵ is probably only due to a certain excess of these chlorids⁶ and does not answer the question whether much smaller quantities could not

¹ Central Bl. Agrikultur Chemie, 1897, p. 802.

² Deutsch. L. Presse, Vol. 23, Nos 91 and 92.

³ Deutsche Landw. Presse 1903, No. 19.

⁴ In regard to the influence of diluted solutions of NaCl upon the crops, *Storff*, also *I. König* in conjunction with *Cosack*, *Boehmer* and *Weigman* have made also investigations (*Biedermann's Centralblatt* Vol. 13, 1884). These authors, however, applied comparatively large quantities of NaCl and observed an injurious action. *König* concludes that a water containing 1 per mille NaCl should be avoided in irrigation.

⁵ *Sjollema*: Bot. Cent. Bl. 1901, No. 33.

⁶ While with maize a certain amount of NaCl depresses, according to *Schimper*, the energy of the assimilation of carbon in the leaves, even a 0.5% solution did not depress it essentially with algae (*A. Richter*, *Flora*, 1892).

act beneficially.¹ *Storpf* observed that a solution of 0.01% NaCl exerts probably a favourable action on the germination; stronger solutions, however, acted injuriously upon that process. *Farius* observed a stimulating action still at 0.4%.² *Pethybridge*³ thinks that sodium chlorid exerts an unfavourable action on the development of root hairs; on the other hand he observed with the wheat plant a favourable action, consisting in the production of a deep green color.⁴ *Blomeyer*⁵ observed a very favorable action on beans by the application of 100 kilo. NaCl per ha., but he would not infer a general recommendation. It seems to me, however, that it is above all necessary to know the quantity of NaCl already present in the soil and to recommend an application only in those cases where that amount was exceedingly small. *Ennenbach*⁶ concludes "Eine geringe Menge Chlor bekommt allen Pflanzen gut. Buchweizen konnte ich z. B. ohne Chlor gar nicht zur Fruchtreife bringen."⁷ This agrees very well with the results of *Nobbe* who inferred from his experiments that.

1. Chlorkalium ist die beste Form der Kalizufuhr.
2. Chlor ist nötig für den normalen Kreislauf des Buchweizens.

¹ According to *Hilgard* the sugar beet can grow well on sandy soil containing besides 0.2% Na₂SO₄ 0.1% NaCl without a depression of the sugar content. *Kossowitsch* (Journal f. exp. Landwirtschaft, 1903, p. 44) reports that flax was much damaged by 0.194% NaCl added to the soil.

² Landw. Versuch stationen, Vol. 32. p. 149 (1886).

³ Bot. Cent. Bl., 1901, No. 33.

⁴ *Wagner* observed a very favorable action of small doses of sodium chlorid with carrots, but not with barley (Die Stickstoffdüngung, p. 233).

⁵ Die Cultur der landw. Nutzpflanzen, I. Bd. p. 330).

⁶ Landw. Jahrbücher XXX. Bd. Ergänzungsband III., 1902, p. 21.

⁷ Quite recently *Gerneck* (Über die Bedeutung der anorganischen Salze für die Entwicklung und den Bau der höheren Pflanzen) made a series of experiments and concluded: "Die Kulturversuche mit *Kresse* haben also ebenso wie die Weizenversuche die Angaben von *Lesage* über die Vermehrung des Palissadenparenchyms durch Kochsalz bestätigt, dagegen nicht seine Angabe, Kochsalz bedinge eine Abnahme des Chlorophyllgehaltes. Meine Maiskulturen ergaben in erster Linie das Resultat, dass Mais sehr wohl 0.5 proc. NaCl verträgt und in dieser Lösung sogar zur Fruktifikation gelangen kann, entgegen den Angaben *Schimpers*, dessen Maispflanzen in 0.5 proc. NaCl nicht über die Anfangsstadien der Entwicklung hinauskamen. Eine Concentration des Kochsalzes von 1 proc. konnten meine Maisexemplare nicht vertragen, trotzdem ich ihnen allen irgend möglichen Schutz angedeihen liess."

*Adolf Mayer*¹ also admits that potassium chlorid is the most favourable form of potassium; it accelerates the ripening of the fruits, by supporting the transportation of carbohydrates from the leaves to the fruits. He ascribes on the other hand the poor development of the buckwheat in absence of chlorids in *Nobbe's* experiments partly to the abundance of nitrates.² *Höstermann* has studied the action of sodium chlorid upon some Gramineæ. The solutions contained from 0.05 to 5% NaCl. He observed that sodium chlorid has already in a small quantity (0.1% NaCl) a depressing influence upon the transpiration, and that the energy of assimilation of carbon decreases already in solutions of 0.05% NaCl, and at 1% concentration it seems to prevent the assimilation altogether. When the NaCl content is so small that it can not cause any damage, the plant will assume gradually the character of a xerophyte, the walls of the epidermis cells become thicker and the mass of the vascular bundles becomes larger, the number and size of the stomata become smaller and the hair formation was increased. Thus far no explanation in regard to the physiological action of sodium chlorid in plants has been given, but it has been observed by *Chittenden* that the vegetable diastase can act more energetically in presence of a small quantity of sodium chlorid (0.24% NaCl) and similar observations have been made recently in regard to the animal diastase by *Wachsmann*.³ *A. Mayer*⁴ found that 1% potassium chlorid retarded the diastatic action, while smaller quantities exerted no decisive effect on the result. A favourable action of sodium chlorid in the living plant might easily find explanation by the observations of *Wachsmann* and of *Chittenden*, since the transport of starch from the leaves to the growing tips might be facilitated by an increase of the diastatic action.

All experience tends to show that favourable effects will result as long as the absolute quantity of sodium chlorid remains below a certain limit and

¹ Journal für Landwirtschaft, Vol. 49, p. 42.

² A recent writer recommends a dose of 20 kilo. NaCl per ha. as very favourable for all kinds of market garden crops.

³ Pflügers Archiv, 1902, Vol. 91, p. 191.

⁴ Journal für Landwirtschaft, Vol. 49, p. 57.

that with the rise above that limit injurious effects will appear and increase. If this is so, then it is above all absolutely necessary to determine analytically the original chlorine content in the soil before the question of application of sodium chlorid on this soil can be taken into consideration, and even then the results might differ according to humidity or dryness of the climate. In order to observe the limits between the beneficial and the injurious influence of NaCl upon certain plants I have made several experiments with a soil that had not been manured for 4 years and had been much exhausted by various crops. This soil contained in 100 parts (air-dry) only 0.0055 gram NaCl or 0.0033 gram Cl.

