東京帝國大學

農科大學

學術報告

第七卷

THE

BULLETIN

OF THE

COLLEGE OF AGRICULTURE,

TŌKYŌ IMPERIAL UNIVERSITY,

JAPAN.

VOL. VII.—8

1906—1908

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Some Catalytic Actions of Platinum Black.

BY

O. Loew and K. Aso.

The catalytic actions of platinum black are doubtless of a peculiar interest since they recall actions of certain enzyms, as of catalase and oxidase, and even some chemical processes thus far observed only in living cells. The fineness of the particles, however, and the absence of certain impurities exert much influence upon the efficacy, as was recognised already by Doebereiner more than fifty years ago. The reduction of platinum chlorid by zinc and hydrochloric acid, further the reduction by a boiling alkaline solution of sodium formate yield less effective products than the reduction by glucose, glycerol or formaldehyd in presence of caustic soda or potassa. Especially the application of formaldehyd in presence of much alkali, a method first proposed by one of us (L.) yields a product of great efficacy.[1]

The catalytic actions of platinum black are of various kinds:

1. Oxydations, by transfering free oxygen.
2. Reductions, by means of hydrogen, as, e.g.,

$$CNH+4H=CH_3\cdot NH_2$$

3. Decompositions, as e.g.:

$$2H_2O_2=2H_2O+O_2$$

4. Reactions between two dissolved compounds. We are able to add now a fifth kind of action, namely:

5. Transformations of labile compounds. We have observed at least one case that here comes into consideration viz., the transformation of *maleic* into *fumaric* acid. This process is easily realised by various circumstances,

[1] *Berichte d. D. Chem. Ges.*, **23**, 289. The great efficacy of this product was recently also pointed out by A. Bringhentis in his treatise: Catalysis and electromotoric power.

also slowly by sunlight, as some time ago was observed by Ciamician and Silver.

A solution of 2 g. maleic acid in 40 c.c. water was mixed with 40 g. platinum black and heated on the waterbath for three hours. The evaporation of the filtrate yielded a mixture of maleic and fumaric acid, from which the former was readily separated by a little water. The undissolved, recrystallised from boiling water, amounted to 0,210g.=10,5% of the maleic acid applied. The low degree of solubility, the form of the crystals, and the sublimation at 200° with partial production of maleic anhydrid confirmed that the product was fumaric acid. In the control test without addition of platinum black only two milligrams of fumaric acid resulted.

A second experiment was made at the ordinary temperature. Five grams maleic acid dissolved in 30 c.c. water were left in contact with platinum black for five days. The production of much carbonic acid caused us to interrupt the action sooner than was originally intented. The fumaric acid, separated like in the first experiment, amounted to 6.8 per cent. of the maleic acid applied.[1]

An experiment was also made with glycose to decide whether it can be transformed into mannose by the activity of platinum black, but our tests with phenylhydrazine to obtain the characteristic mannose-phenylhydrazone failed to show any such transformation, which by dilute alkali is easily accomplished as Lobry de Bruyn has observed.

It was shown formerly by one of us (L.), that by the action of platinum black upon a mixture of glycose and nitrates, ammonia is formed.[2] In this connection we have observed recently, that this reduction also can take place with dilute free nitric acid. A solution of 2 g. glycose in 100 c.c. water was mixed with 2 c.c. of dilute nitric acid containing 0.080 g. NO_3H. After adding 10 g. platinum black and heating for three hours on the waterbath the distillation with magnesia and titration of the distillate showed 0.012 g. NH_3.

[1] We have also repeated this experiment in presence of glycose in order to observe whether by a reducing process succinic acid would result, but such was not the case.

[2] *Ber. D. Chem. Ges.* **23**, 675. I may add to those observations that an intense development of ammonia can also be observed by heating 1 g. glycose with 5 g. magnesium nitrate, 10 g. platinum black and 20 c.c. water for six hours on the waterbath and subsequent addition of soda, L.

In a second mixture consisting of 5 g. glycose, 2 c.c. nitric acid of 1.3 spec. grav. 10 c.c. water and 10 g. platinum black was after remaining for 10 days at the ordinary temperature formed 0.049 g. NH_3.

There can hardly exist any doubt that also in living plant cells the reduction of nitrate is done by means of glycose under the activity of the living protoplasm. By increase of the sugar this process will be accomplished more readily, hence in the leaves the nitrate disappears more quickly than in the stems and roots. The ammonia produced serves either immediately for the production of protein or is intermediately stored up in the form of asparagin.

In our experiments also nitro-benzoic acid, picric acid and related compounds have been treated with glycose and platinum black, but although a reducing action was noticed, a smooth transformation to corresponding amido-compounds did in no case take place.

Similar to nitrates also chlorates, perchlorates and iodates are reduced, whereby chlorid and iodid respectively result.[1] Upon shaking 5 g. platinum black with a solution of 0.2 g. potassium perchlorate and 2 g. glycose in 10 c.c. water for two minutes, a considerable amount of potassium chlorid was formed, as revealed by nitrate of silver. Potassium chlorate behaved in the same way.

Potassium iodate, 0.5 g. were dissolved in 75 c.c. water and warmed after addition of 5 g. glycose for 20 minutes in order to observe whether glycose alone would exert a reducing action upon the iodate which was not the case. However, upon adding 4 g. platinum black, the odor of free iodine became at once perceptible. A brown colored filtrate resulted which by addition of some ammonia was decolorised and yielded now with silver nitrate a yellow precipitate of AgI. Also the reaction with starch showed the presence of much free iodine. The same experiment was repeated in presence of a little potassium carbonate to prevent the action of the free acids produced from sugar. Under this condition a smooth reduction of iodate to iodide took place without the liberation of iodine.

[1] *Abelous* and *Aloy* (1903) report about an enzym in the potato juice which acts on a mixture of salicylic aldehyd and potassium chlorate producing potassium chlorid and salicylic acid. In the above experiment it is the sugar that is oxidised to acids by the potassium chlorate.

As to potassium periodate the case is different, since this is reduced by glycose alone without the assistance of platinum black.

One of us had observed formerly that platinum black moistened with pure caustic potassa solution forms traces of nitrous acid and ammonia from the free nitrogen of the air.[1] Further observations have shown that freshly prepared and well purified platinum black[2] when kept slightly moistened with distilled water in a well closed belljar and in a room in which neither chemical work was carried on nor flames were burning during that time and hence no traces of nitrous acid were produced, showed after two months a strong reaction with diphenylamin, but not the reaction of Griess with sulphanilic acid and α-naphthylamine. This shows that in this case nitric acid but no nitrous acid was present, probably on account of its oxidation to nitric acid. Nesslers reagent gave also in this case a faint reaction upon ammonia.

The attention might also here be called again to a very peculiar action of platinum black on glycose[3] which not only undergoes thereby oxidation to glyconic and saccharic acid but to a very small extent a reduction, as can be recognised by a distinct rancid odor, recalling that of capronic, valerianic and butyric acids. The composition of the silversalt of the volatile acid corresponded to valerianic acid.[4] Hereby an analogy is furnished to the interesting process of the transformation of sugar into fat in living cells, and especially to the production of valerianic acid from sugar in the process of intramolecular respiration of a worm (Ascaris) as Weinland has observed some years ago.[5] He gives the equation :

$$4C_6H_{12}O_6 = 9CO_2 + 9H_2 + 3C_5H_{10}O_2$$

[1] *Ber. D. Chem. Ges.* **23,** 1443. That observation was recently confirmed by *L. Wöhler;* ibid, **36,** 3479.

[2] This platinum black was prepared by means of formaldehyde, as in all other cases mentioned here. The well washed product was dried at 60—70°. Sometimes it was "regenerated" by alkaline glucose solution after use.

[3] Cf. O. L. in *Ber. D. Chem. Ges.* **23,** 866.

[4] The quantity did not suffice for a fractional precipitation of the barium salt with silver nitrate; hence it is not yet decided whether capronic and butyric acid were absent. For a successful study of that interesting process not less than 4—500 grams of platinum black should be applied.

[5] *Zeitschr. Biol.* **24,** 55 [1901].

But as free hydrogen is not formed thereby, the equation of Koenigs is in better accord with the facts:

$$13C_6H_{12}O_6 = 12C_5H_{10}O_2 + 18H_2O + 18CO_2$$

The question as to the cause of activity of platinum black has been answered in different ways. L. Woehler[1] observed that platinum black undergoes easily an oxidation to the hydroxid $Pt<^{OH}_{OH}$ and in absence of water to protoxid. In the latter case the primary formation of $Pt<^{O}_{O}$ is supposed to take place. Since platinum oxids part easily with their oxygen, some oxidation phenomena of platinum black might be ascribed to their intermediate production. But this would certainly not suffice for explaning other actions than oxidations. Engler and Weissberg[2] ascribe the catalytic actions to the unsaturated character of the platinum. But it may be objected that other fine metallic powders as, e.g., *ferrum hydrogenio reductum* should act in a similar way. Various catalytic actions, as the production of valerianic acid from sugar or the reduction of nitrate to ammonia in presence of sugar cannot be explained by the unsaturated character of platinum black.

One of us has endeavored to give a more physical than chemical explanation.[3] The fine particles of platinum black are according to this hypothesis especially suited to transform oscillations of thermical energy into such of chemical energy, i.e., molecular motion into atomic motion, in analogy to the transformation of light-energy into chemical energy by certain compounds. When the particles of platinum black are not exceedingly fine, but rather large and compact, or when the particles are covered by thin films of insoluble compounds the faculty of transformation of energy is at once impaired as can be noticed whenever there is occasion for the formation of a subchlorid of platinum[4] or of sulphide. The formation of sulphide may

[1] *Ber. D. Chem. Ges.* **36,** 3480.

[2] Cf. The interesting publication: Kritische Studien über die Vorgänge bei der Autoxydation, p. 150.

[3] Cf. O. L., *Ber. D. Chem. Ges.* **23,** 677.

[4] Cf. O. L., Ibid. **23,** p. 289, footnote, also p. 1446 ibid. A discussion of catalytic actions is also contained in the publication: Die chemische Energie der lebenden Zellen, von O. Loew, Kap. 5. Stuttgart.

explain the inactivity after treatment with sodium thiosulphate, which Bredig has observed.

Recently Loewenhart and Kastle[1] have also shown that whenever an insoluble compound results which forms a thin film upon the metallic particles, the catalytic action is either stopped and retarded.[2] Thus the catalysis by silver is inhibited by soluble chlorids, bromids, iodids and cyanids but not by fluorids, while the catalysis by metallic thallium is not essentially retarded by cyanids. However, the catalytic actions of other metals than platinum are very few at the ordinary temperature and consist chiefly in the decomposition of hydrogen peroxid.

Summary.

1. Platinum black can change maleic to fumaric acid.

2. Platinum black can transform dilute free nitric acid to ammonia in presence of glycose.

3. Platinum black can by the help of glycose not only reduce potassium chlorate in aqueous solution but also perchlorate and iodate.

4. Platinum black moistened with a little water shows after several months the presence of nitric acid and slight traces of ammonia.

[1] *Amer. Chem. Journ.* **29**, p. 397 [1903]; *Science*, 1904, p. 631.

[2] The expression "poisoned" used by some authors is certainly not in place.

Ueber die Veränderung des Zellkernes durch kalkfällende Mittel.

VON

Oscar Loew.

Es ist von mir früher beobachtet morden, dass neutrales oxalsaures Kali in 0.5–2% Loesung bald den Zellkern der Spirogyren bedeutend verändert, bevor noch irgend ein anderer schädlicher Einfluss beobachtet werden kann.[1] Erst etwas später werden auch die Chlorophyllkörper angegriffen, was dann den Tod des Cytoplasmas nach sich zieht. Jene Veränderung ist ganz auffallend und besteht in einer seitlichen Contraction, die Spindelform des Zellkerns geht so zu sagen in einen Faden über, welcher erstarrt und mit den ebenfalls erhärteten Plasmadiensträngen noch an den Chlorophyllbändern befestigt bleibt. Werden 0.5–0.1% Loesungen angewendet, so sterben die Zellen langsamer ab und es kommt dann häufig jene characteristische Erscheinung nicht mehr zu Stande, der Kern contrahirt sich dann auch in der Längsrichtung mehr oder weniger und wird zu einem unregelmässig gestalteten Klumpen. Stets wenn der Kern nicht momentan abstirbt, sondern eine gewisse, wenn auch immerhin noch kurze Zeit vergeht, findet Contraction in der Richtung der Längsaxe und Annäherung der ursprünglichen Spindelform an die Kugelform statt, wobei die Plasmodienstränge auf der einen Seite abreissen. Man kann das z. B. beobachten beim Töten mit verdünnter Schwefelsäure oder mit Alkohol. Beim Töten mit manchen Anaesthetetica oder Pyridin sieht man den zu einer rundlichen Masse contrahirten Zellkern öfters von einer Blase umgeben, welche vielleicht vom Tonoplasten herrührt.

[1] Flora 1892, S. 375. Andere Salze, wie z. B. weinsaures oder essigsaures Kali hatten durchaus keine derartige Wirkung.

Von den löslichen oxalsauren Salzen kennen wir als characteristische Eigenschaft weiter keine, als die, Kalk selbst bei bedeutender Verdünnung seiner Salze als Oxalat auszufällen. Dieses veranlasste mich (l. c.), die Folgerung zu ziehen, dass der Zellkern Kalk in Verbindung mit den Nucleoproteiden (Plastin, Chromatin) enthält und dass, wenn dieser als Oxalat abgetrennt und durch andere Basen ersetzt würde, eine Veränderung der Imbibitionsfähigkeit statt fände, welche eine Structurstörung und damit den Tod bedinge.[1] War diese Ansicht richtig, so mussten andere Kalk fällende Mittel ebenfalls rasch giftig wirken und bei genügend rascher Wirkung wahrscheinlich auch ähnliche Contractionserscheinungen des absterbenden Kernes hervorrufen, wie das Kalium-Oxalat. Diese Folgerung wurde im vergangenem Jahre von mir für Fluornatrium bestätigt[2] und kürzlich beobachtete ich dieses auch bei dem Anfangsstadium der Einwirkung von Kaliumcarbonat. Dagegen wirkt weder Di—noch Mono—Kaliumphosphat in ähnlicher Weise. Sie trennen den Kalk als Phosphat nicht ab, wahrscheinlich er mit den Phosphorsäurerest der Zellkern-Nucleoproteide schon verbunden ist.[3] Ich habe nun, in Folge irrtümlicher Anschauungen einiger Autoren, die Wirkung verschiedener Salze kürzlich nochmals verglichen. Fäden von *Spirogyra nitida* wurden in die 0.5 procentigen Loesungen bei 8–10° C. (Januar) eingelegt und nach gewisser Zeit microscopisch verglichen, mit folgendem Resultat:

[1] Nur Pilze und die niedersten Algenformen sind ausgenommen; denn für diese sind Oxalate absolut ungiftig; sie bedürfen zur Ernährung auch des Kalkes nicht.

[2] Flora 1905, S. 333.

[3] Beim Monokaliumphosphat in höherer Concentration wäre bei dessen stark saurer Reaction Tod durch Säurewirkung wohl zu erwarten.

	Nach 30 Minuten.	Nach 20 Stunden.
Dikaliumoxalat.	Viele Kerne in ihrer ursprünglichen Lage zu Fäden contrahirt. Sonst noch keinerlei schädliche Wirkungen sichtbar.	Sämmtliche Kerne in Fadenform erstarrt, Chlorophyllkörper contrahirt und oft zerrissen. Turgor verschwundeu, Zellen tot.
Natriumfluorid.	Erscheinungen wie beim Kaliumoxalat; die Zahl der angegriffenen Zellen ist jedoch geringer.	Wie beim Kaliumoxalat; doch haben die erstarrten Plasmodienstränge sich etwas eingezogen und daher eine Einschnürung des Cytoplasmas an deren Anheftungstellen bewirkt.
Dikaliumcarbonat.	Eine mässige Anzahl der Zellkerne zeigt dieselbe Erscheinung, wie beim Oxalat. Turgor noch überall erhalten.	Alle Zellen tot. Inhalt verquollen, Chlorophyll zerstört. Wo der Zellkern noch sichtbar ist, zeigt er oft nur in der Mitte eine Quellung durch die alkalische Reaction.
Magnesiumsulfat	Normal.	Etwa 20 Procent der Zellen angegriffen, Kern in der Längsrichtung contrahirt.
Kaliumnitrat.	„	Normal.
Dikaliumphosphat.	„	„
Monokaliumphosphat.	„	„

Um zu sehen, ab Kaliumnitrat und die Kaliumphosphate vielleicht bei höherer Concentration solche Erscheinungen am Kerne hervorrufen können, wie Kaliumoxalat und Natriumfluorid bei 0.5%, wurden Fäden der gleichen Spirogyra in je fünfprocentige Loesungen jener Salze gelegt. Es trat dann, wie vorauszusehen, Plasmolyse ein, welche nach mehreren Tagen zum Tode führen musste.[1] Nach 24 Stunden wurden folgende Erscheinungen beobachtet: Bei Monokaliumphosphat. In vielen Zellen normale

[1] Nur bei Plasmolyse durch Zuckerloesungen bleiben die Zellen noch lange am Leben, wie aus den interessanten Studien von *KZebs* hervorgeht.