1. *Experiment with pea.*

3 Wagner's porcelain pots, each containing 6.7 kilo air-dry soil, served for the experiment. As general manure for each pot was applied:

Double superphosphate	12.0 grams
Sodium nitrate	5.0 „
Ammonium sulphate	5.0 „
Potassium carbonate ¹	10.0 „

While one pot (A.) served as check, the other two (B. and C.) received 0.1 gram. and 5.0 grams. NaCl respectively.² 15 seeds of pea were sown in each pot on December 19th (1902), and the young shoots reduced to 6 of equal size four weeks later. There was no decided difference in height during the vegetation. The flowering started on April 9th and had ended on the 1st of May. Up to the flowering period the pots received almost every day 300.0 c.c. water, later on 500.0 c.c. The plants were cut on June 2nd and weighed in the air-dry state with the following results:

¹ Potassium carbonate was applied 2 days later.

² Hence one kilo air-dry soil received 0.015 gram. NaCl in B, and 0.75 gram in C., and the percentage of NaCl in each pot was:

A.	B.	C.
0.0055% NaCl. (original).	0.007%	0.0805%

	Total weight,	Weight of seeds,
A. (Control),	115.0 grams.	60.0 grams.
B. (0.1 gram. NaCl).	118.0 „	61.5 „
C. (5.0 „ „	124.6 „	62.1 „

2. *Experiment with buckwheat.*

4 Wagner's pots containing 5.4 kilo. air-dry soil received each the following manure :

Double superphosphate... 9.6 grams

Sodium nitrate 4.0 „

Ammonium sulphate 4.0 „

Potassium carbonate 8.0 „ (separately applied).

One pot A. received 0.015 gram. NaCl, another B. 0.148 gram. and the third C. 0.74 gram sodium chlorid for each kilo air-dry soil, while pot D. served as control.¹ 35 seeds of buckwheat were sown in each pot on March 28th (1903). The young shoots were reduced, to 6 of equal height on April 27th. Length measurement was made on April 30th, with the following results :

	Average length of the stalks,
A. (0.015 gram NaCl for each kilo soil).	52.5 c.m.
B. (0.148 „ „ „ „ „ „).	51.3 „
C. (0.74 „ „ „ „ „ „).	45.8 „
D. (Control).	50.5 „

Although the plants in pot C. were shortest, they had the thickest stems and most branches. On July 10th, the crop was harvested and left to become air-dry. The weight was as follows :

¹ Therefore the percent amount of NaCl in each pot became as follows :

A.	B.	C.	D. (original soil).
0.007%	0.0203%	0.0795%	0.0055

	Total weight.	Weight seeds.
A. 0.0070% NaCl.	118.9 grams.	49.4 grams.
B. 0.0203% „	132.2 „	52.7 „
C. 0.0795% „	137.2 „	58.2 „
D. 0.0055% „	122.5 „	49.0 „

3. *Experiment with rice.*

4 Wagner's pots were filled with 6.2 kilo. air-dry soil taken from the same unmanured field as served for the preceding experiments. As general manure for each pot served :

Ammonium sulphate	4.25 grams.
Sodium nitrate	4.25 „
Double superphosphate.....	17.0 „
Potassium sulphate....	8.5 „

while the amount of NaCl applied was as follows :

Pot A.....	6.4 grams NaCl ¹
„ B.	9.6 „ „
„ C.....	16.0 „ „
„ D. (Control)....	no addition

On July 30th (1903), the young rice plants from the seedbed were transplanted into the pots, each receiving 3 bundles of three healthy individuals of equal size (about 42 c.m. long).

Towards the end of August a decided difference in plant growth was noticed in favour of the control pot. The plants that received NaCl were more or less injured. Moreover it was very noticeable that the plants in C which had received most NaCl had the deepest green leaves, although the height was far behind that of the other plants. On September 25th all

¹ Hence the percent content of NaCl became as follows :

A.	B.	C.	D. (original soil).
0.1087%	0.1603%	0.2635%	0.0055%

plants were in flower, and on November 2nd, the crop was harvested and left to become air-dry.¹

The weight was as follows :

	Number of stalks bearing ears.	Weight of full grains (unhusked), gram.	Weight of empty grains, gram.	Weight of straw, gram.	Total weight, gram.
A. (6.4 grams, NaCl).	40	52.5	3.2	59.5	115.2
B. (9.6 „ „).	55	43.4	1.8	59.0	104.2
C. (16 „ „).	33	11.6	2.2	41.3	55.1
D. (Control).	54	<u>64.2</u>	2.8	70.0	<u>137.7</u>

These experiments with pea and buckwheat show that moderate quantities of NaCl can exert only a beneficial action, while with the increase of NaCl in the experiment with rice in contrary a depression resulted. It is therefore absolutely necessary to take care of the amount of chlorid applied and to consider the original chlorine content of the soil. Further it must be mentioned in regard to the experiment with rice that the depression would not have been so great in the open field, since the rains and irrigation water would have washed out a considerable portion of the salt. Now let us see whether the kainit could act injuriously on account of its chlorine content.

Since the usual doses of kainit do not exceed 800 kilo. per ha,² the chlorine content of this amount would certainly be insufficient to damage the above crops on soils containing but traces of chlorids.

Let us make a calculation on this point.

We obtained the following numbers :

¹ At this time the leaves of most plants had turned yellowish, but the plants in pot C. still showed a green color. This agrees with the observation of *Petchybridge* mentioned above, that chlorids produce a deep green color.

² *Muercker* mentions, however, certain cases in which the enormous dose of 2,000 kilo kainit per ha had been applied. In such a case the possibility of some injurious influence of the chlorids present on certain crops as potatoes could not be denied.

Sodium chlorid was applied in the experiment with

	NaCl kilo per ha.
Pea	1018.2
Buckwheat	1051.8
Rice	1682.9

These amounts of NaCl correspond in regard to the chlorine content to kainit,¹ kilo per ha :

Pea.....	4.402
Buckwheat	4.547
Rice	7.259

We find from these numbers that the chlorine content of even 4,400 kilo. kainit per ha would not be sufficient to yield a depression of the yield with buckwheat or pea, while only the immense dose of 7,259 kilo kainit per ha would lead to a depression with rice. But such an enormous quantity of kainit is excluded from practice altogether. My further experiments with kainit described further below in which this was applied in pot experiments, the doses corresponded to a ratio of 4,980 kilo. kainit per ha, but even at this ratio no depression with rice and pea resulted. In those experiments to be described presently the kainit was applied in following proportion :

	Kainit.	
	Per pot grams.	Per ha kilo.
Buckwheat ²	0.78	= 2,572.0
Phaseolus ³	12.22	= 2,488.0
Rice ³	24.44	= 4,977.0
Pea ³	24.44	= 4,977.0

¹ 100 parts of our air-dry purified kainit (analysed by myself) contained.

CaO.....0	K ₂ O	17.67
MgO	Chlorine, as NaCl	23.125

² Buckwheat was cultivated in a smaller Wagner's pot the surface area of which was = 28 $\frac{1}{3}$ ha.

³ For these three crops served 'larger pots' the surface of which was = 283 $\frac{1}{3}$ ha.

The following experiments were made with the view to decide, whether the *kainit* could act injuriously on account of its magnesium content. Such an injury would have been expected, however, only on soils which are considerably richer in magnesia than in lime. The results of the writer show that a moderate surplus of magnesia over lime would not yet interfere with the beneficial action of the *kainit*, since the magnesia content of the quantities of *kainit* applied per ha is not so large to yield an undue increase of the total magnesia in the soil. Of course there will be some differences noticed when the *kainit* is applied in the autumn or in the spring.

1. *Experiment with Buckwheat.*

4 Wagner's pots, each containing 5.4 kilo air-dry soil received as manure, each :

Sodium phosphate.....	16.0	grams.
Ammonium sulphate.....	4.0	„
Sodium nitrate	4.0	„

Besides, the following compounds were applied :

Pot A....	Potassium sulphate	3.2	grams.
B. ..	Kainit	9.78 ¹	„
C....	{ Potassium sulphate	3.2	„
	{ Magnesium sulphate	5.78	„
D..	{ Kainit	9.78	„
	{ CaO	0.95 ²	„

20 seeds of buckwheat were sown on March 28th (1903) and the young shoots reduced to 6 of equal size on April 27th. Length measurements were made on April 30th and May 17th with the following results :

¹ The chlorid content (as NaCl) of this quantity of *kainit* was 2.262 grams. NaCl. Hence the percentage of NaCl in the soil became as follows :

A. and C.....	0.0055% NaCl (original soil)
B. and D.....	0.0529% „

² This amount of CaO is equivalent to that of MgO contained in the *kainit*.

	Average length of the stalks.	
	April 30 ^h .	May 17 th .
A. (K_2SO_4).	54.0 c.m.	81.4 c.m.
B. (Kainit).	48.1 „	78.9 „
C. ($K_2SO_4 + MgSO_4$).	50.4 „	79.9 „
D. (Kainit + CaO).	50.0 „	78.6 „

The crops were harvested on July 10th and left to become air-dry.

The weight was as follows :

	Total weight.	Weight of seeds.
A. (K_2SO_4).	123.6 grams.	54.8 grams.
B. (Kainit).	132.7 „	56.1 „
C. ($K_2SO_4 + MgSO_4$).	135.0 „	57.0 „
D. (Kainit + CaO).	119.1 „	51.4 „

2. Experiment with *Phascolus*.

4 Wagner's pots each containing 6.7 kilo air-dry soil served for the experiment. General and special manures were applied in the same proportion (per kilo soil) as in the experiment with buckwheat.

15 seeds were sown on March 26th and the young shoots reduced to 4 of equal height on April 21st. The plants had developed very well, starting the flowering on May 17th, but there was no decided difference in height during the vegetation. The plants were cut on July 20th and weighed in the fresh with the following results :

	Weight of fruits.	Weight of seeds.
A. (K ₂ SO ₄).	60.5 grams.	43.5 grams.
B. (Kainit).	66.7 „	<u>50.1 „</u>
C. (K ₂ SO ₄ + MgSO ₄).	70.9 „	<u>49.8 „</u>
D. (Kainit + CaO).	63.0 „	48.5 „

These two experiments show that the action of kainit on our soil did not differ much from that of the artificial mixture of potassium and magnesium sulphate, but the addition of CaO did here not promote the action of kainit. Kainit can very probably act injuriously only on such soils as contain already a considerable excess of magnesia over lime; it would increase the injurious excess of magnesia still more. Only in these cases a simultaneous application of lime with the kainit would be in order and act beneficially. Two more experiments with rice and pea were made :

3. Experiments with rice.

4 Wagner's porcelain pots were filled with 8.2 kilo. air-dry soil. As general manure for each pot served :

Sodiumphosphate	20.0 grams.
Ammoniumsulphate	8.0 „
Sodiumnitrate	5.0 „

and in order to increase¹ the magnesia content of the soil 83.2 grams. finely powdered magnesite² (for each pot) were applied. The amount of compounds applied were as follows :

¹ Thus, the ratio of lime to magnesia in the soil became $\frac{1}{1.6}$. (The analytical data for this calculation will follow below.)

² Magnesite was imported from Germany and contained only minute quantities of impurities.

Pot A....	Potassium sulphate	8.0	grams.
B....	Kainit.....	24.44	..
C...{	Potassium sulphate	8.0	..
C...{	Magnesium sulphate	14.44	..
D..{	Kainit	24.44	..
D..{	CaO	2.364	..

The transplanting of the young rice plants into the pots (each receiving 3 bundles of 3 individuals) took place on June 24 (1903). Although this experiment was carried out in a glass house, the treatment was the same as in the field. The flowering started at the beginning of September. The plants were cut on November 2 and weighed in the air-dry state. The result was a follows :

	Number of stalks bearing ears.	Weight, grams.			
		Total.	Straw.	Full grains (unhusked).	Empty grains.
A. (K ₂ SO ₄).	56	190.8	108.2	78.4	4.3
B. (Kainit).	63	209.4	119.4	<u>86.6</u>	3.4
C. (K ₂ SO ₄ + MgSO ₄).	58	200.1	112.8	<u>84.5</u>	2.9
D. (Kainit + CaO).	67	192.2	106.7	81.6	3.0

Also this experiment did not yield results unfavourable for kainit ; in contrary, the kainit acted better than the equivalent amount of potassium sulphate.

4. *Experiment with pea.*

In this experiment more powdered magnesite was added than in the former case and the ratio of lime to magnesia was now changed in 8 pots to reach $\frac{1}{2}$. The general plan of this experiment will be seen from the following table :—

Number of pots.	Air-dry soil.	Magnesite, ¹ added to make the ratio $\frac{\text{CaO}}{\text{MgO}} = \frac{1}{2}$.	General manure.	K ₂ SO ₄	Kainit.	MgSO ₄ (hyd.)	CaO	NaCl ²
Kainit	I.	7.8 kilo.	106.3 g.	—	24.44 g.	—	—	—
	II.	"	"	—	"	—	—	—
K ₂ SO ₄ + NaCl	III.	"	"	8.0 g.	—	—	—	5.7 g.
	IV.	"	"	"	—	—	—	"
Kainit + CaO	V.	"	"	—	24.44 g.	—	2.364 g.	—
	VI.	"	"	—	"	—	"	—
K ₂ SO ₄ + MgSO ₄ + NaCl	VII.	"	"	8.0 g.	—	14.44 g.	—	5.7 g.
	VIII.	"	"	"	—	"	—	"

On December 9th (1903), 15 seeds of pea were sown in each pot and the young shoots reduced to 8 of equal height on January 28th (1904). The flowering commenced on April 8th and had ended on May 3rd. The treatment (watering, etc.) was not essentially different from that of the first experiment with pea described above. The crops were harvested on May 3rd and weighed in an air-dry state with the following result:—

¹ According to Katayama's analysis (see Bulletin, College of Agriculture, Imperial University, Vol. VI. No. 2. p. 104) our soil from Komaba contains 0.55% CaO and 0.45% MgO.