Plasmolyse mit lebendem Cytoplasma und normalem Zellkern, in einer Zahl von Zellen ist der plasmolysirte Inhalt bereits abgestorben und der Zellkern liegt als rundliche Masse dem Cytoplasma an, in wieder anderen Zellen hat sich der überlebende Tonoplast aus der sich contrahirenden Cytoplasmahülle durch eine entstandene Oeffnung frei gemacht und liegt nun als eine straff gespannte Blase[1] neben der toten cytoplasmatischen Hülle mit dem Zellkern und Chlorophyllkörpern. In dieser Form hatte ich nie zuvor die anomale Plasmolyse beobachtet.

In den fünfprocentigen Loesungen von Dikaliumphosphat und von Kaliumnitrat waren unerwarteter Weise weit mehr der plasmolysirten Zellen abgestorben, als beim sauren Mono-Kaliumphosphat. Nirgends war eine seitliche Contraction des Zellkernes zu sehen, wie bei den 0.5 procentigen Loesungen von Kaliumoxalat und Natriumfluorid. Diese Erstarrung zu einem Faden mit den Plasmodiensträngen *in situ* ist jedenfalls characteristisch für plötzlichen Tod unter besonderen Umständen, denn selbst bei Tötung mit 1 procentiger „Ueberosmiumsäure" wird dieses nicht beobachtet. Es bleibt auch hier zwar Alles *in situ* aber der tote Kern hat sich nicht seitlich contrahirt, er hat seine Spindelform noch beibehalten.

In enger Beziehung zu der Giftwirkung kalkfällender Mittel steht die schon vor langer Zeit beobachtete Giftwirkung von Magnesiumsalzen auf Pflanzen. Nur eine genügende Menge von Kalk kann, wie Atterberg, Ulbricht und der Schreiber dieses unabhängig von einander beobachtet haben, diese Giftwirkung aufheben[2] und die Magnesia kann dann ihre physiologische Function ausüben. Für die niederen Algen und für die Pilze, welche ohne Kalk leben können, für welche desshalb Oxalate kein Gift sind, ist auch ein Ueberschuss von Magnesiumsalzen nicht giftig. Dieser Parallelismus wurde zwar schon von mir hervorgehoben, aber ich musste nochmals darauf zurückkommen, weil in einem kürzlich erschienenem Buche[3] die ganze Frage wieder verdunkelt und von einem einseitigen Parteistandpunkt behandelt

[1] In dieser Blase, welche sich hie und da in zwei geteilt hat, sind öfters körnige Ausscheidungen zu sehen, welche wahrscheinlich aus dem labilen Reserveeiweiss des Zellsaftes hervorgegangen sind.

[2] Eine frühere Notiz von Boehm war unbeachtet geblieben.

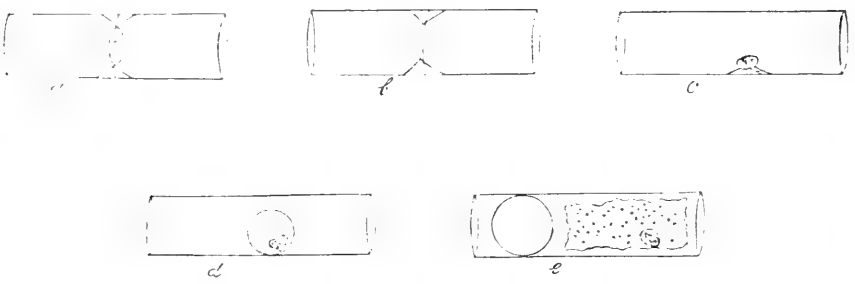
[3] Czapeck, Biologie der Pflanzen, II.

wurde. Es wurde in jenem Buche z. B. betont, „dass auch andere Salze und Salzgemische bei Mangel von Kalksalzen Erkrankungen bei Wasserculturpflanzen erzeugen können, die durch Kalksalzzusatz paralysirt werden können." Beim rechten Lichte betrachtet ist dieses aber gar kein Einwand gegen meine Theorie über die Rolle des Kalks; denn bei Kalkmangel und Gegenwart verschiedener Kalisalze wird eben ein langsames Absterben in Folge mangelhafter Ernährung, also quasi ein Tod durch Verhungern eintreten, welcher nur durch Calciumsalze aber nicht durch Magnesiumsalze aufgeschoben werden kann. In jenem Buche wurde ein ganz wesentlicher Unterschied totgeschwiegen, nämlich der, dass die Wirkung von Magnesiasalzen bei Ausschluss von Kalk eine wahre *Giftwirkung* ist, die gar nicht zu verwechseln ist mit dem ebenerwähnten Tod durch *Ernährungsmangel*. In 0.1 procentigen Loesungen von Magnesiumsalzen sterben z. B. Spirogyren in 4–5 Tagen ab, während bei ebenso starken Loesungen anderer Nährsalze, wie z. B. Kaliumnitrat der Dikaliumphosphat das Leben noch viele Wochen lang dauert.[1] Selbst in 0.5% Loesungen des sauren Monokaliumphosphats mit 0.2% KNO_3 können Spirogyren noch mehrere Wochen fortleben, bei Abwesenheit von Kalk. Aehnliche bedeutende Unterschiede existiren für Phanerogamen.

In dem löblichen Bestreben, nach weiteren Einwänden gegen meine Theorie der Kalkfunction zu suchen, erwähnt der Autor des obencitirten Buches noch Folgendes: „Es steht nicht ohne Analogie da, dass das Calcium-ion entgiftende Wirkungen besitzt. Loeb fand, dass die Eier des Teleostiers Fundulus in reiner, dem Seewasser isotonischer NaCl-Loesung rasch zu Grunde gehen, dass man aber durch eine Reihe mehrwertiger Kationen die schädliche Wirkung des Na-ion (!) äquilibriren kann. 1 Aequ. Ca-ionen entgiften 1000 aequ. Na-ionen." Eine Erklärung, in was denn die „entgiftende Wirkung" des Kalks hier besteht, versucht der Autor gar nicht und doch hält er jene Beobachtung für einen „Einwand." Bei jenen Seetieren handelt es sich jedenfalls darum, dass die Proteinstoffe des Cytoplasmas mit

[1] Das zu diesen Versuchen dienende Wasser muss aus Glasgefässen destillirt sein, d. h. darf keine Spur Kupfer enthalten!

Kalksalzen locker verbunden sind[1] und dass, wenn diese in einer Chlornatriumloesung verdrängt werden, schädliche Quellungsänderungen entstehen. Dieser für Seetiere ganz specielle Fall hat kein Analogon bei Süsswasser -und Landpflanzen, ebensowenig bei höheren Land - und den Süsswassertieren. Man kann *Spirogyra* wochenlang ohne Schaden in einer 0.2 procentigen Chlornatriumloesung aufbewahren. Eine concentrirtere bringt allerdings Schaden, aber dieser kann durch Kalksalze *nicht* verhindert werden. Die Einwände in dem erwähnten Werke sind somit gänzlich unbegründet.



Erscheinungen beim Absterben von *Spirogyra*-Zellen. Die Chlorophyllbänder sind der Einfachheit halber weggelassen.

a. Normaler Zellkern.
b. Zellkern, getödtet durch Kaliumoxalat oder Fluornatrium.
c. Zellkern, getödtet durch verdünnte Schwefelsäure oder Kochen.
d. Zellkern, getödtet durch Schwefelkohlenstoff.
e. Eigenartiger Fall von anomaler Plasmolyse, hervorgerufen durch eine 5% Loesung von Monokaliumphosphat nach 24 Stunden.

[1] Es mag dieses nötig sein um der Magnesiamenge des Meerwassers gegenüber eine genügende Resistenz zu erzielen. Kobert (1903, Sitzgsber. Rostock) hat im Cephalopodenblut locker gebundenen Kalk nachgewiesen.

Injurious Action of Acetates and Formates on Plants.

BY

K. Asō.

Although free acetic and formic acid exert even in considerable dilution injury on lower and higher plants, it was not to be expected that the sodium and calcium salts of these acids would in moderate concentration also exert an injurious action on phaenogams. Various observations have convinced me, however, of this fact. The probable cause is that they undergo easily a hydrolytic dissociation in the living cells, whereby the base is absorbed by proteids and the acids are set free.[1] The behavior of nitrate is evidently different and becomes dissociated in the cells only in the measure as the nitric acid can be reduced and its nitrogen assimilated. Moreover, the following observations show that the effect of various acetates and formates is very different from that of oxalates and further that there exists quite a remarkable difference in the behavior to acetates and formates between the phaenogams and the algae.

Experiment with an Alga.

In the first experiment, a few threads of Spirogyra were put in 1% solutions of potassium oxalate, calcium acetate and calcium formate. The result was :

[1] Calcium acetate as a source of lime may nevertheless be applied successfully in high dilution to plants growing in soil deficient in lime, as experiments by S. Suzuki in this college have shown.

Time.	Potassium oxalate 1%	Calcium acetate 1%	Calcium formate 1%
After 5 minutes.	Chlorophyll bands contracted, and its spiral form destroyed. Nucleus in most cells contracted to a thin thread.	The whole appearance of all threads was quite normal. Nucleus was also quite normal.	The whole appearance was quite normal, also nucleus normal.
1½ hour.	Phenomena essentially the same as before.	Quite normal.	Quite normal.

In the next experiment, 0.5% and 0.1% solutions of potassium oxalate, calcium acetate and calcium formate were applied at a temperature of 10–16°C with the following results:

Time.	Potassium oxalate.		Calcium acetate.		Calcium formate.	
	0.5%	0.1%	0.5%	0.1%	0.5%	0.1%
After 1 hour.	In most cells, chlorophyll-band contracted.	Normal.	Normal.	Normal.	Normal.	Normal.
2 hours.	Almost all chlorophyll-bands and nuclei contracted.	„	„	„	„	„
4 days.	All cells died, spirals contracted, the color became paler.	But few threads still alive.	„	„	Most cells alive, only a few threads dead.	„
6 weeks.	All dead.	All dead.	„	„	„	„

The threads of Spirogyra in 0.1% and 0.5% solutions of the acetate and the formate contained all the time much starch and labil reserve protein as the reaction with coffeïn revealed. From these results, it will be clearly seen that calcium acetate and formate are not poisonous for Spirogyra, while potassium oxalate is a decisive poison, which had been observed long time ago by Loew.

Experiments with Shoots.

The results obtained with shoots of Sorghum, barley, onion and pea are seen from the following tables:

SHOOTS OF SORGHUM, 30 c.m. long.

Time.	Calcium formate.			Calcium acetate.			Sodium acetate.			Sodium sulphate (control)
	1%	0.5%	0.1%	1%	0.5%	0.1%	1%	0.5%	0.1%	1%
After 2 days.	Brown spots appeared on leaves, tips dried up.	Tips of leaves withered.	Tips of leaves commenced to wither.	The leaves lost turgor.	The leaves commenced to lose turgor.	Normal.	The leaves withered.	Normal.	Normal.	Normal.
5 days.	Dead.	Dead.	Tips dried.	Dead.	Dead.	,,	Dead.	,,	,,	,,
8 days.			,,			,,		Dead.	,,	,,
13 days.			All leaves became brown.			,,			,,	,,

SHOOTS OF BARLEY, 15 c.m. long.

Time.	Calcium formate.			Calcium acetate.			Sodium acetate.			Control.	
	0.5%	0.1%	0.05%	0.5%	0.1%	0.05%	0.5%	0.1%	0.05%	Sodium sulphate, 0.5%	Calcium nitrate (anhydr.) 0.5%
After 5 days.	Leaves became yellowish.	The color of leaves became paler.	Normal.	Leaves changed to yellowish.	The color of leaves paled a little.	Normal.	Leaves became a little parler.	Normal.	Normal.	Normal.	Normal.
8 days.	Dead.	Dead.	,,	Dead.	Dead.	,,	Dead.	,,	,,	,,	,,
11 days.			,,			,,		The tips became a little yellow.	,,	,,	,,
20 days.			Still alive.			Stil. alive.		Still alive.	Still alive.	Still alive.	Still alive.

K. Aso.

SHOOTS OF ONION, 5 c.m. long.

Time.	Calcium formate.				Calcium acetate.			Sodium acetates.		Calcium nitrate. (control)
	0.5%	0.1%	0.05%	0.5%	0.1%	0.05%	0.5%	0.05%	0.5%	0.5%
After 1 day.	Leaves lost turgor.	Normal.	Normal.	Some leaves lost turgor.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal
5 days.	Dead.	,,	,,	,,	,,	,,	Tips of the leaves become yellowish.	,,	,,	,,
8 days.		,,	,,	Almost dead.	,,	,,	Dead.	,,	,,	,,
11 days.		,,	,,	Dead.	,,	,,		,,	,,	Withered a little.
20 days.		Developed a little.	Developed a little.		Developed a little.	Developed a little.		Developed a little.	Developed a little.	Developed a little

SHOOTS OF PEA, 12 c.m. long. (DEPRIVED OF COTYLEDONS).

Time.	Calcium formate.		Calcium acetate.		Sodium acetate.		Control.	
	0.5%	0.1%	0.5%	0.1%	0.5%	0.1%	Calcium nitrate (anhydr.) 1%	Sodium sulphate. 1%
After 5 days.	Lowest leaf withered.	Normal.	Dead.	Normal.	Dead.	Normal.	Normal.	Normal.
8 days.	,,	,,		,,		,,	Withered a little.	Withered a little.
11 days.	Almost dead.	,,		,,		,,	Dead.	,,
20 days.		,,		,,		,,		Still alive.

From three results, it is quite clear that the injurious action on shoots of 0.5% solution of formates and acetates is not due merely to the concentration itself, since sodium sulphate at 1% concentration exerted no such noxious influence.

In the next experiments, the influence of oxalate, acetate, formate and nitrate of sodium upon shoots of barley and pea was compared. The results are shown in the following tables :

SHOOTS OF BARLEY, 10 c.m. long.

Time.	Sodium oxalate.		Sodium acetate.		Sodium formate.		Sodium nitrate. (control)
	0.5%	0.1%	0.5%	0.1%	0.5%	0.1%	0.5%
After 2 days.	Tips of leaves killed.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal.
5 days.	Dead.	Yellowing of leaves commenced.	Much injury.	,,	Much injury.	,,	,,
7 days.		One leaf dried up, another nearly dried up.	Dead.	Tip of a leaf commenced to yellow.	Dead.	Tips of a leaf commenced to yellow.	,,
4 weeks		Dead.		One leaf still alive and a young shows its tip.		Dead.	One leaf became yellowish.

SHOOTS OF PEA, 5 c.m. long, DEPRIVED OF THE COTYLEDONS.

Time.	Sodium oxalate.		Sodium acetate.		Sodium formate.		Sodium nitrate. (control)
	0.5%	0.1%	0.5%	0.1%	0.5%	0.1%	0.5%
After 2 days.	Leaves withered.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal.
5 days.	Dead.	„	Leaves withered.	„	Leaves withered partly.		Some leaves withered.
7 days.		Lower leaves lost turgor.	Dead.	„	Almost dead.	„	„
2 weeks.		Dead.		„	Dead.	„	Still alive.

These results show evidently that the injurious action of oxalates on phaenograms is much more pronounced than that of acetates and formates. Further the phenomenon was noticed that the plants killed by the 0.5% oxalate solution had still preserved the green color, while the plants which died in the acetate and the formate solutions had turned light brown. In this case the withering proceeding more slowly gave the oxidases time to act on a chromogen.

Experiments with branches.

Young branches of *Quercus acuta*, *Photinia glabra* and *Capsicum longum* were placed in the solution mentioned with the following results :

QUERCUS ACUTA.

Time.	Calcium formate.		Calcium acetate.		Sodium acetate.		Sodium sulphate. (control)
	1%	0.5%	1%	0.5%	1%	0.5%	1%
After 2 days.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal.
5 days.	Brown color appeared in the veins of leaves.	Brown color appeared in the veins of leaves.	Brown spots appeared on some leaves.	,,	Brown spots appeared on some leaves.	,,	,,
8 days.	Almost dried up.	Almost dried up.	Brown spots appeared on all leaves.	,,	Brown spots appeared on all leaves.	,,	,,
13 days.	Dried up.	Dried up.	Dried up.	Dried up.	Dried up.	Brown spots appeared on some leaves, but still alive.	Still alive.

PHOTINIA GLABRA.

Time.	Calcium formate.		Calcium acetate.		Sodium acetate.		Sodium sulphate. (control)
	1%	0.5%	1%	0.5%	1%	0.5%	1%
After 2 days.	Normal.	Normal.	Normal.	Normal.	Nor .	Normal.	Normal.
5 days.	Midribs of leaves became dark brown.	,,	Youngest leaf became dark.	,,	Brown spots appeared on some leaves.	,,	,,
8 days.	Almost dried up.	Petioles became dark.	,,	,,	Brown spots appeared on all leaves.	,,	,,
13 days.	Dried up.	Still some leaves alive.	Lower leaves still alive.	Still alive.	Dried up.	Brown spots appeared on some leaves, but still alive.	Still alive.

CAPSICUM LONGUM.

Time.	Calcium formate.		Calcium acetate.		Sodium acetate.		Calcium nitrate. (control)
	0.5%	0.1%	0.5%	0.1%	0.5%	0.1%	0.5%
After 2 days.	Commenced to lose turgor.	Normal.	Normal.	Normal.	Normal.	Normal.	Normal.
5 days.	Lost turgor.	,,	Withered,	,,	Withered.	Leaves withered a little.	,,
8 days.	Dead.	,,	Dead.	,,	Dead.	,,	,,
2 weeks.		,,		,,		Withered.	,,

QUERCUS ACUTA.

Time.	Sodium oxalate.	Sodium acetate.	Sodium formate.	Sodium nitrate. (control)
	0.5%	0.5%	0.5%	0.5%
After 2 days.	Brown color appeared on veins of certain leaves.	Normal.	Normal.	Normal.
5 days.	All veins of the leaves became brown.	The color of petioles turned brown.	The color of petioles turned to brown.	,,
8 days.	Almost dried up.	,,	,,	,,
2 weeks.	Dried up.	Veins of leaves became brownish.	Veins of leaves became brown, and brown spots appeared on leaves.	,,

An injurious effect of acetates and formates on young branches is therefore distinctly recognised; the injurious action of the formates, however, was still more marked than that of the acetates. The action of both these salts, however, is much weaker than that of the oxalates.

CONCLUSION.

1. Acetates and formates of alkali metals and calcium act injuriously on phaenogams in solutions of 0.5% and over, while they are under the same conditions not injurious for higher algae, as *Spirogyra*. This forms a marked contrast to the action of neutral potassium oxalate which at the same concentration is not only a more powerful poison for phaenegams but exerts the same poisonous character also upon the higher algae, as Spirogyra.

2. The poisonous action of acetates and formates very probably is caused by the hydrolytic dissociation of these salts into acid and base in the living cells, whereby the base is absorbed by proteids and the acid set free injures the living protoplasm.

Können kleine Dosen Kupfer eine chronische Kupfervergiftung hervorrufen?

VON

M. Toyonaga.

Verschiedene Versuche haben gezeigt, dass kleine Dosen von Kupferverbindungen dem tierischen Körper nicht schaden und Lehmann berichtet, dass selbst 20–30mg. Kupfer pro Tag nach Monaten ihm nicht geschadet hätten. Doch spricht sich Tschirch[1] dahin aus, dass, um defintiv zu entscheiden, ob es eine chronische Kupfervergiftung giebt, Versuche Jahre lang fortgesetzt werden müssten und dass „manche Widersprüche noch der Lösung harren." Für die Ungiftigkeit des Kupfers scheint auch zu sprechen, dass manche Tiere, sowohl Mollusken als Arthropoden, besonders aus dem Meere ein kupferhaltiges Haemoglobin, das sogenannte Haemocyanin, im Blut enthalten. Dhéré fand in 100 c.c. Blut von Octopus vulgaris 18.0–23.5 mg. Kupfer und in dem von Astacus fluviat. 4,0–8,0 mg. Kupfer. Kobert fand ferner, dass dieses Haemocyanin ohne Giftwirkung für Kaninchen ist. Ich beabsichtigte, Kaninchen Jahre lang mit kleinen Dosen Kupfer zu behandeln und falls sie dabei am Leben blieben, das Blut auf kupferhaltiges Haemoglobin zu untersuchen und begann meine Versuche mit vier Tieren, von denen zwei als Controll-Tiere und zwei als Kupfer-Tiere dienten. Jedes Tier erhielt vom 15 April ab pro Tag 50 g. Gerste, befeuchtet mit 10 g. Wasser. Bei den Kupfer-Tieren enthielt dieses Wasser anfangs 5 mg. Kupfer, welches in der Form von kohlensaurem Kupfer (Kupferchlorid mit kohlensaurem Natron in equivalenter Menge versetzt) dargereicht wurde. Jeden Tag um 12 Uhr wurde die übrig gelassene Nahrung gewogen. Jeden zweiten Tag wurde das Körpergewicht bestimmt und die Faeces von Zeit zu Zeit auf Spuren von

[1] Das Kupfer. Stuttgart 1893, S. 114.

Kupfer geprüft. In den ersten Monaten wurde indessen selbst mit der so empfindlichen Ferrocyan-Reaktion kein Kupfer in der Asche der Faeces gefunden; erst als später (von 15 September ab) das eine Kupfer-Tier alltäglich 20 mg. Kupfer erhielt, wurde bald darauf Kupfer im Kote gefunden. Das zweite Kupfer-Tier erhielt von September ab 10 mg. Kupfer pro Tag. Der Tod sämmtlicher Tiere erfolgte leider zu früh, wahrscheinlich im Folge von Erkältung. Die Autopsie ergab bei den Kupfer-Tieren ebenso wenig etwas Abnormales als bei den Controll-Tieren. Eine Prüfung der Leber ergab einen geringen Kupfergehalt, dagegen war kein Kupfer im Gehirn vorhanden. In folgender Tabelle sind die täglichen Wägungen für das Monat berechnet zusammenfasst:

	(A) Controll-Tier.		(B) Controll-Tier.		(C) Kupfer-Tier.		(D) Kupfer-Tier.	
Monat.	Nahrungsaufnahme.	Körpergewicht am Ende jedes Monats.	Nahrungsaufnahme.	Körpergewicht am Ende jedes Monats.	Nahrungsaufnahme.	Körpergewicht am Ende jedes Monats.	Nahrungsaufnahme.	Körpergewicht am Ende jedes Monats.
April. (15–30)	506 g.	1508 g.	655 g.	1498 g.	502 g.	1398 g.	689 g.	1658 g.
Mai.	1421	1557	1336	1587	1490	1557	1600	1827
Juni.	1680	1677	1706	1727	1806	1697	1761	1887
Juli.	1596	1697	1639	1747	1950	1757	1865	2007
Aug.	1229	1547	1319	1617	1492	1617	1665	1917
Sept.	565	1557	604	1117	1237	1537	643	1427
Oct.	579	1160	Am 14. verendet.		875	1320	99	1040
Nov.	872	1200			1121	1180	Am 7. verendet.	
Dec.	Verendet am 10.				Am 3. verendet.			

Das Tier C hatte im ganzen aufgenommen 2035 mg. Cu,
und das Tier D „ „ „ 1040 mg. Cu.

Eine chronische Kupfervergiftung ist somit durch das Kupfercarbonat nicht herbeigeführt worden; denn selbst bis kurz von dem Tode zeigten die Tiere keine Vergiftungssymptome.

Ein Mangan-Versuch.

Zum Schluss sei auch noch ein Versuch kurz erwähnt, in welchem Kaninchen fast elf Monate lang Manganchlorid erhielten. Ich hatte die Vermutung, dass dadurch der Effect der oxidierenden Enzyme im Tier vermehrt würde und wollte ferner prüfen, ob das Haemoglobin dieser Tiere manganhaltig würde[1]; denn Kobert erwähnt, dass das Pinnaglobin der Steckmuschel Mangan statt des Eisens enthält. Doch vor allem wollte ich zuerst versuchen, ob die Kaninchen durch die Manganbehandlung vielleicht etwas widerstandsfähiger gegen Infectionskrankheiten werden könnten, aber ein Versuch mit Milzbrandinfection entschied nicht in diesem Sinne. Versuche in Beziehung auf einen Mn-Gehalt der roten Blutkörperchen hoffe ich später wieder aufzunehmen. Ein Mangantier erhielt in elf Monaten im ganzen 27 g Manganchlorid ohne irgend eine abnorme Erscheinung zu zeigen.

Die folgende Tabelle giebt die Fütterungsdaten,[2] sie dürfte wohl zeigen, dass eine chronische Manganvergiftung per os nicht existiert.

[1] Es wird Mangan nur in sehr geringen Mengen aus dem Darm resorbiert, aber von Riche wurden doch bis 2,5 milligramm Mn_3O_4 in 100 g. normalem Rindsblut gefunden. Nach Debierre (1885) wird durch Mangan die Zahl der roten Blutkörperchen vermehrt. Mangan-Eisen-Pepton wird in neuester Zeit therapeutisch verwendet.

[2] Die täglich abgewogenen Futtermengen waren hier nicht immer gleich

Monat.	(A) Control-Hase (männlich)			(B) Control-Hase (weiblich)			(C) Mangan-Hase (weiblich)			(D) Mangan-Hase (männlich)		
	Nahrungsaufnahme.	NaCl.	Körpergewicht am Ende jedes Monats.	Nahrungsaufnahme.	NaCl.	Körpergewicht am Ende jedes Monats.	Nahrungsaufnahme.	$MnCl_2$. 4 aq.	Körpergewicht am Ende jedes Monats.	Nahrungsaufnahme.	$MnCl_2$. 4 aq.	Körpergewicht am Ende jedes Monats.
Oct. 26–30	248.5 g.	0.8 g.	1865 g.	370 g.	1.0 g.	2342 g.	220.7 g.	0.8 g.	2237 g.	329.5 g.	0.9 g.	1750 g.
Nov.	2013.8	2.8	2075	2187	2.7	2570	2265	2.6	2415	2118.8	2.8	1965
Dec.	1742.5	2.6	1960	1977.4	2.6	2425	2160.2	3.1	2310	1843.9	2.9	1860
Jan.	1132.3	2.0	1608	992.5	1.9	1982	1467	2.5	1995	1350.3	2.3	1565
Feb.	1346.4	2.5	1730	2038.5	1.9	1900	1466.7	2.5	2170	1287.2	2.2	1710
März.	1693.5	2.9	1810	1567	2.6	1910	1958.5	3.0	2250	1708.2	2.8	1701
April.	1453.5	2.5	1955	1437.7	2.6	2100	1651	2.6	2445	1468	2.5	1890
Mai.	1970.5	3.5	2175	2371	3.0	2590	1856	3.1	2680	2056	2.9	2145
Juni.	1666	2.0	2130	1937	2.7	2470	1549.8	2.6	2380	1703.5	2.8	2055
Juli.	Am 2. Verendet.			1529.5	2.6	2595	1182	2.2	2490	1349	2.2	2120
Aug.				1685.4	2.7	2650	1352	2.6	2515	1489.5	2.7	2160

On the Formation of Anthokyan in the Stalk of Barley.

BY

S. Suzuki.

During a series of experiments with barley in pot culture the writer had repeatedly observed that in certain pots the stalks of the barley plants showed a red coloration which according to the tests made with alkali and acid was due to the anthokyan. In other pots manured differently, however, the stalks were all of a normal green color. In the former case it was further observed that the lower leaves in dying off gradually did not show a normal yellow straw color, but a more reddish color here and there with violet spots. These phenomena were not restricted to one variety of barley but observed with four different varieties, some of the two lined and others of the six lined character, viz. Goldenmelon and the Japanese varieties Minoguro, Hozoroi and Kobizen. While not denying the possibility that there exist varieties which under all conditions produce normally some anthokyan in the stalks, it was in the cases I observed certainly an effect of the composition of the manure and therefore a special series of experiments was instituted in order to find the conditions which cause the formation of anthokyan. My observations had led me to the inference that it must be a deficiency of some manuring compound which brought on the formation of anthokyan, since these plants yielded always a smaller harvest than these plants which has a nice green stalk. I experimented with two kinds of soil, the one a sandy soil[1] of great natural fertility, the other a loamy soil[2] of poor natural fertility. My determinations gave the following numbers :—

[1] This soil came from Kawasaki, a place about 12 miles south of Tokyo.

[2] This soil came from Komaba, a suburb of Tokyo.

The sandy soil contained 97.8% of fine earth (<0.5 m.m.) and in the air-dry soil was found :

Total nitrogen		0.092%
Potassa, soluble in hot conc. HCl		0.211 ,,
Phosphoric acid	Soluble in hot conc. HCl.	0.261 ,,
	Soluble in 1% citric acid solution.	0.100 ,,

and in the loamy soil :

Fine earth (<0.5 m.m.)		97.47 %
Total nitrogen		0.609 ,,
Potassa, soluble in hot conc. HCl		0.280 ,,
Phosphoric acid	Soluble in hot conc. HCl.	0.541 ,,
	Soluble in 1% citric acid solution.	0.044 ,,

These soils were partly applied as such and partly mixed with various quantities of purified quartz sand in order to diminish the amount of available phosphoric acid in some pots. While as general manure served 2.4 g. potassium sulphate, 1.7 g. sodium nitrate and 2.3 g. ammonium sulphate for each pot, the phosphoric acid was applied as double superphosphate[1] in different quantities. Since the ammonium sulphate is a physiologically acid compound and the amount of sodium nitrate applied was much smaller, the general reaction of the manure was therefore weak acid. The plan of this experiment will be seen from the following table :

[1] This sample contained 42.12% of soluble P_2O_5.

No. of pots.	Kind of soil.	Quantities of.		Weight of mixtures.	General manure per pot.	Phosphoric acid added.	Amount of available phosphoric acid (soluble in 1% citric acid solution) in each pot.	
		Soil.	Purified sand.				absolute.	percentage.
		g.	g.	g.		g.	g.	g.
I.	Sandy soil.	260	2250	2510	Potassium sulphate ..... 2.4 g. Sodium nitrate ..... 1.7 „ Ammonium sulphate ..... 2.3 „	0	0.260	0.010
II.	„	433	2083	2517		„	0.434	0.017
III.	„	650	1875	2525		„	0.651	0.026
IV.	„	1300	1250	2550		„	1.301	0.051
V.	„	1600* (orig. soil)	0	2600		„	2.602	0.100
VI.	„	„	„	„		0.04	2.645	0.102
VII.	„	„	„	„		0.22	2.817	0.108
VIII.	„	„	„	„		2.15	4.750	0.183
IX.	Loamy soil.	292	2083	2375		0	0.128	0.005
X.	„	438	1875	2313		„	0.192	0.008
XI.	„	875	1250	2125		„	0.384	0.018
XII.	„	1750 (orig. soil)	0	1750		„	0.768	0.044
XIII.	„	„	„	„		0.02	0.781	0.045
XIV.	„	„	„	„		0.04	0.811	0.046
XV.	„	„	„	„		0.22	0.982	0.056
XVI.	„	„	„	„		2.15	2.916	0.167
XVII.	Sandy soil.	2600 (orig. soil)	„	2600	Unmanured.		2.602	0.100
XVIII.	Loamy soil.	1750 (orig. soil)	„	1750	„		0.768	0.044

10 seeds were sown per pot on Jan. 13 (1905), and the young shoots reduced to 3 of nearly equal size on Febr. 3. On Febr. 9 (27 days after sowing) some difference was perceptible which gradually became still more marked. The stalks of those plants grown in pots with a small amount of phosphoric acid showed a red color and the less the content of phosphoric acid in a pot the deeper was also the coloration. Indeed such plants showed a sickly appearance—a phenomenon of phosphoric acid hunger—they re-

* 2600 g. of the sandy soil, 1750 g. of the loamy soil and 2500 g. of the sand had almost the same volume.

mained short, the formation of new shoots was retarded, and the tips of the older leaves died off gradually with a reddish yellow color. The details of observations in different periods and the final harvest are shown in the following table :—

No. of pots.	Kind of soil.	Percent of available P_2O_5 in the soil.	Coloration of stalk.			No. of stalk Febr. 21.	Average length of stalk Febr. 21. c.m.	Total air dry harvest. g.	Weight of seeds. g.
			Febr. 9.	Febr. 21.	March 10.				
I.	Sandy soil	0.010	red	red	red	3	9.9	2.0	0
II.	,,	0.017	slightly red	,,	,,	7	12.4	3.0	0
III.	,,	0.026	,,	,,	,,	5	12.3	6.0	0.6
IV.	,,	0.051	,,	,,	,,	3	9.5	7.5	2.0
V.	,,	0.100	,,	slightly red	,,	9	15.1	23.7	6.4
VI.	,,	0.102	green	,,	,,	9	18.9	22.2	6.6
VII.	,,	0.108	,,	green	reddening set in	14	21.6	23.1	6.6
VIII.	,,	0.183	,,	,,	green	10	20.4	24.8	7.3
IX.	Loamy soil	0.005	red	red	red	3	9.1	1.0	0
X.	,,	0.008	,,	,,	,,	,,	9.1	1.6	0
XI.	,,	0.018	,,	,,	,,	,,	8.9	2.3	0.3
XII.	,,	0.044	,,	,,	,,	,,	9.9	0.8	0
XIII.	,,	0.045	,,	,,	,,	,,	10.0	3.7	0.4
XIV.	,,	0.046	,,	,,	,,	,,	9.8	7.7	1.5
XV.	,,	0.056	,,	,,	,,	6	12.9	11.5	2.2
XVI.	,,	0.167	green	deep green	slightly red	12	21.0	19.0	5.7
XVII.	Sandy soil	0.100	red	red	red	6	11.9	6.6	2.0
XVIII.	Loamy soil	0.044	,,	,,	,,	3	9.7	0.2	0

The observations do not quite agree with similar observations of Clausen on the stalk of oats. He observed on a very peculiar soil[1] that with the increase of phosphoric acid the number of stalks was increased but not the weight of grains and further that in these cases of the depression of the yield

[1] A black peaty sandy-soil.

in grains also the stalks showed a bluish red color.[1] In order to observe whether also the deficiency in nitrogen or potassa can cause the formation of anthokyan in the stalk some further experiments were made, the plan of which is seen from the following table :

	No. of pots.	Kind of soil.	Quantities of. Soil. g.	Quantities of. Sand. g.	General manure, per pot.	Relative amount of available N in the soil.
Without nitrogen manure.	XIX.	Sandy soil.	2600	0	5.1 g. Double superphosphate. 2.4 „ Potassium sulphate.	Sandy soil, 1
	XX.	„	650	1875		„ „ 0.25
	XXI.	„	433	2083		„ „ 0.16
	XXII.	Loamy soil.	1750	0		Loamy soil, 1
	XXIII.	„	438	1875		„ „ 0.25
	XXIV.	„	292	2083		„ „ 0.16

	No. of pots.	Kind of soil.	Quantities of. Soil. g.	Quantities of. Sand. g.	General manure, per pot.	Relative amount of available potassa in the soil.
Without potassa manure.	XXV.	Sandy soil.	2600	0	5.1 g. Double superphosphate. 1.7 „ Sodium nitrate. 2.3 „ Ammonium sulphate.	Sandy soil, 1
	XXVI.	„	650	1875		„ „ 0.25
	XXVII.	„	433	2083		„ „ 0.16
	XXVIII.	Loamy soil.	1750	0		Loamy soil, 1
	XXIX.	„	438	1875		„ „ 0.25
	XXX.	„	292	2083		„ „ 0.16

[1] Journal für Landwirtschaft, 1905, p. 225.