² NaCl was added here in the same ratio as in the kainit, for check.

		Total weight, grams,	Weight of total fruits, grams,	Weight of one fruit, grams,	Number of fruits,	Weight of total seeds, grams,	Weight of one seed, grams,	Number of seed,
Kainit	1.	85.0	53.7	1.1	49	45.6	0.220	205
	2.	80.0	51.2	0.97	53	43.1	0.211	204
	average.	82.5	52.5	1.04	51	44.4	0.216	205
K ₂ SO ₄ + NaCl	3.	61.2	45.9	1.15	40	37.9	0.217	174
	4.	64.9	46.3	1.27	37	37.8	0.223	169
	average.	63.1	46.1	1.21	39	37.9	0.220	172
Kainit + CaO	5.	76.7	49.7	1.15	43	41.9	0.212	198
	6.	75.7	48.2	1.07	45	40.5	0.216	180
	average.	76.2	49.0	1.11	44	41.2	0.214	194
K ₂ SO ₄ + MgSO ₄ + NaCl	7.	69.5	44.1	1.13	39	36.6	0.230	153
	8.	79.4	48.8	1.25	39	39.2	0.233	168
	average.	74.5	46.5	1.19	39	37.9	0.236	161

In all our experiments therefore *kainit* has always acted very favorably and no case was observed in which the chlorine content or the magnesia content would have interfered with the production. Therefore I am inclined to believe that cases of a depression by kainit can only be restricted to soils which contain quite an undue percentage of chlorids or of magnesia. Only in the latter case, however, a simultaneous application of lime would counteract the depressing effect of kainit.



On the Influence of Various Ratios of Phosphoric Acid to Nitrogen on the Growth of Barley.

BY

Rana Bahadur

FROM

NEPAL.

Numerous investigations have shown that according to different manuring, the ratio of N : P₂O₅ in the plant changes considerably. According to *Atterberg* the oat grain showed a ratio of N : P₂O₅ = 100 : 50 to 55., when nitrogen and phosphoric acid are present in the soil in a favorable ratio. *Stahl-Schröder*,¹ however, could not always observe this ratio. With an excess of phosphoric manure only, the ratio 100 : 52 was observed. Soils not rich in phosphoric acid gave in one case the ratio 100 : 34 ; in others 100 : 20 to 30. This author concludes that for the grains from his soil the proper ratio was 100 : 35 to 40. If more phosphoric acid was found in the grains, it would indicate need of nitrogen in the soil or excess of phosphoric acid. When, in the contrary, the amount of phosphoric acid is smaller, it would indicate a need of phosphoric acid or an excess of nitrogen in the soil.

Atterberg observed for oats the following limits :

	Grains.		Straw.	
	Max.	Min.	Max.	Min.
P ₂ O ₅ .	1.1	0.44	0.8	0.28
N.	3.87	1.3	0.44	0.25

¹ Journ. f. Landw. Vol. 52, p. 80.

He believes that the ratio of nitrogen to phosphoric acid can vary between the extremes 100 : 15 and 100 : 83.

Now the question seemed to me of some special interest, whether on the loamy humus soil of our college farm, manuring with nitrogen in different forms and in certain ratios to phosphoric acid, would yield also the same ratio in the grains, and further to observe which was the best ratio of N : P₂O₅ in the manure for barley on our soil. It was probable from the outset that nitrogen in form of ammonia would not yield quite the same result as the nitrogen in the form of nitrates. According to *Wagner* one gram ammoniacal nitrogen can produce in average 56.97 grams oat straw and 41.33 grams oat grains; while one gram nitrate nitrogen 58.03 grams oat straw and 42.53 grams oat grains, under otherwise favorable conditions. In applying nitrogen in the form of ammonium salts, it was further to be considered on the one hand the nitrification of it in the soil, and on the other hand that a certain excess of ammonium salts can act injuriously.

Prof. *Aso* of this college has recently shown also this injurious property for rice and he kindly permitted me to make use of his data for this article:—

Three pots, each containing 8 kilo soil which contained 11% of humus of weak acid reaction were manured as follows per kilo :

	A.	B.	C.
K ₂ O.	0.8 gram as K ₂ SO ₄ .	0.8 gram as K ₂ CO ₃ .	0.8 gram as K ₂ SO ₄ .
P ₂ O ₅ .	1.0 gram as Na ₂ HPO ₄ .	1.0 grams as Double Superphosphate.	2.0 grams as Double Superphosphate.
(NH ₄) ₂ SO ₄ .	2.5 grams.	2.5 grams.	5.0 grams.

Three bundles of rice shoots, each bundle consisting of three individuals, were planted in each pot July 13. The difference in growth became soon very marked, and the injury by an excess of ammonium sulphate¹ is also

¹ The total quantity of ammonium sulphate in pot C was as much as 40 g.

plainly shown by the plate No. XXXIII. The harvest on November 6 yielded the following numbers.

	A. grams.	B. grams.	C. grams.
Total harvest (air dry)	279.0	337.5	35.0
Straw	151.0	195.0	9.0
Full grains	121.5	137.0	7.0
Empty	6.5	5.0	19.0

A very interesting account of injury by an excess of ammonium salts has also been published by *A. D. Hall* in the Rothamstead Experiment Station in his publication: "The Continuous Growth of Mangels" 1903. He writes on page 12 as follows:—

TABLE IV.—INCREASE OF CROP PRODUCED BY 1 lb. OF NITROGEN.

Series.	Supplied in.	Plots 4, with minerals only. tons.	Plots 1, with dung. tons.
A	400 lb. ammonium salts (=86 lb. N.)	0.110	0.050
N	550 lb. nitrate of soda (=86 lb. N.)	0.147	0.085
C	2,000 lb. rape cake (=98 lb. N.)	0.163	0.067
AC	{ 2,000 lb. rape cake, and 400 } (= 184 lb. N.) { lb. ammonium salts. }	0.109	0.036

"The injurious effect of the very large amounts of nitrogen added to some of the plots is very manifest wherever there is more nitrogen than the plant can properly deal with. The leaves have a dark green appearance, are much curled and crinkled, and show an increased tendency to variegation, the chlorophyll collecting into dark green or almost black blotches on the lighter background of the leaf. The leaf stalks are often much more coloured and become a bright orange yellow."