All other conditions were the same as in the first experiment. The result is seen from the following table :—

	No. of pots.	Kind of soil.	Relative amount of available N in the soil.	Feb. 9.	Coloration of stalks.			No. of stalks.	Average length of stalks. c.m.	Total air-dry weight.	Weight of grains.
					Febr. 16.	Febr. 21.	March 10.				
Without nitrogen manure.	XIX.	Sandy soil	1	No difference was noticed.	green	red	red	9	20.0	5.3	0.7
	XX.	„	0.25		„	„	„	5	14.3	3.0	1.0
	XXI.	„	0.16		ambiguous	„	„	7	14.0	2.3	0.5
	XXII.	Loamy soil	1		slightly red	distinctly red	„	6	15.0	1.1	0.3
	XXIII.	„	0.25		red	„	„	3	13.5	1.6	0.6
	XXIV.	„	0.16		„	„	„	5	11.4	0.9	0.2

	No. of pots.	Kind of soil.	Relative amount of available K_2O in the soil.	Feb. 9.	Coloration of stalks.			No. of stalks.	Average length of stalks. c.m.	Total air-dry weight.	Weight of grains.
					Febr. 16.	Febr. 21.	March 10.				
Without potassa manure.	XXV.	Sandy soil	1	No difference was noticed.	green	green	green	10	20.8	23.4	8.5
	XXVI.	„	0.25		„	„	„	9	16.6	16.0	4.3
	XXVII.	„	0.16		„	„	„	7	15.4	16.2	4.2
	XXVIII.	Loamy soil	1		„	„	„	10	21.9	12.7	2.6
	XXIX.	„	0.25		„	„	„	8	15.9	7.0	0.3
	XXX.	„	0.16		„	„	„	6	13.5	3.0	0

From this table it will be seen that the deficiency of nitrogen may also cause the formation of anthokyan, while the deficiency of potassa has not such an influence.[1] But, since it might be objected that there existed in the original soils no deficiency of available potassa and that there was even sufficient potassa in the mixtures of these soils with sand, a further experiment with water culture was instituted. The solution had the following compositions :

I. Without nitrogen.
   1.61 per mille Magnesium sulphate (anhydr.).
   1 ,, ,, Calcium sulphate (,,).
   2 ,, ,, Monopotassium phosphate.
   2 ,, ,, Precipitated tricalcium phosphate.
   Few drops of ferric chlorid.

II. With nitrogen.
   Solution I+2 per mille Calcium nitrate (anhydr.).
   0.5 ,, ,, Potassium nitrate.

III. Without phosphoric acid.
   3 per mille Calcium nitrate (anhydr.).
   1 ,, ,, Potassium nitrate.
   1.61 ,, ,, Magnesium sulphate (anhydr.).
   Few drops of ferric chlorid.

IV. With phosphoric acid.
   Solution III+1 per mille Monopotassium phosphate.
   0.02 ,, ,, Ferric phosphate.

V. Without potassa.
   3 per mille Calcium nitrate (anhydr.).
   1.61 ,, ,, Magnesium sulphate (,,).

[1] If in an unheated glass house during cold winter months young barley plants show some anthokyan even on well manured soil, this is easily explained by the low soil temperature while the air temperature in the house can reach on sunny days 16–12° C. In a case I observed the pots holding 8 kilo soil showed in the afternoon in the center 15 c.m. below the surface only 6° C, which naturally depresses the function of the roots, becoming incapable to provide the shoots with a sufficient amount of mineral food. The root is too cold for the shoot exposed to the warm air.

2 per mille Monocalcium phosphate (hydr.).

Few drops of ferric chlorid.

VI. With potassa.

Solution V + 1 per mille Potassium nitrate.

0.5 „ „ Monopotassium phosphate.

VII. Knop's solution.

3 per mille Calcium nitrate (anhydr.).

1 „ „ Potassium nitrate.

1.61 „ „ Magnesium sulphate (anhydr.).

1 „ „ Monopotassium phosphate.

0.02 „ „ Ferric phosphate.

Young barley shoots were placed in these solutions[1] on Jan. 14 (1905) and kept in a glass house. From time to time a current of air was passed through the flasks. Seventeen days afterwards a very striking difference was noticed. The plants which could develop normally in the full nourishing solution, had also a deep green stalk. The plants in the incomplete culture solutions could of course not develop beyond a very limited size, but anthokyan was shown only in the case of deficiency of nitrogen[2] and of phosphoric acid, but not in the case of the deficiency of potassa. In this latter case, however, the first leaves dying off gradually developed some brown spots, a phenomenon which also was regarded by Wilfarth as characteristic for the deficiency of potassa.[3] Finally not only these soil experiments were repeated with several varieties of barley with essentially the same results, but also a similar experiment was made with lettuce, which growing on poorly manured soil showed an abundant formation of anthokyan in the leaves and after transplanting in richly manured soil they developed soon a deep green color.

My observations with barley will, I think, permit the conclusion :

[1] During the first three weeks, I used dilute solutions, adding the same volume of distilled water.

[2] The shoots developed in absence of nitrogen were characterised by their unusually long main roots.

[3] Journal für Landwirtsch; 1903, p. 129.

The formation of anthokyan in the stalks of barley can be regarded as a sign of deficiency of available phosphoric acid or nitrogen or of both in the soil.

On the Influence of the Reaction of the Manure upon the Yield.

BY

K. Aso and Rana Bahadur (Nepal).

Various reasons have been adduced for the fact that on some soils sodium nitrate is superior to ammonium sulphate, while on other soils both forms of nitrogen are equally satisfactory. On some light soils again ammonium sulphate was found superior to sodium nitrate.

A loss of ammonia may be caused by certain kinds of soil bacteria which more readily assimilate ammonia than nitrate, rendering thus the ammoniacal nitrogen partly insoluble in the form of bacterial protoplasm. Some loss of ammonia is further ascribed to the transformation of the sulphate into carbonate and volatilization of the latter, a process that may take place on soils rich in calcium carbonate (Wagner).

Nitrification with following leaching may also cause losses; finally nitrate-nitrogen may be lost by denitrification. Some soils and manures favor one process more than another, hence the differences in the yield according to the forms of nitrogen can be partly accounted for.

But there exist still other reasons. Loew has called attention to the fact that for the assimilation of nitrates[1] in the leaves a certain concentration of sugar is required, i.e., for the reduction to ammonia needed for the protein-formation. This condition is not so readily fulfilled in cool seasons and with clouded skies, than in warm sunny weather. In cool rainy seasons ammonia therefore will be better utilized than nitrates, ceteris paribus.[2]

[1] The absorption from the soil by the roots is sometimes called erroneously assimilation.

[2] The absence of sufficient sugar in etiolated leaves kept in darkness accounts for the observation of Laurent, that such leaves assimilate much more readily ammonium salt than nitrate.

Another and powerful factor for the efficacy of one or the other form of nitrogen is the reaction of the manure. A mixture of superphosphate with the physiologically acid ammonium sulphate is generally unfavourable unless some carbonate of lime is applied to counteract the evil effect of the acid reaction on the roots (Wagner). In order to avoid the formation of too acid a reaction by the application of ammonium sulphate, it is further recommended to use a mixture of it with the physiologically alcaline sodium nitrate (Kossowitsch).

Such considerations have led us to compare the effects of the (neutral) disodium phosphate with the (acid) monosodium phosphate and with calcium superphosphate in presence of ammonium sulphate or of sodium nitrate in sandculture and in soilculture; the former was carried out by K. Aso, the latter by Rana Bahadur.

Sandculture with pea and barley.

Each pot containing 2.5 kilo well purified sand was manured as follows:

Potassium sulphate ..... 0.73 g.
Sodium chlorid ..... 0.10 „
Finely powdered limestone ..... 3.6 „
„ „ magnesite ..... 4.2 „
Concentrated emulsion of ferric phosphate..... 5. c.c.

The special manure consisted of 0.4 g. nitrogen and 0.2 g. phosphoric acid in the following compounds (grams)[1]:

	A.	B.	C.	D.	E.
Disodium phosphate	1.01	0	1.01	0	0.505
Monosodium phosphate.....	0	0.27	0	0.27	0.135
Sodium nitrate	2.49	0	0	2.49	1.214
Ammonium sulfate	0	1.88	1.88	0	0.942

[1] The amounts of the sodium phosphates refer to the crystallized salts Na_2HPO_4+12 aq. and $Na_1H_2PO_4+4$ aq.

On March 23 shoots of pea and barley were transplanted, three in each pot. During the development the water content of the sand was kept at 60% of the water holding capacity of the sand. Towards the time of harvesting a considerable difference was noticed: the pea plants in A and the barley plants in B were far behind the plants in the other pots. A contained the most alkaline, B the most acid mixture.

The plants were cut, June 21, with the following weight in the air-dry state:

PEA.

	Total yield.	Fruits.	Stalks.
A.	12.7 g.	0	12.7 g.
B.	Killed by an accident.	Killed by an accident.	Killed by an accident.
C.	28.2 g.	4.0 g.	24.2 g.
D.	37.3	0.8	36.5
E.	34.2	6.0	28.2

On examining the pea-roots, there were no nodules.

Barley was harvested also on the same day as pea and weighed in the air-dry state:

BARLEY.

	Total yield.	Ears.	Straw.	Full grains.
A.	32.0 g.	9.3 g.	22.7 g.	5.3 g.
B.	22.4	7.7	14.7	4.1
C.	33.0	10.4	22.6	5.0
D.	35.2	10.0	25.2	5.0
E.	33.7	11.0	22.7	7.2

From these results it is clearly shown that *the combination of several acid manures or of several alkaline manures is not favorable, while a*

mixture of acid with alkaline salts acts beneficially on the yield. The great depression in A with barley is surprising, since there was added $CaCO_3$ to the sand.

The application of sodium nitrate together with monosodium phosphate enhanced especially the straw-production, while ammonium sulphate together with sodium nitrate, monosodium phosphate and disodium phosphate was favorable for grain production.

Sandculture with Rice.

On July 11, a bundle of young paddy rice plants was transplanted in each pot, each bundle consisting of three plants.[1] Water was continuously present a little over total water capacity of the sand. On Sept. 28, aphotograph was taken (see Plate I) and on Nov. 10, the plants were cut and weighed in the air-dry state with the following result in grams :

	Total yields.	grains.	Straw.	Number of stalks.
A.	1.3	0	1.3	4
	1.0	0	1.0	3
B.	26.0	5.5	20.5	13
	20.0	6.0	14.0	13
C.	29.9	8.5	21.5	15
	22.0	6.5	15.5	12
D.	2.0	0.2	1.8	3
	0.8	0	0.8	3
E.	11.2	2.8	8.4	10
	4.7	9	4.7	8

[1] Two series of the experiment were made in this case.

This table shows that sodium nitrate is not a suitable source of nitrogen for rice, as had already been proved by Prof. Nagaoka for rice and other paddy plants.

CONCLUSION.

1. The reaction of the manuring compounds is of very great influence.

2. The combination of ammonium sulphate and disodium phosphate yielded the best result in the case of paddy-rice, while the mixture of sodium nitrate and monosodium phosphate produced the highest yield with barley and pea.

Soilculture with onion and barley.

The pots held 8 kilo of a loamy soil. In these experiments two forms of potassa were applied, the neutral K_2SO_4 and the alkaline K_2CO_3.

2 Pots A, each received:		2 Pots, B, each received:	
		Na_2HPO_4 12 aq.	11.94 g. (equivalent)
Double superphosphate	5 g.	K_2CO_3	10 „
Potassium sulphate	10 „	Na_2SO_4	5 „
Sodium „	5 „	$FeSO_4$	1 „
Ferrous „	1 „	$CaCO_3$	1.1 „ (equivalent to CaO in 5 g. superphosphate)
A_1 5 g. $(NH_4)_2SO_4$		B_1 5 g. $(NH_4)_2SO_4$	
A_2 6.4 „ $NaNO_3$ (equiv.)		B_2 6.4 „ $NaNO_3$	

Experiment with Onion.

Onion seeds were sown, twenty in each pot, Nov. 8., and the plants were some weeks later on, thinned to 14 in each pot. As the plants made very little growth during the winter months, the first measurement was made March 14. The plants were cut May 22. The measurements, and the weight of the harvest in the fresh state are shown in the following table :

		Average height in c.m.		Fresh weight of plants.	Relative harvest. $B_2 = 100$
		March 14.	May 17.		
A_1	Potassium sulphate. Superphosphate. Ammonium sulphate.	7.4	44.0	146 g.	113.5
A_2	Potassium sulphate. Superphosphate. Sodium nitrate.	6.4	42.8	142.2	110.5
B_1	Potassium carbonate. Disodium phosphate. Ammonium sulphate.	11.8	45.7	207	160.9
B_2	Potassium carbonate. Disodium phosphate. Sodium nitrate.	6.0	41.7	128.6	100

From this table it will be learned that *ammonium sulphate produced a far better result than sodium nitrate, when phosphoric acid was offered as disodium phosphate and potassa in the form of potassium carbonate*, in other words, when care was taken to neutralize the acidity produced gradually by ammonium sulphate or to convert a ammonium sulphate into ammonium carbonate.[1] In comparing A_1 with B_1 an enormous difference in yield will be noticed, chiefly due to the difference in the reaction of the manure and partly also to the presence of sodium in B_1.

The observations of various authors, recently again made by W. Krüger in a series of extended investigations, viz., that *nitrification is not necessary* and that *ammonia* under proper conditions *is as efficacious* as nitrate, is confirmed by our results.

[1] Some care has of course to be taken that ammonium carbonate remains sufficiently diluted, as it becomes sooner injurious by higher concentration than the ammonium sulphate does.

Experiment with barley.

A second experiment was made with two sided barley under exactly the same conditions as above mentioned. Twenty seeds were sown December 12, and a selection was made Jan. 17, leaving 10 plants of nearly equal height in each pot. The average height of the plants measured every month, is shown in the following table:

	Jan. 23	Feb. 27.	Mar. 20.	Apr. 18.	May 17.
A_1 Potassium sulphate. Superphosphate. + Ammonium sulphate.	12.0	23.2	31.4	64.8	87.7
A_2 Potassium sulphate. Superphosphate. + Sodium nitrate.	13.7	25.5	33.0	63.2	90.3
B_1 Potassium carbonate. Sodium phosphate. + Ammonium sulphate.	17.2	28.5	33.6	64.6	85.0
B_2 Potassium carbonate. Sodium phosphate. + Sodium nitrate.	16.6	31.5	41.1	65.4	81.0

The plants were harvested June 15, and weighed in the air dry state. The result was as follows:

	Number of stalks.	Total number of ears.	Number of undeveloped ears.	Weight, g.	
				Whole plants.	Seeds.
A1	19	19	4	67	15.6
A2	18	18	1	64	20.6
B1	25	25	1	71	24.1
B2	29	29	0	79	26.5

This result shows that, as regards the production of seeds, the three mixtures which contained sodium compounds, viz. A_2, B_1, and B_2 gave a better result than the mixture A_1 containing no sodium salt. Further it will be observed that the two mixtures B_1 and B_2 containing disodium phosphate produced a better yield than the two mixtures containing superphosphate, and finally it will be noticed that the alkaline mixture B_2 which in case of the onion produced the poorest result, proved the best for barley, at least on the loamy humus soil serving for this test.[1]

As a general conclusion, however, it may be mentioned that small changes in the reaction of the manure have often a much greater influence on the yield than might be presumed, and that the effects differ with different crops.

[1] Also in the above described sand culture with barley the mixture of disodium phosphate with sodium nitrate produced a good result, not far behind the best. Onion and the pea are not favored by such a decisively physiologically alkaline mixture.

A B C D E

A: $Na_2HPO_4 + NaNO_3$

B: $NaH_2PO_4 + (NH_4)_2SO_4$

C: $Na_2HPO_4 + (NH_4)_2SO_4$

D: $NaH_2PO_4 + NaNO_3$

E: $NaH_2PO_4 + NaNO_3$ / $Na_2HPO_4 + (NH_4)_2SO_4$

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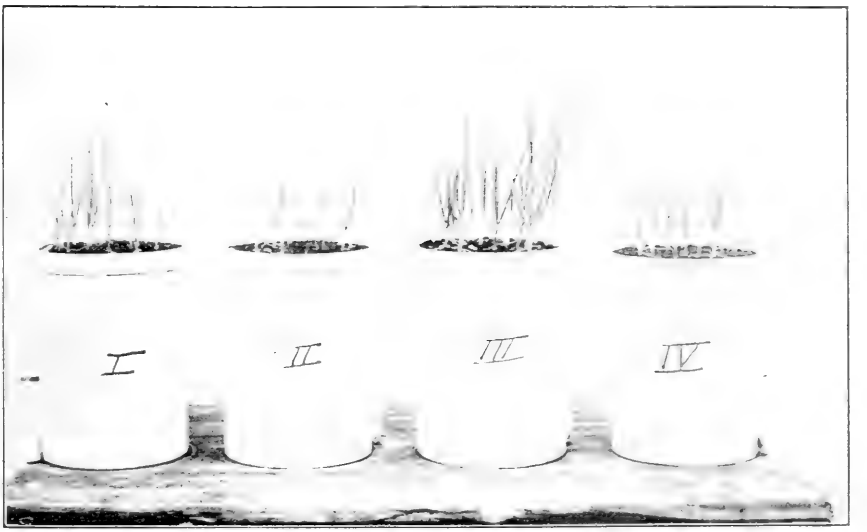

Influence of the Reaction of the Manure on Onion.

I. Sodium nitrate + Superphosphate.
II. Sodium nitrate + Disodium phosphate.
III. Ammonium sulphate + Disodium phosphate.
IV. Ammonium sulphate + Superphosphate.

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On the Manurial Value of Calcium Cyanamide.

BY

K. Asō.

Since the manufacture of calcium cyanamide had been established in Berlin, various authors have reported on the results of manurial experiments with this compound. M. Gerlach and P. Wagner[1] were the first who concluded that it has a manurial value similar to that of chilisalpeter for oats, barley, mustard and carrot, and that it did not injure plant growth in the quantity applied. Later on, Wagner[2] compared the action of chilisalpeter, ammonium sulphate and calcium cyanamide upon turnips and observed that calcium cyanamide yielded the best when applied before sowing but it exerted a very unfavorable action when applied in form of topdressing. A. Frank[3] also concluded that the manurial value of nitrogen in the form of calcium cyanamide is almost equivalent to those of ammonium salts and nitrates. But Br. Tacke[4] made a series of pot experiments with a peaty soil and noted an injurious action of calcium cyanamide on mustard on applying it 8 days before sowing, and that the injury was not observed on applying it $2\frac{1}{2}$ months before sowing. The manurial value on that kind of soil was behind that of chilisalpeter. Steglich[5] observed a retarding action of calcium cyanamide on mustard when applied 7 days before sowing, but not when applied 17 days before sowing; the result was even better than with chilisalpeter and ammonium sulphate.

[1] Landw. Presse. 1903. 30, 367.

[2] Ebend. 1904. 30, 493.

[3] Ill. Landw. Zeit. 1903. 23, 491.

[4] Mitt. d. Ver. z. Förder. d. Moorkultur. i. D. R. 1903. 21.

[5] Tätigkeitsber. d. Versuchsstation Dresden. 1903.

R. Otto[1] found that the value of calcium cyanamide is equivalent to those of nitrates and ammonium salts for spinach, lettuce, white cabbage and maize, and concluded that this manure seems to be suitable for garden plants, provided that it is applied a week or two before planting or else dug into a depth of 13–26 c.m. H. von Feilitzen[2] observed that this new fertilizer gave poorer results than nitrate of soda and was also not equal to ammonium sulphate on certain soils with barley, oats, wheat and potatoes. Zielstorff[3] showed by pot experiments, that when calcium cyanamide was applied at sowing time, its value was 88.4% of that of chilisalpeter and when the seed was sown 10 days after the application of the manure, the value of the latter was equal to 92.8%. A. D. Hall[4] concluded that calcium cyanamide is an effective nitrogenous manure, though more extended experiments are necessary to decide whether the unit of nitrogen is worth more or less than in sulphate of ammonia. Most recently Haselhoff[5] published a series of the experimental results made in Marburg and arrived at the conclusion that calcium cyanamide has a retarding action on the germination of seeds, and as soon as the cyano-compounds are decomposed, all nitrogen contained in this manure assumes the form of ammonia and its value is almost equal with nitrogen in chilisalpeter. He noted also that the time required for the complete decomposition of this cyanamide is different according to the qualities of soils. It was for us of special interest to carry on similar manurial experiments with crops especially cultivated in Japan.

One series of experiments was made in large zinc cylinders open on both ends, which were sunk into the ground and filled with a soil unmanured for six years. The area of each cylinder corresponded to $\frac{1}{30000}$ hectar. For manuring the following ratios were applied: $K_2O = 80$ kg. per ha. as potassium sulphate, $P_2O_5 = 100$ kg. per ha. as double superphosphate; further N in the form of calcium cyanamide in cylinder I at the ratio of 100 kg. N

[1] Gartenflora, 53, 1903. No. 20. 524–538.

[2] Abstr. in Exp. stat. Record. Washington. Vol. XVII. No. 1. 17.

[3] Bied. Centr.-Blt. f. Agrik.-chem. 1905. 34. 217–218.

[4] Journ. of Agric. Science. Vol. I. Part I. 1905. 146–148.

[5] Landw. Jahrbücher XXXIV. 1905. 597–616.

per ha., while cylinder II received the equivalent quantity of N in the form of ammonium sulphate and cylinder III in the form of chilisalpeter.

The calcium cyanamide was the commercial crude product, a fine black powder, containing 19.2% N. On April 19, 17.2 grams of calcium cyanamide were mixed with the soils to about 5 c.m. depth, while the other manures were applied April 28. Ten days after the application of calcium cyanamide, some buckwheat seeds were sown in each cylinder, which germinated well but were injured afterwards by insects. Hence, on June 19, 18 seeds of upland rice were sown and later on the young plants reduced to nine per cylinder, all of about equal height. On Oct. 13 the plants were harvested with the following result :

Cylinder.	Form of N.	Number of shoots.	Weight of grains air dry.	Weight of straw air dry.	Total yield air dry.
I.	Calcium cyanamide	48	91.5 g.	120.5 g.	212.0 g.
II.	Ammonium sulphate	39	68.5	92.0	160.5
III.	Sodium nitrate	42	70.5	80.2	150.7

An analogous experiment was made with Sesamum. The seeds were sown May 25, two weeks after the application of calcium cyanamide and the plants in each cylinder were reduced to nine. On Sept. 15, the plants were harvested, but unfortunately all the leaves had already been dropped at the ripening stage. The result was as follows :

Cylinder.	Form of N.	Average length of each plant.	Weight of seeds air dry.	Weight of stems without leaves.	Total yield.
I.	Calcium cyanamide	45.5 cm.	13.0 g.	54.0 g.	67.0 g.
II.	Ammonium sulphate	41.1	14.8	59.5	74.3
III.	Sodium nitrate	42.1	4.5	43.2	47.2

In a third experiment, hemp seed was sown in such cylinders on June 26, six weeks after the application of calcium cyanamide. Later on, the

young plants were reduced to six of equal size in each cylinder. On Oct. 13, the plants were harvested with the following result :

Cylinder.	Form of N.	Weight of leaves air dry.	Weight of stalks air dry.	Total yield.
I.	Calcium cyanamide	47.5 g.	36.5 g.	84.0 g.
II.	Ammonium sulphate	29.9	34.1	64.0
III.	Sodium nitrate.....	31.7	39.2	70.9

It is therefore quite evident that calcium cyanamide was generally superior to ammonium sulphate and to chilisalpeter, in the three experiments.

Since the manurial value of calcium cyanamide is different according to the kind of soils, as several authors have shown, a series of pot experiments with two different soils was made, one of which was an alluvial sandy soil from Kawasaki near the river Rokugō, containing only 1% of humus, while the other was a diluvial loamy soil from Komaba, containing about 10% of humus. The experiments were carried out in pots, each containing about 8 kg. air dry soil and of an area of $\frac{1}{2000}$ hectar. The quantities of manures applied May 25 were as follows :

Common superphosphate	15 grm.	General manures.
Kainit	10 grm.	
Calcium cyanamide.....	2.6 grm.	Special manures.
Ammonium sulphate	2.4 grm.	
Sodium nitrate.....	3.03 grm.	

Experiment with upland rice.

Fifteen seeds of uplandrice were sown in each pot May 30, which were afterwards reduced to six. On August 23 the plants commenced to blossom. The harvest took place Oct. 28, with the following result :

SANDY SOIL.

Form of N.	Weight of straw air dry.	Weight of grains air dry.	Total yield.
Calcium cyanamide	59.0 g.	16.7 g.	75.7 g.
Ammonium sulphate.....	55.0	18.2	73.2
Sodium nitrate	53.2	16.5	69.7

LOAMY SOIL.

Form of N.	Weight of straw air dry.	Weight of grains air dry.	Total yield.
Calcium cyanamide	34.4 g.	22.2 g.	56.6 g.
Ammonium sulphate	32.2	24.1	56.3
Sodium nitrate	40.2	17.7	47.9

Experiment with paddy rice.

On July 10, young rice plants from the seed bed were transplanted in each pot, three bunches in each, each bunch consisting of three plants, a week after the application of the manures. The plants were cut Nov. 10. The result obtained was as follows :

SANDY SOIL.

Form of N.	Weight of straw air dry.	Weight of grains air dry.	Total yield.
Calcium cyanamide	32.5 g.	13.0 g.	45.5 g.
Ammonium sulphate.....	25.0	11.0	36.0
Sodium nitrate	27.3	10.7	38.0

LOAMY SOIL.

Form of N.	Weight of straw air dry.	Weight of grains air dry.	Total yield.
Calcium cyanamide	31.0 g.	17.2 g.	48.2 g.
Ammonium sulphate.....	33.0	20.7	53.7
Sodium nitrate	37.5	20.0	57.5

Experiment with Hemp.

On June 8, 10 seeds of hemp were sown in each pot, and the young plants later on reduced to six, all of equal size. Sowing took place two weeks after manuring. Flowering commenced Aug. 28. The plants were cut Sept. 11. The development of plants in the pot manured with ammonium sulphate was a little behind other two, so that these plants were harvested four days after. The result was:

LOAMY SOIL.

Form of N.	Average height.	Weight of stems.	Weight of leaves.	Total yield.
Calcium cyanamide	88.8 cm.	16.8 g.	10.7 g.	27.5 g.
Ammonium sulphate.....	80.3	20.5	14.0	34.5
Sodium nitrate	87.7	16.5	10.0	26.5

All the results mentioned show distinctly that calcium cyanamide is an effective nitrogenous fertilizer, the only not satisfactory result being that with paddy soil. That soil was rich in humus and closely related to moor soils, which according to Tacke and Feilitzen yield not such satisfactory results with calcium cyanamide as other soils do.

As a general conclusion, I can confirm that calcium cyanamide is not inferior to ammonium sulphate and to chilisalpeter.

The Efficacy of Calcium Cyanamide under Different Conditions.

BY

R. Inamura.

It has been repeatedly observed that under certain conditions ammonium sulphate will give a better result than sodium nitrate, while under other conditions the latter source of nitrogen has been found superior to the former. The new nitrogen manure of commerce, the calcium cyanamide or „Kalkstickstoff" (Lime-nitrogen) has not yet been tested under all possible conditions although various valuable observations have been made which prove that „Kalkstickstoff" can act as favorably as nitrate or ammonium sulphate.

Since the reaction of the manure has surely a great influence upon the utilisation of the nitrogen compound applied, I have made some experiments to observe how the acid superphosphate and the neutral disodium phosphate would modify the efficacy of calcium cyanamide, which we must consider as an alkaline manure, because it yields on decomposition in the soil calcium carbonate and ammonia.

$$Ca=N-C\equiv N+3H_2O=CaCO_3+2NH_3$$ [1]

While sodium nitrate is physiologically alkaline and ammonium sulphate is physiologically acid, „Kalkstickstoff" must be classed with *de facto* alkaline manures.

Löhnis has observed 8 kinds of bacteria which can accomplish the decomposition of this compound. Since ammonia and ammonium carbonate have a strong alkaline reaction, while ammonium sulphate although of neutral reaction is physiologically acid, it became probable that the best conditions for the efficacy of both these ammonium compounds are not

[1] This ammonia would, however, soon be transformed into carbonate in the soil.

exactly the same. Therefore I have applied together with lime-nitrogen two different sources of phosphoric acid, namely the acid double superphosphate and the neutral disodium phosphate.

The lime-nitrogen was applied to the soil two weeks before the other manure was applied and the mixture kept sufficiently moist in order to have the above decompositions thoroughly realized.

8 pots each holding 8 kg. soil served for the experiment. The general manure was per pot:

Calcium cyanamide ..... 4 g.
K_2SO_4 ..... 6 „
$FeSO_4$ ..... 1 „

Two pots A received double superphosphate 5 g., while two pots B 10 g. crystallized disodium phosphate, the equivalent in P_2O_5.

Each of these pots received 10 seeds of *Brassica chinensis* Oct. 10, and when a height of the young plants of about 15 cm. was reached, they were thinned to 4 per pot, all of equal size.

The plants in pots B showed in the beginning a better development and were later on somewhat darker green but nevertheless the plants in the pots A had finally a greater weight. The plants were cut Dec. 10 and weighed in the fresh state with the following result:

	Produce per pot.			
	Four plants of Brassica chinensis.			
	Average length of leaves. cm.	Leaves. grams.	Roots. grams.	Total. grams.
A.	28	174	23.5	416.2
A.	29	187.7	31	
B.	29	168	25.3	382.0
B.	31	171	17.7	

The result shows that ammonia in the form of calcium cyanamide or rather more correctly of ammonium carbonate behaves different from the form of the sulphate in as much as in combination with superphosphate it acts superior than in the combination with neutral sodium phosphate.[1] Ammonium sulphate together with superphosphate is in absence of calcium carbonate not by far as favorable. It follows also that for *Brassica* the neutral reaction is most favorable, the alkaline reaction of the ammonium carbonate was certainly neutralized by the superphosphate.

[1] The reverse was observed by Uchiyama to take place with ammonium sulphate; and Bahadur has observed this also in an experiment with onion.

On the Lime Factor for Flax and Spinach.

BY

S. Namikawa.

The lime factor, i.e. the best ratio of lime to magnesia differs with different plants, and even with different organs of plants.

In regard to the leaves this ratio is larger than for all the other organs. With the flax it is principally the stem, while with other plants the fruit, with others again the leaves (cabbage and tobacco) which are the most valuable parts of the harvest. Hence also the question of the lime factor depends to some extent on the special organs to be chiefly developed.

In regard to flax the question was which ratio of CaO : MgO is the most favorable for the production of the strongest fibre ?

For my experiments served the same soil from our College farm that served for other investigations mentioned in this Bulletin. This soil had been examined recently again by T. Katayama, whose determinations of the available amount of lime and magnesia yielded the following data :

	In the fine earth :	
Fine Earth of the soil = 76.33%	Lime.	Magnesia.
	0.60%	0.49%

Hence, the original soil contained

Lime .....0.458% Magnesia.....0.374%

The Wagner pots used held 10 kg. soil. This amount contained therefore

45.8 gr. CaO and 37.4 gr. MgO.

By addition of finely powdered calcium carbonate and magnesite were procured the following ratios :[1]

I $\frac{CaO}{MgO} = \frac{1}{1}$; 8.4 gr. MgO = 17.5 gr. $MgCO_3$

II $\frac{CaO}{MgO} = \frac{2}{1}$; 29.0 gr. CaO = 51.8 gr. $CaCO_3$

III $\frac{CaO}{MgO} = \frac{3}{1}$; 66.4 gr. CaO = 118.5 gr. $CaCO_3$

Each pot received further the following general manure :

5 gr. potassium sulphate
10 gr. double superphosphate
10 gr. ammonium sulphate
5 gr. sodium nitrate.

20 seeds per pot were sown on Nov. 1. The number of young plants when 10–12 cm. high was reduced to 10 per pot, all of nearly equal height. The plants showed gradually a considerable difference in development, which will be noticed also from the photograph taken April 12 and reproduced on Plate II. On May 20, the plants were cut and left to become air dry.

My observations were as follows :

$\frac{CaO}{MgO}$	Jan. 11.		March 27.		June 3.			
	Height cm. average.	Number of branches.	Height cm. average.	Number of branches.	Height cm. average.	Number of branches.	Weight (g) of fruits.	Weight (g) of total crop.
$\frac{1}{1}$	12	14	51	20	92	20	19	47
$\frac{2}{1}$	10	0	35	20	83	20	13	37
$\frac{3}{1}$	8	0	34	20	80	20	2	37

1 Former experiments with flax by I. Kawakita had shown here that an increase of magnesia over lime produced a depression of yield.

It will be seen that the ratio $\frac{CaO}{MgO}=\frac{1}{1}$ was the most favorable. The increase of lime beyond that ratio depressed the total production from 47 gr. to 37 gr. $=21\%$. Since phosphoric acid was applied here as double superphosphate, this depression cannot be due to a decrease of the availability of phosphoric acid in the soil, as is the case when bone dust serves as phosphatic manure.

After removal of the brittle parts the fibres were subjected to a rough comparison as to strength and it was thus easily recognized that the fibers grown with the ratio $\frac{CaO}{MgO}=\frac{3}{1}$ were much weaker than those grown with the ratio $\frac{1}{1}$. The general result agrees well with the statement of Blomeyer[1] that soils rich in lime are not favorable for flax.