How manuring with nitrogen in different doses can effect the yield has been shown by *Wagner*. He found that for 100 parts of straw can thus result grains:

with oats	51-87 parts
„ rye	46-53 „
„ wheat	33-63 „ ¹

Voelker and others have shown that an excess of nitrogen can only be utilized for protein production when phosphoric acid (in some cases also potassa) is applied in increased quantities.

The ratio $N : P_2O_5$ given in the manures varies very much with different authors, where however, we must distinguish between field experiment and pot experiment, since in the former the ammonia supplied by the rain has to be taken into account.

Since nitrogen is the most expensive plant nutriment, rational manuring with nitrogen has to be paid careful attention to. Every excess of this element is not only a direct loss of money but also may lead to a depression of the harvest. *Godlewsky* has further shown that when potassa is present in too small quantities, the smaller harvest obtained may extract as much nitrogen from the soil as a much larger harvest with normal quantities of potassa in the soil. This author applied in his field experiment the ratio $N : P_2O_5$ as 2 : 1; *Reitmayer* in some cases 3 : 1; in others 1.5 : 1; *Schreiber* the ratio 1 : 1; *Wagner* used frequently the ratio 2 : 1; while *Hellriegel* very nearly 1 : 1.²

In my experiment four *Wagner's* pots were used each holding 8 kilo soil.³ This received as a general manure 5 grams double superphosphate⁴ and 10 grams potassium sulphate.

Further the nitrogen was varied in the form of ammonium nitrate in such proportions that the ratio of $P_2O_5 : N$ was in pot

No. I.	as	3 : 1.
No. II.	as	3 : 3.
No. III.	as	3 : 6.
No. IV.	as	3 : 9.

¹ Hence the quotient of yield depends to a great extent upon the amount of nitrogen in the manure.

² Untersuchungen über das Stickstoff bedürfniss der Gerste.

³ This soil had not been manured for four years.

⁴ The double superphosphate contained 41.8990% P_2O_5 .

Consequently pot No. I received

ammonium nitrate = 1.995 grams.

No. II. 5.985 „

No. III. 11.970 „

No. IV. 17.955 „

Twenty seeds were sown in each of the pots on October 9. On October 29, the plants were thinned out, leaving 10 plants in each pot of about equal height.

The plants in pot II were the first to show the ears on March 7, pot III developed ears on the 9th and pot I a week later, but it was only on the 23rd that pot IV showed ears.

The soil which was applied in these experiments had not been manured for four years, as already mentioned, but nevertheless, it was necessary to make one more control experiment, in order to show that there was very little available nitrogen present. Therefore one pot holding 8 kilo of this soil was manured in exactly the same quantities of superphosphate and potassium sulphate as in the first experiment, but no nitrogen in any form was applied to this pot.

Twenty seeds previously soaked in water were sown April 25, and May 16 the seedlings thinned out to 10.

The plants remained in this case very small, formed no ears, and measured, June 5, only 23.15 c.m. in average. After cutting, June 23, the plants were dried at 100° and weighed. The total weight of 10 plants was 3 g. This proves that there were mere traces of available nitrogen present in that soil.

Special experiments had already shown previously that this soil contained but a very insignificant quantity of available phosphoric acid. Most of the phosphoric acid is present in form of an organic compound in the humus of that soil.

During the further development of our barley plants, the greatest height was observed in pot III.

The following Table gives the average height in c.m.

	Nov. 9.	Dec. 7.	Jan. 14.	Feb. 16.	March 23.	April 18.
No. I.	32.1	49.31	54.54	56.58	76.16	85.4
No. II.	32.1	52.11	58.03	62.38	79.02	87.2
No. III.	32.1	50.96	60.25	64.22	83.89	99.2
No. IV.	29.9	47.71	53.05	54.58	77.96	86.7

The plants were harvested June 6, and straw and grains weighed after becoming air dry with the following result :

	Ratio of P_2O_5 : N.	Total Number of stalks.	Number of still green stalks.	Number of Ears.	Weight of Ears in grams.	Weight of grains in grams.	Weight of straw in grams.
No. I.	3 : 1	30	0	30	23	19	49
No. II.	3 : 3	37	9	33	33	25.5	59.5
No. III.	3 : 6	28	3	27	30.5	23.3	66.7
No. IV.	3 : 9	21	8	21	19.0	12.5	45.5

In order to observe the ratio of N : P_2O_5 in the grains of the best harvest in the grains of barley, an analysis was made of the grains of No. II with following result :

	Ratio in the seed.	Ratio in the manure.
N : P_2O_5 .	100 : 37.3	100 : 100

which agrees very closely with the numbers given by *Stahl-Schröder* for the oat grains grown under favorable conditions (see above).

Although the ratio N : P_2O_5 in the seed is about 3 : 1, yet a manuring in this ratio did not produce the best results. It is again confirmed for our soil-conditions that the physiological requirements are not always identical with the manuring requirements.

In order to collect some further information, a second series of experi-

ment was made with barley, in which the absolute quantity of ammonium nitrate was constant and was the same as No. IV of the first series, but the amount of phosphoric acid was here the variable quantity. Three pots manured each with 10 grams K_2SO_4 and 18 grams NH_4NO_3 received:

- I. 5 grams double superphosphate.
- II. 7.3 " " "
- III. 10 " " "

Further a control pot served for the comparison in which the ratio of $P_2O_5:N$ and the absolute quantities of superphosphate and ammonium nitrate were the same as in No. III of the first series = 12 grams NH_4NO_3 + 5 grams Double-superphosphate + 10 grams K_2SO_4 .

Seeds were sown March 21. On April 12 a selection was made leaving ten plants of about equal height in each pot. The following table shows the average height in c.m. of the plants of the four pots:

	April 12.	May 2.	June 5.
I.	16.2	42.15	63.85
II.	16.3	47.08	72.66
III.	15.9	46.84	71.65
IV.	16.3	42.80	76.38

The plants were cut here before flowering, since danger from fungi threatened, on June 7, with the following result:

	Manure.			Ratio.	Straw, air-dry, grams.
	Superphosphate, grams.	NH_4NO_3 , grams.	K_2SO_4 , grams.	$P_2O_5:N$.	
I.	5	18	10	3:9	42
II.	7.3	18	10	3:6	60
III.	10	18	10	3:4.5	66
IV.	5	12	10	3:6	61.5

This second but incomplete experiment shows only that also here the ratio $P_2O_5 : N = 1 : 3$ in the manure was not so favorable as the ratio $1 : 2$ and further that the dose of 18 grams ammonium nitrate per pot was not yet injurious.¹

This would have been different probably with ammonium sulphate.



Bahadur: Plate showing the injurious effect of an excess of ammonium sulfate on rice.



Can Aluminium Salts Enhance Plant Growth?

BY

Y. Yamano.

Although alumina has frequently been observed in the ashes of plants, its presence was and is still considered as merely accidental. But although the plants have no physiological need for alumina some further tests seemed necessary to decide, whether moderate doses of alumina might exert a depressing or enhancing effect upon the development. Of course the alumina absorbed by the roots would in the plant cells be transformed into salts, most probably with organic acids and if any effect would result, it would be due to this form of alumina.