In order to determine the best ratio of lime to magnesia for the growth of spinach, a sand culture served in which the lime and magnesia were applied in the form of very fine powder of the natural carbonates in such proportions that the ratios

	I	II	III	IV
$\frac{CaO}{MgO}=$	$\frac{1}{2}$	$\frac{1}{1}$	$\frac{2}{1}$	$\frac{3}{1}$

were obtained.

The total quantity of CaO in pots No. I and II was two grams for $2\frac{1}{2}$ kilo. purified sand. Hence the proportions of powdered marble and powdered magnesite became as follows :

I. $\frac{CaCO_3}{MgCO_3}=\frac{3.57\text{ g.}}{8.36\text{ g.}}$

II. $\frac{CaCO_3}{MgCO_3}=\frac{3.57\text{ g.}}{4.18\text{ g.}}$

III. $\frac{CaCO_3}{MgCO_3}=\frac{7.14\text{ g.}}{4.18\text{ g.}}$

IV. $\frac{CaCO_3}{MgCO_3}=\frac{10.71\text{ g.}}{4.18\text{ g.}}$

1 Die Cultur der landwirtschaftlichen Nutzpflanzen, II, p. 333.

Each pot received the following general manure :

0.6 g. K_2SO_4

1.0 g. Double superphosphate.

3.5 g. NH_4NO_3

2.0 g. ferric hydrate.

On April 24, ten seeds were sown in each pot and the young plants reduced to four of equal size after one month. The plants were cut June 24, and weighed in the fresh state.

The results were as follows :

Limefactor.	Height, cm.	Number of stalks.	Total weight (g).
$\frac{CaO}{MgO} = \frac{1}{2}$	11	16	8.9
	11	16	
	10	8	
	12	7	
$\frac{CaO}{MgO} = \frac{1}{1}$	11	17	13.2
	13	16	
	11	10	
	11	8	
$\frac{CaO}{MgO} = \frac{2}{1}$	10	14	7.9
	8	11	
	8	10	
	7	8	
$\frac{CaO}{MgO} = \frac{3}{1}$	10	10	5.1
	8	7	
	7	5	
	7	5	

It will be seen that the true limefactor for spinach = 1 ; this ratio produced the best results, like with flax.

Regeneration of Overlimed Soil.

BY

S. Maki and S. Tanaka.

It has been repeatedly pointed out in these Bulletins, that a certain ratio of lime to magnesia entering into the plant body is among other things necessary to insure the best growth, and the experiments of Aso, Daikuhara, Furuta, Suzuki, Namikawa and Katayama here have furnished ample confirmation of the former experiments of Loew and May.

Recently Nakamura[1] operated with a soil that contained seventeen times more lime than magnesia (1,76% CaO and 0,11% MgO). The absolute amount of magnesia would have been sufficient for a series of crops, but the ratio to lime was unfavorable. By manuring with magnesia the yield of barley in pot culture was increased by 69%.

Since it occurs sometimes that poor sandy soils are injured by a heavy dose of lime it was of value to observe the restitution of overlimed soil to its former state by manuring with magnesia compounds.

For the culture of cereals lime and magnesia should be present in equal quantities, provided that both these bases are present in such forms that their availability for the plant roots is equal.

To this end the best form of magnesia for manuring an overlimed soil would be magnesite, since it is in regard to solvents similar to the lime compounds present in most soils.

But this material is often difficult to procure and not easily pulverized. The cheapest soluble magnesium salt is the sulphate, but much less of the magnesia in this form is required than in the form of magnesite since this salt represents a much more available form of magnesia. It was therefore

1 Bulletin of the Imp. Central Experiment Station at Nishigahara, Tokyo, No. 1.

of importance to determine how much of this salt would be required for counteraction of the overliming. For the test served a loamy soil with 0.6% CaO and 0.5% MgO. It was mixed with twice as much lime as was present originally, in order to overlime it in relation to barley, and would require now an addition of 1.3% MgO in the form of magnesite to procure the ratio 1 : 1 which had been also nearly the ratio in the original soil. But in the form of magnesium sulphate much less was required. Hence the overlimed soil was mixed with various quantities of crystallised magnesium sulphate in order to find the most favorable dose. The lime was applied as quicklime which was slaked before mixing it with the soil, and the magnesium sulphate was added after this lime had passed completely into carbonate and no trace of an alkaline reaction was perceptible.

For the experiment served nine pots, each containing eight kilo soil.

Pot I. contained the original soil, the other pots the overlimed soil.

Pot II. received no dose of magnesium sulphate while the others rising doses of this salt, viz :

III., $\frac{1}{5}$ of the calculated amount of magnesia in the form of magnesium sulphate.

IV., $\frac{1}{10}$; V., $\frac{1}{20}$; VI., $\frac{1}{30}$; VII., $\frac{1}{40}$; VIII., $\frac{1}{50}$; IX., $\frac{1}{100}$ of that calculated amount of magnesia in the form of magnesium sulphate. These doses corresponded to :

III. = 120.78 grams or $MgSO_4 + 7aq.$
IV. = 60.39 „ „ „ „
V. = 30.18 „ „ „ „
VI. = 20.13 „ „ „ „
VII. = 15.09 „ „ „ „
VIII. = 12.08 „ „ „ „
IX. = 6.04 „ „ „ „

The magnesium sulphate was added in high dilution, well mixing it with the soil.

The general manure per pot was

5 gr.....Double superphosphate.
10 gr.....Potassium sulphate.
10 gr.....Sodium nitrate.

This was applied after the addition of magnesium sulphate.

Twenty seeds of sixsided barley were sown on Oct. 25 and after the young plants had reached 10—12 cm. in height, the number was reduced to 6 per pot, all of equal size.

Early in April the plants began to show ears. The plants were cut June 17 and weighed in the air dry state with the following results.

		Number of Ears.	Number of Stalks.	Mean Height of Stalk.	Total Harvest.	Weight of Ears.	Weight of Straw.	Weight of Grains.
				cm.	g.	g.	g.	g.
	I. (Original soil.)	34	33	63.5	76	30	46	25
Overlimed soil.	II. ( )	29	29	61	59	23	36	18
	III. ($\frac{1}{5}$ mgO.)	30	34	62.5	66	27	39	22
	IV. ($\frac{1}{10}$ „)	38	40	62.5	68	29	39	23
	V. ($\frac{1}{20}$ „)	36	40	62.5	70	30	40	24
	VI ($\frac{1}{30}$ „)	30	27	62.5	65	27	38	21
	VII. ($\frac{1}{40}$ „)	24	25	62	63	25	38	20
	VIII. ($\frac{1}{50}$ „)	25	27	62	60	25	35	19
	IX. ($\frac{1}{100}$ „)	27	29	62	59	23	36	18

In comparing I. with II. it will be seen that overliming has depressed the yield in barley and in comparing II. with the other pots, that the addition of certain quantities of magnesia in the form of sulphate had a counteracting effect. The best result was obtained when $\frac{1}{20}$ of the theoretically necessary amount of magnesia as magnesite was added in the form of the crystallised sulphate. The harvest in grains did here most approach that on the original soil.

In comparing therefore this amount of magnesium sulphate with the calculated amount of magnesite, we find that 14 parts of the former have accomplished as much as 100 parts of the latter; in other words: the agronomical equivalent of the crystallized magnesium sulphate on this soil is = 14.

While the former experiment was carried out on a rather poor loamy

soil it was a sandy soil of great natural fertility from Kawasaki which served for the next trial with two sided barley.

This soil was overlimed by adding so much lime as slaked lime that the total quantity of the lime in the soil became three times as high as the amount of MgO present.[1]

In order to produce now for barley the most favorable ratio, the MgO was applied in the form of crystallized magnesium sulphate in the following quantities :

	No. I.	Original soil.	
	No. II.	Overlimed soil ; addition of 124g. CaO.[2]	
	No. III.	$\frac{1}{5}$ of MgO as sulphate	= 134.6g. $MgSO_4+7H_2O$.
Overlimed soil.	No. IV.	$\frac{1}{10}$,, ,, ,,	= 67.3g. ,,
	No. V.	$\frac{1}{20}$,, ,, ,,	= 33.7g. ,,
	No. VI.	$\frac{1}{30}$,, ,, ,,	= 22.4g. ,,
	No. VII.	$\frac{1}{40}$,, ,, ,,	= 16.8g. ,,
	No. VIII.	$\frac{1}{50}$,, ,, ,,	= 13.4g. ,,
	No. IX.	$\frac{1}{100}$,, ,, ,,	= 6.7g. ,,

This magnesium sulphate was added to the soil after the lime had passed completely into carbonate.

The general manure per pot of 10 Kilo soil was

5 g.....Double superphosphate.
10 g.....K_2SO_4.
10 g.....$NaNO_3$.

On January 15, 20 seeds of barley were sown. After about three weeks, the number of the young plants was reduced to 6 per pot, all of equal size.

When the ears of barley developed, a great difference in the height was observed. Hence a photograph of some of the pots was taken, May 22, which is reproduced on plate II.

1 This soil contained 68.8% fine earth in which was contained 0.63% CaO and 0.80% MgO, soluble in 10% HCl. Ten Kilo soil contained therefore 43.4g CaO + 55.0g MgO in available form.

2 In order to restitute the best ratio for barley $\left(\frac{CaO}{MgO}=\frac{1}{1}\right)$ it would have been necessary to added 113g MgO in the form of finely powdered magnesite.

At that time the height was measured with the following results.

	Height.		Height.
I.	95 cm.	VI.	109 cm.
II.	95 ,,	VII.	107 ,,
III.	96 ,,	VIII.	98 ,,
IV.	116 ,,	IX.	98 ,,
V.	116 ,,		

The plants were cut June 22 and after becoming airdry they yielded the following weights :

		Total Harvest.	Ears.		Seeds.
			Number.	Weight.	
Overlimed soil.	I. Original soil.	154 g.	52	62.5 g.	50.5 g.
	II. no MgO.	136 ,,	52	62.0 ,,	50.0 ,,
	III. $\frac{1}{5}$ of MgO.	165 ,,	57	73.5 ,,	60.0 ,,
	IV. $\frac{1}{10}$,,	195 ,,	63	80.0 ,,	65.5 ,,
	V. $\frac{1}{20}$,,	195 ,,	63	88.5 ,,	73.5 ,,
	VI. $\frac{1}{30}$,,	189 ,,	62	85.0 ,,	68 0 ,,
	VII. $\frac{1}{40}$,,	187 ,,	62	83.5 ,,	67.0 ,,
	VIII. $\frac{1}{50}$,,	173 ,,	62	78.0 ,,	65.0 ,,
	IX. $\frac{1}{100}$,,	155 ,,	54	64.0 ,,	52.6 ,,

It will be seen that $\frac{1}{20}$ of MgO as sulphate compared with MgO in the form of magnesite brought the best harvest ; a decrease as well as an increase depressed the yield again. Hence the results on a sandy soil with two sided barley agree with the above result on a loamy soil with six sided barley.[1]

1 A peculiarity of this soil is however, that the increase of lime and magnesia together increased the yield above that on the original soil.

II. Overlimed Soil.
IV. Overlimed Soil + $\frac{1}{10}$ of the calculated amount of MgO as sulphate.
V. Same + $\frac{1}{20}$ of MgO as sulphate.
IX. Same + $\frac{1}{100}$ of MgO as sulphate.

To page 64.

$\frac{CaO}{MgO}$ =	3	2	1
	1	1	1

To page 58. On the Limefactor for Flax.

The Manurial Value of Different Potassium Compounds for Barley and Rice.

BY

K. Asō.

Many authors have compared the manurial effect of potassium chlorid, potassium sulphate and kainit. Several authors also compared the action of potassium silicate with other potassium compounds and Nobbe has already in 1870 compared potassium chlorid, sulphate and nitrate in their action on buckwheat in waterculture. The general result was that the same amount of potassa in different forms have not the same action with different crops. Thus, while a certain amount of potassium chlorid can depress the starch content of potato, it seems not to depress the starch content of the barley grain. Further, the chlorid[1] acts in other cases more favorable than the sulphate, and in combination with chlorids of sodium and magnesium in the form of kainit it has been found superior to the 40% potassium salts. The effect of potassium chlorid on the sugar-content of the sugar-beet is reported by several authors to be unfavorable. The nature of the soil and the absolute amount of potassium chlorid will however somewhat influence the result. Sebelien observed (1901) that potassium chlorid acts especially favorably on grain-production while potassium sulphate more on that of straw.

Thus far, I have not encountered in the literature an experiment in which at the same time the four potassium compounds, namely, the carbonate, the sulphate, the chlorid and the silicate were applied to the same plants and further no comparisons at all have been made for plants growing

[1] Wagner observed that potassium chlorid is not more easily absorbed by the plants than potassium sulphate as has been asserted.

in paddy soil. I have therefore compared these four salts in three consecutive years in their manurial effect on barley and paddy rice.

Experiment with Barley.

Four pots containing 8 kilo air-dry soil were manured with 20 g. ammonium sulphate and 50.4 g. sodium phosphate ($Na_2HPO_4 + 12$ aq.) while the other four pots of equal size received 20 g. ammonium sulphate and 18.1 g. double superphosphate, the quantity of P_2O_5 being equal in each case. The potassium compounds were used in equivalent quantities:

	Per pot.
Potassium carbonate	9.6 g.
Potassium sulphate	12.1 g.
Potassium silicate[1]	23.4 g.
Potassium chlorid	10.4 g.

On Nov. 27, 1903, 15 seeds of barley, soaked in water and heated at 45° C. for ten minutes,[2] were sown in each pot, and the young plants later reduced to eight of an equal size on Jan. 8, 1904. In the first stage of development notable differences were not present; but later, in April the plants in the pots containing potassium silicate showed the best growth. The flowering commenced at first in the pots containing potassium chlorid and on April 13 the number of flowering stalks was counted:

	Potassium Chlorid.	Potassium Silicate.	Potassium Carbonate.	Potassium Sulphate.
With Sodium phosphate, ...	29	7	7	4
With Double superphosphate	29	11	13	7

On June 3, the plants were cut; the yield, weighed in the air-dry state, is shown below in the table.

In the next experiment, 20 seeds were sown Oct. 26, 1994 and later on the young plants reduced to five per pot, all of an equal size. The growth in

[1] This preparation contained 28% K_2O.

[2] This was done to prevent the smut disease of barley.

the pots with potassium chlorid was at first a little behind the other plants, but nevertheless the ears appeared earlier. On June 8, 1905 the plants were cut and the harvest weighed in the air-dry state.

RESULTS.

1904.

		Total.	Straw.	Ears.	Grains.	Quotient of Yield.
		g.	g.	g.	g.	
With Sodium phosphate.	Potassium Sulphate	124	77.0	47.0	31.0	40.3
	Potassium Carbonate ...	119	74.0	45.0	30.5	41.2
	Potassium Chlorid.....	136	65.5	70.5	59.5	90.8
	Potassium Silicate.....	134	85.5	48.5	32.5	38.0
With Double Superphosphate.	Potassium Sulphate	133	80.0	53.0	38.5	48.1
	Potassium Carbonate ...	131	76.0	54.5	38.5	50.4
	Potassium Chlorid.....	138	67.0	71.0	59.5	88.8
	Potassium Silicate.....	127	73.0	54.0	41.0	56.2

1905.

		Total.	Straw.	Ears.	Grains.	Quotient of Yield.
		g.	g.	g.	g.	
With Sodium phosphate.	Potassium Sulphate	116.5	71.5	45.0	52.6	73.6
	Potassium Carbonate ...	129.5	70.0	59.5	50.5	72.1
	Potassium Chlorid.....	131.5	68.0	63.5	53.0	78.0
	Potassium Silicate.....	158.5	91.3	67.2	52.5	57.5
With Double Superphosphate.	Potassium Sulphate	129.7	67.9	61.8	35.0	51.5
	Potassium Carbonate ...	126.5	68.5	58.0	48.0	70.1
	Potassium Chlorid	111.0	48.0	63.0	52.2	108.7
	Potassium Silicate.....	121.0	61.5	59.5	52.3	85.0

Experiment with Paddy Rice.

Experiments with rice were conducted under essentially the same conditions as with barley, except as to watering. On July 13, 1903, shoots of paddy rice were transplanted, three plants in a bunch and three bunches in each pot. The plants exhibited towards the end of August 31 great difference in development; above all, it may be mentioned that the pots which received potassium carbonate together with calcium superphosphate produced much better plants than the pot that received potassium carbonate with the secondary sodium phosphate. Evidently, the alkaline reaction is in the latter case much stronger than in the former, which is unfavorable for the roots. On Sept. 9 the plants commenced to flower and on Nov. 6, they were cut and later weighed in the air-dry state.

Another experiment was carried out in the summer of 1905 with paddy as well as upland rice under the same manuring conditions. On July 11, the young plants of paddy rice were transplanted. Of the upland rice 20 seeds were sown in each pot, and the plants reduced later on to eight. On Nov. 15, the plants were cut.[1]

RESULTS.

1903.