*G. Smith*¹ observed a very high content of alumina in the ash of *Orites excelsa*, a tree growing in New South Wales and Queensland. In the central portion of the trunk were contained deposits of succinate of alumina and the peripheral parts yielded an ash containing 36-43% alumina, in one case even 79.66%.²

Quite recently peculiar, cupshaped, colorless bodies chiefly consisting of aluminium salts were discovered by *L. Radlkofer*³ in various kinds of *Symplocos*. It is of interest that the leaves of such trees served long ago as mordants in place of alum wherefore these plants were named *arbor aluminosus* as early as 1690 by *Rumphius*.

Lycopodiaceæ have long been known to contain notable quantities of alumina. *Adolf Mayer* found 6.8% Al_2O_3 in the ash of *Lycopodium clavatum*,

1 Chem. News 33, p. 135 [1903].

2 In one case also cobalt was observed, besides aluminium and manganese compounds.

3 Ber. D. Botan. Ges. 22, p. 216 [1904].

but he calls attention to the possibility that the alumina mentioned in some former analyses of plant ashes might partly have been derived from soil particles adhering to the vegetable objects. Nevertheless we mention some further data extracted from Wolffs Tables of plant ashes :

Plant.	Organ.	Al ₂ O ₃ in the ash, %
Fagus sylvatica.	Trunk.	0.77
Salix.	„	0.22-0.42
Ulmus campestris.	„	0.27
„ „	Leaves.	0.29
Secale cereale.	„	3.49
Castanea vulgaris.	Fruit.	6.36
Prunus domestica.	Flesh of fruit.	0.87
Pinus Pumilio.	Bark.	0.37
Sphagnum.	—	3.6-5.8
Boletus.	—	3.73

Some preliminary experiments had demonstrated last year in our laboratory that 0.2% of alum acted injuriously after 3 weeks upon young barley plants in water culture, the oldest leaf being completely and the next partially dried up while the third and youngest was still alive. While in this case there was observed a moderate growth for a time, this was not the case when the amount of alum was increased to 0.8%; here the young plants (6 cm. high) were killed within a few days. In soil culture the injurious action was much weaker, however.

In order to observe whether in certain quantities a stimulating action can take place the following pot experiments were made.

I selected ammonium alum $\text{Al}(\text{SO}_4)_2(\text{NH}_4) + 12 \text{ aq.}$ in the following doses :

- A. 0.2 gr. per kilo soil.
- B. 1 „ „
- C. 2 „ „

In the control pots A', B', C' a separate dose of $(\text{NH}_4)_2\text{SO}_4$ was added corresponding to the same amount contained in the alum and further so

much mono sodium sulphate that its sulphuric acid corresponded to the aluminium sulphate present in the ammonium alum. The acid sodium sulphate was selected in order to reach about the same acid reaction which the alum shows.

The general manure per pot containing 2 kilo soil was :

1 gram double superphosphate of lime.

1 „ NaNO_3 .

1 „ K_2SO_4 .

The control pots received :

A'. 0,027 gr. $(\text{NH}_4)_2\text{SO}_4 + 0,03 \text{NaHSO}_4$.

B'. 0,145 „ + 0,152 „

C'. 0,291 „ + 1,305 „

Barley and flax served for the experiments, 15 seeds being sown on January 14 into each pot, which were kept in the greenhouse. On March 18 the young barley plants were singled out, leaving only plants of equal size, five in each pot, while the young flax plants were thus singled out to four per pot on April 12. A favorable influence of alumina¹ became gradually evident, and the measurements of May 24 as well as the final harvest June 8 confirmed that conclusion :

BARLEY.

		Height, cm. May 24, average.	Straw air dry, grams.	Grains air dry, grams.
Alum	A. 0.2 grams.....	54.2	5.8	3.4
	B. 1.0 „	54.0	5.4	4.2
	C. 2.0 „	54.8	6.7	4.0
Control.	A'	46.6	3.3	1.8
	B'	53.4	4.2	1.0
	C'	52.3	5.0	2.4

¹ The alum applied was naturally changed to other aluminium salts in the soil, as phosphate humate, etc.

FLAX.

		<i>Height, cm, May 24,</i> average.	Straw air dry, grams.	Seeds air dry, grams.
Alum.	A. 0.2 grams.....	79.8	6.0	3.2
	B. 1 ".....	76.5	6.3	3.6
	C. 2 ".....	80.8	7.9	4.1
Control.	A'.....	66.3	5.3	3.0
	B'.....	71.8	4.8	2.8
	C'.....	69.5	4.7	2.9

These results leave no doubt that aluminium salts can exert in moderate doses a stimulating influence upon plant development.

On the Application of Freezing in the Preparation of Certain Articles of Food in Japan.

BY

T. Katayama.

There are, in Japan, prepared three commercial food articles by the application of frost and subsequent drying. Since this procedure is not practiced in other countries as far as I was informed, some observations in this line may be welcome. The three articles in questions are: Kōri-Konnyaku, Kōri-Tōfu and Kōri-Mochi.¹

I. *Kōri-Konnyaku.*

Kōri-Konnyaku is made from the powdered root of the Konnyaku plant (*Amorphophallus Rivieri* or *Conophallus Konnyaku*) which consists to the greater part of mannan.² The common Konnyaku is prepared by boiling the powdered root with some lime water which destroys the sharp taste of the crude root. The product is formed in tables resembling in its consistency gelatinized agar or stiff starch paste. This product contains a high percentage of water, and is, therefore at summer time easily attacked by bacteria.

When the gelatinous masses are subjected to freezing, a large quantity of water is forced out chiefly in form of ice and one can easily observe that after thawing up the physical state has completely changed, a very porous

¹ Kōri is the Japanese word for ice.

² This was discovered in our laboratory by C. Tsuji; Cf. these Bulletins Vol. II, No. 2.

condition having been created, the elasticity having decreased, also the thickness; while the feeling to the touch is no longer smooth but rather rough.

For my trial served a piece of Konnyaku of tabular shape 6.8 c.m. in width 1.2 c.m. thickness and 15.4 c.m. in length and weighing 200.5 grams. The loss of water by the first freezing during exposure in a cold night of January amounted to 86 grams.

After three consecutive freezing operations, the weight was reduced to 38.6 grams. It was now placed in an incubator at 18°C., where the weight diminished in two days to 9.8 grams. After three days more, the weight was 7.4 grams. There was no growth of microbes noticed. The piece was now full of minute pores and measured 5.5 c.m. in width 0.5 c.m. in thickness and 11 c.m. in length.

The control piece lost at room temperature daily 4-7 grams of water by evaporation. When it was placed in the incubator at 18°C. for 2 days the piece weighed still 125.1 grams and was covered with numerous colonies of bacteria and mould fungi. After 3 days more the piece weighed still 37.1 grams, was hard and hornlike on the surface but still very soft in the interior. No pores were present. The action of the freezing process becomes evident at once when two such pieces are compared.

11. *Kōri-Tōfu.*

The tōfu consists of the principal protein of the soy bean¹ and is prepared by mixing the hot aqueous extract with liquids containing calcium and magnesium salts; hereby the soluble alkali phosphates holding the protein in solution are decomposed and the protein, probably also combined with some lime or magnesia, is precipitated. It is then brought into tabular form.² It contains like the Konnyaku a very large amount of water (ca.

¹ According to Osborne (Journ. Amer. Chem. Soc., vol. 20, p. 416) the chief protein constituent of the soy bean is *glycinin*, a globulin similar in properties to legumin, but containing more sulphur and 0.5 per cent. less nitrogen than this.—

² Cf. These Bulletins, vol. II, No. 4.

89%) and thus being very liable to putrefaction, it has to be prepared freshly every day. A durable form is prepared by freezing during the winter, and is called then *Kōri-Tōfu*, an air dry, very porous and light product.

For my observation served half a piece of fresh *Tōfu* of tabular form, 25 c.m. in width, 2.4 c.m. in thickness and 12.7 c.m. in length, weighing 119.0 grams. It was exposed to the air in January at -5°C .

In the following morning, numerous ice needles were found piercing through the whole piece and water more or less frozen had been secreted. After thawing up and wiping dry the surface with some filter paper, the weight of the piece was now only 76.5 grams. On repeating that process the piece weighed now only 43 grams. This piece was kept now in the incubator at 18°C ., whereby the porous mass diminished in weight rapidly, and became hard but remained porous. After 5 days the weight was 15.2 grams and no trace of fungi and microbes had developed on the surface.

The control piece lost at room temperature every day only 4 grams of water by evaporation, and when placed after 2 days in the incubator at 18°C ., it acquired on the one hand soon a putrid odor and on the other it commenced on the edges to become hornlike and very compact and hard. After 5 days, it had only decreased to 25.3 grams.

Now, if the *Kōri-Tōfu* is compared with this dried *Tōfu*, a very great difference in the volume is noticed. The *Kōri-Tōfu* which had numerous pores by the formation of ice needles in the interior shows now a larger volume inspite of its containing less water than the control piece of unfrozen *Tōfu*. The beneficial action of the freezing process is evident at a glance. The drying can be accomplished quickly without any putrefaction and the digestibility of the product is insured by the great porosity by which it resembles baked bread.

III. *Kōri-Mochi*.

Mochi is prepared from glutenous rice and has a consistency of thick starch paste. The durable *Kōri-Mochi* is prepared from this by the freezing process. The frozen product must not be allowed to become

too warm in drying it, since the pasty character would be more or less reproduced.¹

It is kept in the shade at low temperature exposed to the wind whereby a very porous snow white brittle mass results. A fresh tablet of 25 grams in weight was after thorough freezing kept at 20°C., whereby it decreased to 15.2 grams in 3 days.

The control piece weighed at this time 17.8 grams, but it was horny and very hard at the surface and on the edges. Such a condition renders the product very difficult to digest.

Finally it may be mentioned that also the so-called *Kanten*, known in other parts of the world as *agar-agar*, which consists of galactan and is prepared from marine algæ is in the fresh state subjected to the freezing process, whereby it assumes the well known highly porous condition. Since this galactan, as well as the mannan above mentioned, serve as an article of food in Japan, it is very probable that there exist in the human intestines galactase and mannose which transform these polysaccharides into the respective sugars.

CONCLUSION.

The freezing process of articles of food, rich in water, for the purpose of subsequent drying renders the products highly porous which condition is not only essential for digestion but makes possible such a rapid drying that changes by microbes and fungi are excluded.

¹ Glutinous rice contains dextrin besides starch. It is well known that starch paste loses its pasting properties completely by freezing.

Note on the Detection and Determination of Fusel Oil.

BY

T. Takahashi.

The quantitative determination of the fusel oil leaves still much to be desired, since its quantitative composition varies to some extent. As a comparatively satisfactory method is considered that of *Röse*. Recently, *D. Komorowsky*¹ has suggested a new method, which is based upon the red color produced by salicyl-aldehyd (ortho-oxybenzaldehyd) and sulphuric acid.

The writer has substituted for the salicylaldehyd benzaldehyd and various other aldehyds in this reaction in form of a 1-2% alcoholic solution and tested commercial fusel oil, as well as pure isoamylalcohol, isobutylalcohol, isopropylalcohol and common propylalalcohol. A satisfactory reaction for fusel oil and these alcohols however, was obtained only by application of benzaldehyd, anisaldehyd, and vanillin.² It was of course necessary to compare their behavior to SO_4H_2 alone, in absence of fusel oil, to the reaction in the presence of fusel oil. After many tests and comparisons which may be here omitted, the writer recommends the following way:

To 4-6 c.c. of the fluid to be tested in the test tube add 5-10 drops of 1-2% alcoholic solution of benzaldehyd, anisaldehyd, or ortho-oxybenzaldehyd³ and pour carefully about an equal volume of SO_4H_2 and observe

¹ Chemikerzeitung, Nr. 88 and "Die deutsche Essigindustrie" 1903, VII. Jahrg. Nr. 49, S. 385.

² Formaldehyd, propylaldehyd, thio-oxybenzaldehyd and chloral gave unsatisfactory reactions.

³ *Komorowsky's* test with ortho-oxybenzaldehyd requires especial care, since this aldehyd produces a certain red color with sulphuric acid alone.

the color appearing. If fusel oil is present, the following colorations will appear :

With benzaldehyd :—Redish color above a yellow layer.

With anisaldehyd :—Brownish yellow, below green, and yellow layers, the former altering to red, the green to blue and the yellow to brownish-purple and violet.

When after some time the fluid is shaken, it shows a purplish red color.

With ortho-oxybenzaldehyd :—Purplish layer above a red layer.

With vanillin the method is a little different. About 5 c.c. of a 1% alcoholic solution of vanillin is added to an equal volume of concentrated SO_4H_2 , and well shaken. After the generation of greenish yellow coloration an equal volume of the testing fluid is added. If the fluid contains fusel oil, the color turns to blue.

For a tolerably satisfactory quantitative colorimetric estimation, I propose to proceed as follows :

Take 10 c.c. of the testing fluid and fusel oil of known strength contained in 15% alcohol and pour each into a small cylinder. Then add well mixing 2 c.c. of 1% alcoholic solution of anisaldehyd and next carefully add 20 c.c. of strong SO_4H_2 , and compare the *purplish tinges* produced by the fluids.