		Total.	Straw.	Grains.	Full grains.	Empty grains.	Quotient of Yield.
		g.	g.	g.	g.	g.	
With Sodium phosphate.	Potassium Chlorid.....	178.0	97.5	80.5	76.0	4.5	82.6
	Potassium Carbonate ...	268.5	145.5	123.0	118.0	5.0	84.5
	Potassium Sulphate	279.0	151.0	128.0	121.5	6.5	84.7
	Potassium Silicate	299.5	160.5	139.0	134.0	5.0	86.6
Potassium Carbonate + Double Superphosphate		337.5	195.5	142.0	137.0	5.0	71.1

[1] The weather was too cool for the growth of rice plants through the whole summer in 1905, so that the ripening process was very much retarded, especially in the case of upland rice.

1905.

		Paddy Rice.						Upland Rice.			
		Total.	Straw.	Grains.	Full grains.	Empty grains.	Quotient of Yield.	Total.	Straw.	Grains.	Quotient of Yield.
		g.	g.	g.	g.	g.		g.	g.	g.	
With Sodium phosphate.	Potassium Chlorid ...	95.9	70.9	25.0	19.1	5.9	35.2	77.0	73.8	3.2	4.3
	Potassium Carbonate.	104.0	80.8	23.2	18.0	5.2	28.7	76.5	72.0	4.5	6.3
	Potassium Sulphate...	120.0	85.0	35.0	29.5	5.5	41.2	80.0	75.0	5.0	6.7
	Potassium Silicate ...	107.0	78.0	29.0	23.8	5.2	37.2	74.0	64.2	9.8	15.3
With Double Superphosphate.	Potassium Chlorid ...	98.2	71.2	27.0	21.0	6.0	37.9	87.5	83.6	3.9	4.7
	Potassium Carbonate.	114.8	85.8	29.0	23.0	6.0	33.8	85.0	76.5	8.5	11.1
	Potassium Sulphate...	118.0	87.6	30.4	24.5	5.9	34.7	83.0	78.8	4.2	5.3
	Potassium Silicate ...	110.4	77.9	32.5	25.0	7.5	41.7	105.0	90.0	15.0	16.7

From the results the following conclusions can be drawn:

1. While the chlorid accelerated the flowering process and enhanced grain production, the quotient of yield being highest in the case of barley, it retarded in the same quantity the yield with the rice plant.
2. The manurial value of the silicate was highest in several cases[1] and martellin may be called a favorable potassa manure for the Gramineæ.
3. While the chlorid acted very favorably for the production of grains,

[1] Ritthausen and Wolff proved that the physiological rôle of silica in the leaves is to cause the dying off of the leaves during the ripening. Thus their phosphoric acid and other mineral nutrients become available for the ripening seeds.

the sulphate more favored that of straw, in accord with Sebelien's former observations.

4. Carbonate was inferior to sulphate in all cases, when it was applied together with secondary sodium phosphate, a physiologically alkaline manure.

On the Effect of Various Potassic Manures on the Growth of Colocasia antiquorum.

BY

S. Namikawa.

Thus far woodash represented the chief potassa manure in Japan. But since the supply of this material is insufficient to meet the demand, various salts from Stassfurt have recently been imported. For the information of our farmers it was of some interest to compare the efficacy of woodash with Kainit and the 30 percent potassa-salt from Stassfurt on some Japanese crop, as, e.g., *Colocasia antiquorum*. The bulbs of this plant are rich in starch and serve under the name of sato-imo (sugar-potato) extensively as a food material.

The three potassa-manures mentioned were applied at the rate of 100 Kilo potassa per ha, on a rather poor loamy soil. Four plots, each of 28 square meters, were manured with ammonium sulphate at the rate of 50 Kilo N per ha, further with double superphosphate at the rate of 100 Kilo per ha. These plots were also supplied with slaked lime at the rate of 500 Kilo per ha several weeks before the general manure was applied. The liming was carried on for several reasons:

1. The combined application of ammonium sulphate with superphosphate—yielding an acid reaction—is unfavorable, when no neutralisation can take place in the soil.
2. The soil contained nearly equal amounts of lime and magnesia, while Colocasia, as a plant of extensive foliage requires at least 2–3 times as much lime than magnesia for its best development, to judge from analogy.

Each plot received accordingly:

Burnt lime	1400 g.
Double superphosphate	683 „
Ammonium sulphate	695 „

Further Plot A received 933 g. of the 30% potassium salt.

„ „ B „ 2333 g. woodash.

„ „ C „ 2333 g. Kainit.

Plot D served as check.

The woodash of the Japanese manure market contains from 12–13% potassa,[1] like the common Kainit.

On May 20, tubers of sato-imo were planted, 84 on each plot, containing six furrows.

During the vegetation no great differences in the height of the plants were observed, only those on the check-plot appeared not so luxuriant as those on the other three plots. No disease or dammage was noted.

The harvest on December 10 yielded the following amounts in bulbs (fresh weight):

A, 30% K-salt .....44,20 Kilo.

B, Woodash .....40,68 „

C, Kainit .....44,47 „

D, Check-plot, ...30,20 „

The result shows that on the limed loamy soil serving for the experiment Kainit and the 30% potassium salt acted equally well and were somewhat superior to woodash. There may however exist soils in Japan which are more benefitted by Kainit than by the 30% potassium salt (for equal amounts of potassa) since Kainit can act on certain soils beneficially also by its content of chlorids and magnesium salts, namely on soils devoid of sodium chlorid and on soils relatively poor in magnesia.[2] On certain soils in Europe Kainit was found superior to the 40 percent potassium salt of Stassfurt under the condition that lime was applied in conjunction with these potassa manures.

[1] The price in Japan of woodash is 2,68 *yen* per 100 Kilo, while for imported Kainit 3,00 *yen* per 100 Kilo.

[2] Barley is further benefitted by chlorids, but potatoes not.

On the Application of Chilisalpetre as Top-dressing for some Japanese Crops.

BY

K. Asō.

Although chilisalpetre is extensively used as top-dressing in America and Europe, and favorable results have been observed generally it seemed neverthless of some value to test its effect also on some Japanese crops, since the results differ somewhat with certain plants. Vorhees found it best in many cases to apply chilisalpetre at the rate of 300 pounds per acre in three doses as top-dressing (400 K. per ha.). Wolff states "Bei Kartoffel u. Turnip-rübe ergab sich, dass selbst neben einer reichlichen Düngung von Stallmist, die Zufuhr von Chilisalpeter die Ernte noch bedeutend zu steigern vermochte." The favorable action of chilisalpetre consists not only in the supply of a suitable form of nitrogen, but also to some extent on the favorable action of soda. In some cases chilisalpetre acted more favorably than calcium nitrate, which may be due perhaps to the fact that the soil was already somewhat rich in lime. Some authors explained this by the greater capacity of diffusion of Chilisalpetre. Some cases however were recently published, in which calcium nitrate acted more favorably than the sodium nitrate.[1]

My experiments were made with upland rice, Sesamum and Colocasia antiquorum (sato imo). Two plots, each of 20 sq. metre were used for each crop. Each plot received 20 K. barnyard manure and 2 K. superphosphate on May 19. Besides, one plot was manured with chilisalpetre as top-dressing while the other served as control. 84 bulbs of Colocasia were planted in each plot on May 19, further 180 c.c. of seed of upland rice, while 50 c.c.

[1] Ampola: Chem. Centrbl, 1905. No. 2. Calcium nitrate was suppo-ed by him to resist denitrification better than sodium nitrate.

of Sesamum seeds were sown on June 15. The young Sesamum plants were afterwards reduced to eighty-three in each plot. The application of 400 g. chilisalpetre[1] took place twice as top-dressing for upland rice and Colocasia on June 15 and July 17, and only once for Sesamum on July 17. The following tables show the results obtained :

UPLAND RICE.

Harvested on Oct. 25 and weighed in air-dry state.

	Control.	With Chilisalpetre.
Total yield.....	15.641 K.	16.301 K.
Grains.....	5.925 K.	6.005 K.
Straw	9.716 K.	10.296 K.

COLOCASIA BULBS.

Harvested on Dec. 14 and weighed in the fresh state.

Control.	With Chilisalpetre.
50.10 K.	55.49 K.

SESAMUM.

Harvested on Sept. 25 and weighed in airdry state.

	Control.	With Chilisalpetre.
Total yield.....	1.340 K.	1.320 K.
Seeds	174 g.	174.5 g.
Stalks	1.166 K.	1.145 K.

A decided increase of harvest was therefore obtained with *Colocasia antiquorum*.

[1] 400 g. Chilisalpetre were dissolved in about 15 L. water.

On the Stimulating Action of Manganese upon Rice. III.

BY

M. Nagaoka.[1]

In former Bulletins (vol. V, No. 4 and VI, No. 2) an experiment with rice was described, which showed, that the application of cryst. Manganese sulphate at the rate of 77 Kilo per ha (=25 Kilo Mn_2O_3) increased the yield of rice 37 percent (in 1902) and that one year afterwards there was on that plot still an after-action observable amounting to 8%, while 16% increase on the plot that had received the year before manganese sulphate at the rate of 92 Kilo per ha (30 Kilo Mn_2O_3). These experiments will be continued in more or less modified form on the same paddy soil. In the following lines the main results in the years 1904 and 1905 are communicated.

In the year 1904 the experiment was repeated on the same plots with the same doses of manganese sulphate as in the year 1902. The manure, number of plants and other circumstances were the same, not so however the conditions of the weather which were so exceptionally favorable in 1904 that the average yield of rice was surpassed in various provinces by 20 percent. Since the maximum development was thus reached, a considerable further stimulation by manganese could not be expected. The weight of the harvest (air-dry) is shown in the following table:

[1] In absence of Prof. Nagaoka, this work was continued by Prof. Toyonaga and Prof. Aso.

No. of Frames.	Mn_2O_3 per ha kg.	Full grains gr.	Empty grains gr.	Straw. gr.	Average. Full grains.	Average. Empty grains.	Average. Straw.	Total.	Comparative increase.
2	No Mn_2O_3	421.0	5.5	521.3					
14		420.0	4.0	519.3	453.3	5.5	553.6	1012.4	100
26		519.0	7.0	620.2					
3		437.0	4.5	586.3					
15	10	507.0	5.5	613.4	485.3	5.5	603.3	1094.3	108
27		476.0	5.5	610.2					
4		529.0	8.5	659.3					
16	15	443.5	5.0	572.8	482.2	6.3	605.5	1094.0	108
28		474.0	5.5	584.3					
5		442.0	5.0	590.0					
17	20	489.0	8.0	587.3	473.0	6.3	587.2	1066.5	105
29		487.0	6.0	584.3					
6		516.0	6.0	684.8					
18	25	505.0	9.5	589.3	532.3	8.0	626.3	1166.6	115
30		477.0	8.5	604.8					
7		552.0	6.0	635.3					
19	30	506.0	7.0	605.3	510.3	6.5	606.5	1123.3	111
31		473.0	6.5	578.5					
8		478.0	9.0	551.3					
20	35	535.0	6.0	637.3	495.5	6.7	561.3	1163.5	115
32		483.0	5.0	595.3					
		491.0	5.0	558.3					
21	40	516.0	7.0	620.8	500.3	6.7	586.5	1093.5	108
33		494.0	8.0	580.3					
10		422.0	5.0	501.8					
22	45	546.0	8.0	568.3	481	6.1	536.4	1023.4	101
34		575.0	5.5	539.3					

No. of Frames.	Mn_2O_3 per ha kg.	Full grains gr.	Empty grains gr.	Straw gr.	Average. Full grains.	Empty grains.	Straw.	Total.	Comparative increase.
11		399.0	5.0	525.8					
23	50	569.0	11.5	705.3	451.0	7.0	553.1	1011.1	100
35		385.0	4.5	428.3					
12		430.0	5.5	561.3					
24	55	424.0	4.5	517.8	446.8	6.0	563.5	1016.3	100
36		486.5	8.0	611.3					

The stimulating action led in this case to an increase of only 15 percent by the same dose of manganese that led two years before to one of 37%. This increased growth had of course drawn upon the store of mineral nutrients of soil and manure, hence the control plots were now in a much better soil condition, and in the interest of a fair experiment it would now have been necessary to procure equal soil-conditions again, before a fresh trial with manganese would be started. But it was the intention to compare in the following year the effect of manganese on the partially exhausted plots with the yield on the control plot.

In the year 1905 the experiment was carried out in the same frames and under the same general manuring conditions as before.[1] But the doses of manganese were no longer varied from 10–55 Kilo Mn_2O_3 per ha; only one dose, namely that in the ratio of 25 Kilo Mn_2O_3 per ha, which had proved to be the most favorable in the first year was applied, but in three different forms, namely as $MnSO_4+4$ aq.; $MnCl_2+4$ aq., and $MnCO_3$ respectively, corresponding per frame to 6 g., 5 g. and 3,06 g. The yield (air-dry) is shown in the following table:

[1] The manure was applied at the following ratio per ha: 100 Kilo N as ammonium sulphate 100 Kilo K_2O as K_2CO_3 and 100 Kilo P_2O_5 as superphosphate.

No. of Frames.	No Manganese. Total.	Grains.	Straw.	No. of Frames.	Manganous Sulphate. Total.	Grains.	Straw.
	g.	g.	g.		g.	g.	g.
2	829	289	540	3	806	258	548
14	877	352	525	4	656	215	441
26	855	291	564	5	647	243	404
40	801	291	510	6	760	270	490
41	798	288	510	7	582	210	372
42	886	315	571	8	715	249	466
43	793	282	511	9	537	211	326
44	888	325	563	10	590	200	390
45	853	312	541	11	612	182	430
46	813	293	520	12	816	298	518
Average.	**839.3**	**303.8**	**535.5**	Average.	**672.1**	**238.6**	**438.5**

No. of Frames.	Manganous Chlorid. Total.	Grains.	Straw.	No. of Frames.	Manganous Carbonate. Total.	Grains.	Straw.
	g.	g.	g.		g.	g.	g.
15	834	325	509	27	753	275	478
16	580	193	387	28	836	308	528
17	831	330	501	29	780	257	523
18	843	290	553	30	821	280	541
19	893	339	554	31	855	303	552
20	851	302	549	32	994	355	639
21	803	301	502	33	862	302	560
22	748	270	478	34	945	313	632
23	670	228	442	35	708	308	400
24	773	278	495	36	885	290	595
Average.	**782.6**	**285.6**	**497.0**	Average.	**843.9**	**299.1**	**544.8**

The summer 1905 was very unfavorable for rice, on account of the prolonged rains. There was however no damage by fungi observed on our

plots. Some small damage by animals (rats) was reported by the assistant but unfortunately the number of the frame was not noted. It will be noticed that manganese sulphate and chlorid had this time depressed the yield, the greatest depression taking place on such plots as had in 1902 yielded a considerable plus-yield. Taking now inconsideration that the frames with manganese carbonate did not share in the depression, although a number of these frames had in 1902 received also large doses of manganese sulphate and had produced a considerable plus-yield, it will be save to conclude that the depression on the other manganese plots partly was due to increased acidity in the soil, which on account of its high humus content was somewhat acid from the outset but could not be neutralised by the dose of potassium carbonate applied in the manure.[1] Superphosphate, ammonium sulphate and manganese sulphate united in increasing the acidity. The further fact, that manganese carbonate did not lead to an *increase* by stimulation must be ascribed to the partial exhaustion of the soil by the former three plus-yields. In continuing the experiments on the same plots, small doses of lime will be applied as a remedy of the increased acidity and care will be taken to restore all plots to the same manuring conditions.

[1] Indeed the aqueous extract of these soils showed a strong acid reaction on litmus paper, more so than the control plot. The aqueous extracts also gave a stronger reaction for chlorine and sulphuric acid respectively than the carbonate and control plots did.

Stimulating Influence of Sodium Fluorid on Garden Plants.

BY

K. Asō.

Three pots each holding 8 Kilo air-dry soil were manured each with :

KCl	5 g.
K_2CO_3	7 „
$NaNO_3$	6 „
$(NH_4)_2SO_4$	6 „
Common superphosphate	18 „

One pot received 0.02 g. sodium fluorid and the other 0.2 g. of it ; the third served for control. On March 14, seeds of Helichrysum bracteatum and Pedicellaria viscide were sown, and later on the young plants of both species reduced to three of equal size.

Gradually a difference in height became noticeable. On July 2, a photograph was taken (see plate III.) and the following height observed :

PEDICELLARIA.

Control.	0.02 g. NaF.	0.2 g. NaF.
cm.	cm.	cm.
78	75	80
65	87	69
75	91	80
Average 73	84	76

A stimulating effect of sodium fluorid had therefore taken place. The flowers appeared first in the pot with 0.02 g. sodium fluorid. In regard to the size of flowers, however, there was hardly any difference observed.[1]

As to Helichrysum, the influence of sodium fluorid was not so marked. Also here the size of the flowers was not affected.

[1] In a former paper was mentioned by the writer that the size of the flowers of the plum-tree was considerably reduced under the influence of a certain amount of sodium fluorid. Small twigs with flower buds of plum-trees had been placed in a solution of 0.001% sodium fluorid. The flowers, that opened after twenty one days, were all very much smaller than those of the control case. When the buds, however, are too far developed before they are placed into the fluorid solution, this peculiar effect can not be noticed.

On a Stimulating Action of Calcium Fluorid on Phaenogams.