Fusel oil, 0.001%.	Fusel oil, 0.01%.	Fusel oil, 0.1%.	Fusel oil, 1%.
Peach color aft. 30 S.	Peach colored layer	Peach and below	Yellowish layer after 10
Peach violet aft. 6 m.	aft. 20 S. Violet below	yellow colored layer	S. Red and below yellow
Faint peach colour	yellow layer aft. 3 m.	after 20 S. The former	lay after 30 S. <i>Purplish</i> ,
when shaken after 10 m.	A yellowish peach	alters to violet after	below crimson and yellow
	color when shaken	1 m., <i>purplish</i> -red	layers after 1 m. <i>Crimson</i>
	after 10 m.	after 3 m. <i>Violet</i> when	<i>red</i> after shaken in 10 m.
		shaken aft. 10 m.	

Is Germination Possible in Absence of Air?

BY

T. Takahashi.

In a former experiment¹ I had made in regard to the intramolecular respiration of seeds of pea, the germination of the seeds could not take place in absence of air although the intramolecular respiration was carried on continuously for a number of weeks. This observation was also made previously by *Godlewski*.² This author declares that a germination of seeds in absence of air never had been observed ;³ only with lupine seeds he observed that in cane-sugar solution some of them showed a primary state of germination in which the rootlets (plumula?) reached finally a length of only 6 m.m. and died off soon afterwards.

I have observed, however, with rice seeds recently that these can germinate in plain water without any sugar and in absence of air, which shows that intramolecular respiration is capable to furnish so much energy that germination becomes possible with certain seeds. The rice plant is accustomed to grow in swampy soil in which the existence of an extensive

1 These Bulletins, vol. V, No. 2.

2 Bulletin de l'Academie des Sciences de Cracovie, Avril 1901 et Mars, 1904.

3 This is erroneous. Yokoi (these Bulletins vol. III, No. 5) writes as follows: "It has already been shown by F. Haberland that some kinds of seed germinate under water deprived of air. (Der allgem. landwirthschaftl. Pflanzenbau. Wien, 1879). Our experiments have also shown that rice seed is one of them. Indeed, rice seed germinates freely under water with or without air." Yokoi further states the remarkable fact that in germination under water only the plumule develops noticeably, while the rootlets remains minute, which is just the reverse from the germination in presence of air. (This seems to me to indicate the presence of some *zymase in the plumule*, while in the rootlets it is probably almost absent).

bacterial flora has consumed more or less the oxygen present in a certain depth, to which the root of the rice plant extends also the germination of rice naturally takes place in presence of very little oxygen, since the seed-bed is frequently set under water. Gramineæ growing on dry land can not germinate in absence of air.

In my experiment I placed 97 rice seeds, previously treated with 1 p.m. sublimate solution for one hour and a half, in an Erlenmeyer's flask of 300 c.c. capacity, which was completely filled with water previous boiled to expel the absorbed air. The flask was connected with a bent glass tube also filled with boiled water and shut off by mercury. The flask remained in the room with a general temperature of 18-21.5°C., from Oct. 6 to Nov. 24. On Oct. 11 the first sign of germination was observed and the young plumula developed gradually until an average length of 3 c.m. was reached, whereupon further growth seemed to stop.

During the first five weeks there was no development of gas observed which can not be surprising since carbonic acid can appear only in bubbles after the liquid is saturated with it. The first appearance of bubbles was observed on Nov. 11, and this development increased gradually. On Nov. 24 the flask was opened and at first the seeds examined as to adhering bacteria. The examination proved bacteria to be present, which was unexpected on account of the previous treatment with corrosive sublimate (1 p. mille solution), application of a sterilised glass vessel and sterilised water. This water had remained also perfectly clear to the end of the experiment. These bacteria were for control inoculated into sugar bouillon and thus proved that they were incapable of producing alcoholic fermentation.

The water of the flask was subjected to repeated fractional distillation with addition of potassium carbonate and thus the presence of alcohol could be qualitatively shown by the iodoform test.

Of the 97 seeds all had germinated except one, they were weighed again after removing the shoots, in the air dry state.

The original weight of the grains was	grams. 2.3966
The weight of the grains after germination and freed from the shoots	1.9213

Weight of the shoots.....	grams.	0'0230
The weight of the dissolved substance in water		0'1161
" " " (organic)		0'0992
" " " (inorganic).....		0'0169

Hence loss 0.4354 grams starch, which was consumed by intramolecular respiration after conversion into glycose.

Godlewski still adheres to his opinion, that the *zymase* is not only the cause of the *intramolecular* but also of the *normal respiration*, but has modified now his opinion that alcohol is the first primary stage in the consumption of sugar for the common respiration; he says¹ "Ich denke mir also den Zusammenhang der intramolekularen mit der normalen Atmung in der Weise, dass durch *Zymasewirkung* der Zusammenhang zwischen den Atomgruppen der Glykosemoleküle erschüttert wird, indem in denselben bestimmte Umlagerungen der Atomgruppen, welche zur Alkohol- und Kohlensäurebildung führen, eintreten. *Bevor aber noch diese Atomgruppen zum Alkohol zusammentreffen*, werden sie teils durch Sauerstoffwirkung *oxydiert*, teils zur Bildung von neuen Baustoffen bei dem Wachstum der Zelle verwertet."

From this opinion I must also differ, since the amounts of *zymase* are generally so small as to be incapable to produce such an extensive oxidation as we observe it with plant and animal cells under normal condition. The amount of alcohol produced immediately after withholding the air should correspond to the intensity of normal respiration if *Godlewski's* opinion would be correct, which however never is the case except in some fungi.

I hold it for much more natural to ascribe the *normal respiration process to the living protoplasm itself*, instead of a special enzym, the *zymase*. Even in the case of the germinating rice seed it is evident, that only the normal respiration produces sufficient energy to cause a normal growth; here the amount of *zymase* is *too small* to do such an amount of work as in the yeast cell. In this regard the opinion of *Mazé* deserves

¹ l. c. (1904) page 138.

attention.¹ *Maximow*² also appears to go far beyond the line of the admissible conclusions from his experiments with *Aspergillus* when he writes: Zugleich beweisen diese Versuche, wie mir scheint, endgültig, dass die Sauerstoffaufnahme und die Kohlensäureausscheidung durch zwei von einander unabhängige Enzyme bewirkt werden.

¹ Compt. rend. 138. (1904).

² Ber. D. Botan. Ges. XXII, Heft. 4, 1904.



ERRATA.

In vol. VI No. 3 of these Bulletins read :

Page 196 ; 11 lines from top ; 4000 kilograms instead
400.

Page 233 ; 4 lines from below ; read *aluminium* phos-
phate, instead of *calcium* phosphate.

Page 244 ; 8 lines from top ; read proportion of the
time, instead of *lime*.



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