BY

K. Asō.

I have shown in former communications that sodium fluorid acts on the one hand as a strong poison[1] on seeds and seedlings and on the other hand that it acts as a stimulant of development when highly diluted. The fact, however, that in soil cultures the sodium fluorid can easily pass into calcium fluorid which is exceedingly little soluble in water viz. 1 : 26000[2] renders probable the supposition that the stimulating compound in soil cultures is not sodium fluorid, but calcium fluorid. This salt can of course hardly exert any poisonous action, not only on account of being very difficultly soluble, but also because it can not precipitate the absorbed lime necessary in the cells. Indeed young onion plants remained alive for several weeks when they were placed in a concentrated suspension of well washed precipitated calcium fluorid.

In order to observe whether calcium fluorid can exert any stimulating action, the following experiments were made.

Experiment with Pea in Waterculture.

On October 1, pea shoots (about 5 cm. long) were placed in the following suspensions of precipitated CaF_2 :

[1] Of the enzyms, zymase and oxidases are much more easily injured than the other enzyms.

[2] The freshly precipitated gelatinous calcium fluorid may show a somewhat higher degree of solubility, especially in presence of certain other salts.

a. Calcium fluorid 0.1%
b. „ 0.01%
c. „ 0.001%
d. „ 0.0001%
e. Check.

These plants had to rely for the time being on the reserve stores of the cotyledons.

On October 18, the following observations were made :

	Length of each plant.	Fresh weight of each plant.
	cm.	grm.
a.	38.5	1.52
b.	34.5	1.40
c.	35.0	1.45
d.	37.7	1.60
e.	34.5	1.25

This result made some stimulating action of calcium fluorid on the growth of pea-plants probable.

Experiment with Pea in Soilculture.

Porcelain pots, each holding 1.5 kg. soil served here for the experiments. Each pot received the following general manure :

1 g. .....Double superphosphate.
1 g. .....Potassium sulphate.
2 g. .....Sodium nitrate.

Besides these compounds calcium fluorid was added in the following quantities :

a. .....0.006 grm.
b. .....0.010 „
c. .....0.050 „
d. .....0.200 „
e. .....Check.

On October 19, 1904, five pea-seeds were sown in each pot and afterwards reduced to three of nearly equal size. On February 13, a photograph was taken (see plate III.) and the following observation made :

	Average-height. cm.
a.	135
b.	121
c.	101
d.	105
e.	104

Although the increase in height in *a* and *b* was striking, there was otherwise not any marked difference as to the development generally. On May 5, the ripe plants were harvested with the following result :

	Number of pods.	Fresh weight of fruits.	Fresh weight of straw.
		grm.	grm.
a.	19	10.0	6.5
b.	17	8.5	6.0
c.	21	11.0	6.5
d.	23	12.9	7.1
e.	18	8.9	6.2

Some stimulating action in regard to fruit formation seems therefore probable to have taken place in pots *c* and *d*.

Experiment with Barley in Waterculture.

On October 18, a pair of barley shoots (about 10–11 cm. long.) were placed in the following solution :

Calcium nitrate	0.2 %
Potassium nitrate	0.15%
Monopotassium phosphate	0.05%
Magnesium sulphate	0.05%
Ammonium sulphate	0.05%
Ferrous sulphate	Trace.

Calcium fluorid freshly precipitated and well washed was added in the following proportions :

a. 0.1%
b. 0.05%
c. 0.01%
d. 0.001%
e. .....Check.

These solutions were renewed from time to time. The following observations were made April 20 :

	Average length.	Total fresh weight.
	cm.	grm.
a.	108	170.0
b.	107	202.0
c.	102	221.5
d.	100	216.5
e.	94	154.5

These results show that calcium fluorid can indeed exert a moderate stimulating action.

The continuous application of the small dose of 100 grms. sodium fluorid per hectar would very probably even after many years not exert any injurious action whatever, as it passes into the calcium fluorid in the soil.[1]

It may in this connection be of some interest that Wein, in comparing Wiborgs phosphate with common superphosphate, observed a much more favorable action of the former. This beneficial influence may to some extent be due to the presence of 1% fluorine in Wiborg phosphate.

[1] Whether potassium iodid, by application in the same ratio, would ever accumulate to an injurious amount is also doubtful, since rains would leach out the small doses left after harvesting. Very small doses only might be retained in the soil by its absorptive powers.

A favorable action of calcium fluorid on the yield was observed recently also by Ampola.[1] But his explanation can hardly be accepted. He assumes that calcium fluorid would be decomposed by carbonic acid, or weak organic acids with liberation of hydrofluoric acid, which then would act on complex silicates of the soils and render the potash assimilable. Calcium fluorid is however not altered at all by carbonic acid and other weak acids. We must therefore assume that calcium fluorid being soluble a little in water can act as a stimulant of plant growth under favorable conditions, and that this salt is formed in the soil when sodium fluorid is applied.[2]

[1] Gazzetta chim. ital. 1904, 34, ii, 156–165. He applied CaF_2 at the ratio of 100 kilo. per ha.

[2] There is not always an effect perceptible when sodium fluorid is applied in form of topdressing after the plants had reached a certain height, as an experiment with upland rice treated at the rate of 200 g. NaF per ha has shown.

Control. Pedicellaria. 0,20g. NaF
0,02g. NaF.

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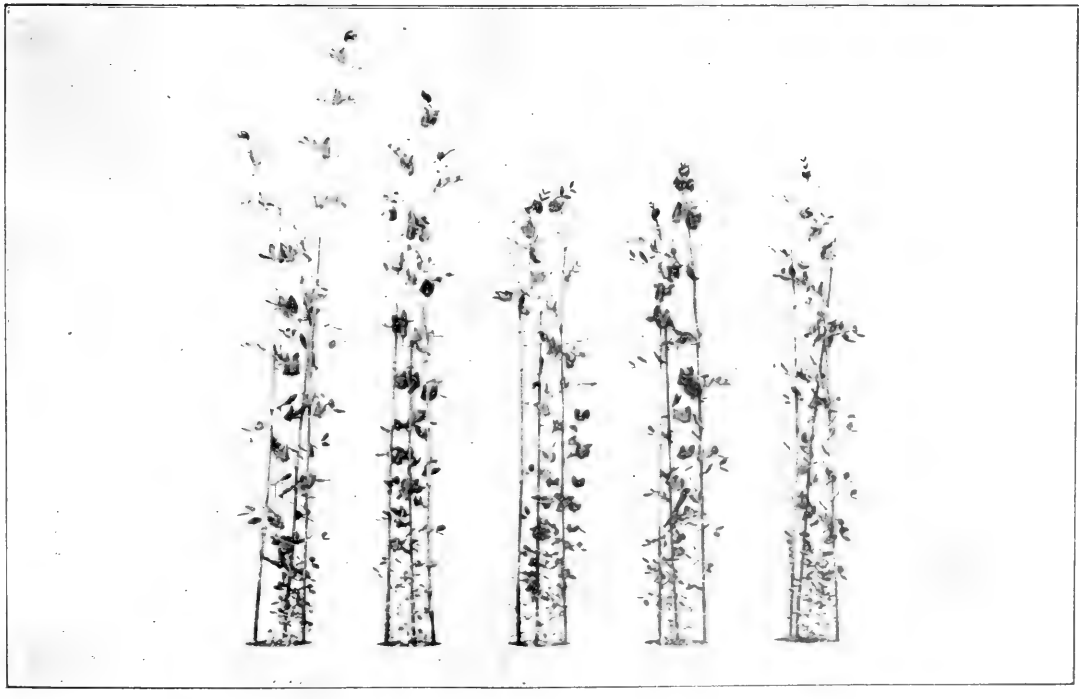

CaF_2 : 0,006g. 0,01 0,05

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On the Degree of Stimulating Action of Manganese and Iron Salts on Barley.

BY

T. Katayama.

Former observations in this college with oats, upland rice,[1] barley and wheat have shown that the stimulating effect of manganese salts on these Gramineæ is not so powerful as on the leguminous plants. Thus, an application of 0.015% manganous sulphate upon the soil has led with the pea to 50% increase of straw and 25% increase in seeds, while neither that amount of the sulphate nor a further increase to 0.04% produced more than about 10% total increase with cereals in pot culture.

It seemed therefore of some value to determine that dose of a manganese salt which would produce also with the common cereals such a favorable result as was obtained with the pea. The doses were therefore further increased, namely to

I. 0.01%
II. 0.05% } ..... $MnSO_4+4aq.$
III. 0.10%

For comparison, the action of ferrous sulphate in the same doses[2] was observed, and also a simultaneous application of a mixture of both those sulphates.

[1] With paddy rice in field culture the result was much more favorable; several reasons might be given for this difference observed on dry and swamp land.

[2] Several authors have recommended 200 kilo. green vitriol per hectar as a favorable dose, others 65–350 kilo. per hectar, but on unmanured soil Larbalétrier and Malpeau (Ann. Agr. 1896, p. 20) have not observed any effect of a dose of 150 kilo. green vitriol per hectar.

The salts were applied in the form of top dressing in fractional doses in high dilution (Dec. 7th and Jan. 25th).

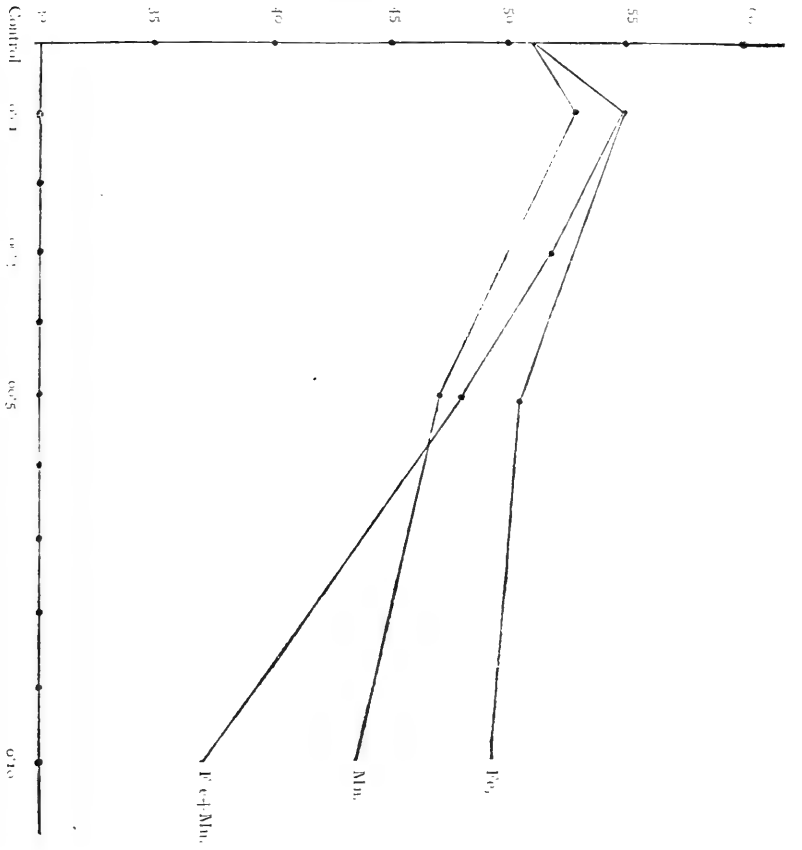
The pots contained 8 kilogramm soil which had not been manured for 5 years and was partly exhausted by yearly crops. The general manure for each pot was: double superphosphate 8 gr., kainit 5 gr., ammonium sulphate 10 gr., sodium nitrate 5 gr.

25 seeds of barley were sown Nov. 13 and when the young plants had reached 12–18 cm., they were thinned, taking care that the remaining plants, 8 per pot, were all of nearly equal size (17–18 cm. high) on Jan. 24th.

The plants were cut May 26 and weighed in the air-dry state, with the following results:

	Straw.	Grains.
Control.	grm. 90.5	grm. 51.3
0.01% $MnSO_4$, 4aq. + 0.01% $FeSO_4$, 7aq.	96.2	55.0
0.03% $MnSO_4$, 4aq. + 0.03% $FeSO_4$, 7aq.	82.2	52.5
0.05% $MnSO_4$, 4aq. + 0.05% $FeSO_4$, 7aq.	85.5	48.1
0.1% $MnSO_4$, 4aq. + 0.1% $FeSO_4$, 7aq.	79.2	37.5
Control.	93.9	50.7
0.01% $FeSO_4$	96.5	55.0
0.05% $FeSO_4$	100.0	50.7
0.1% $FeSO_4$	85.7	49.5
0.01% $MnSO_4$	89.0	53.1
0.05% $MnSO_4$	107.0	47.7
0.1% $MnSO_4$	95.0	44.0

Weight of Grains.



These results show that 0.01% of manganese and iron sulphates produced a moderate increase in harvest, 6.21% in straw, 7.21% in seed, and further that the increase of sulphates of manganese and iron beyond 0.01% of the soil led to a general decrease of the yield.

On the Formation of Humus.

BY

S. Suzuki.

Many authors have examined the influence of calcium carbonate on the formation and decomposition of humus. But the results do not fully agree. Hilgard believes that lime promotes the decomposition of humus.[1] Kossowitch and Tretjakoff, however, believe that the experiments of Petersen and Wollny are not very convincing and infer that the calcium carbonate in the majority of cases exerts a retarding influence on the decomposition of organic matters.[2]

These Russian authors filled a glass cylinder of 0.5 litre capacity and of 34 cm. height with oak leaves or hay with and without addition of 0.5% and 10% $CaCO_3$. Each glass cylinder received 80 g. leaves moistened previously with 160 cc. water and was infected with 1 g. of a humous soil. From time to time a current of purified air was sucked through the apparatus, which was connected with a flask containing potassa, thus determining the CO_2 developed during the humification process. The experiments lasted from 97 to 112 days. Almost in every case it was found that calcium carbonate retarded the humification process. This result can be easily explained by assuming that the fungi necessary for the humification were not provided with soluble phosphates, since the excess of lime present must have rendered insoluble all phosphoric acid originally present in the organic matter, hence development of fungi was retarded.

[1] Forschungen der Agricultur-Physik 1892, p. 400.

[2] The decrease of organic matter by the humification process was found by Ramann and Kostytcheff to amount to 55% in the first year, while by Henry in one year only 15%. Various conditions can of course have influence on the progress of the changes.

In the soil, the fungi producing the humus[1] find all their necessary mineral nutrients in a more or less available state. Since lime is not absolutely required by fungi, while magnesia is absolutely necessary and secondary magnesium phosphate is also more easily soluble than the calcium phosphate, it seems to me of interest to repeat such experiments under more favorable condition for fungi.

Therefore potassium phosphate and magnesium sulphate were added in small quantities and the effect of calcium carbonate was now compared with that of magnesium carbonate upon the process of humification. My experiments were made in the following way: Some Erlenmeyer flasks (a. 1200 cc.) were filled with dry coarsely powdered (<6 mm.) leaves of *Quercus serrata*, Thunb. which commonly serve in Japan as litter. Each flaskcontained 100 g. of these leaves, previously moistened with 200 cc. of water and mixed with 1 grm. of humous soil taken from the fields of our college. The first flask served as check. To the second flask was added 5 g. precipitated basic magnesium carbonate previously mixed with some water to a fine milk and further 0.5 g. K_2HPO_4. To the third flask 5 g. $CaCO_3$, 0.5 g. K_2HPO_4 and 0.5 g. $MgSO_4$ were added, while to the fourth only 5 g. precipitated $CaCO_3$ to compare the observations of Kossowitch with the behavior of the leaves under more favorable conditions. The second, third and fourth flask were infected with 1 g. soil as in the control case. From time to time about 10 litres of purified moistened air was sucked by an aspirator through the vessels and then through a Liebig's bulb filled with caustic potassa, and the carbonic acid in this air thus determined. More importance than the determination of carbonic acid was the transformation of organic matter into black humus. Therefore from time to time a small portion of the leaves was examined under the microscope. Into another flask (V) prepared like the flask II. was introduced the mycelium of a peculiar kind of Penicillium (Schokoladenfarbener Schimmelpilz[2]) after sterilisation of the flask. This fungus has the peculiarity of producing a black substance when cultivated in koji-extract.

[1] On the destruction of organic matter by soil bacteria compare also: O. Bail, Centralbl. f. Bacteriol. II. Abt. Vol. IX. The formation and destruction of humus was here not a special object.

[2] Cf. Lindner, Mikroscopische Betriebscontrolle, p. 243.

The general plan of the experiment is shown in the following table :

No. of flasks.	Amount of powdered air-dry leaves.	Water added.	Humous soil.	$MgSO_4$ (anhyd.)	Precipitated $MgCO_3$	Precipitated $CaCO_3$	K_2HPO_4	Fungus.
	g.	cc.	g.	g.	g.	g.	g.	
I.	100	200	1	—	—	—	—	—
II.	„	„	„	—	5	—	0.5	—
III.	„	„	„	0.5	—	5	0.5	—
IV.	„	„	„	—	—	5	—	—
V.	„	„	„	—	5	—	0.5	fungus.

The experiment was started on Febr. 7, 1905. Until the end of Nov. 36 determinations of carbonic acid were made with the following result :

AMOUNT OF CO_2, g.

Date.	I. Soil alone.	II. Soil + $MgCO_3$ + K_2HPO_4.	III. Soil + $MgSO_4$ + $CaCO_3$ + K_2HPO_4.	IV. Soil + $CaCO_3$.	V. Soil + MgO_3 + K_2HPO_4 + Fungus.
Febr. 14.	0.4394	0.4116	0.4832	0.3428	0.0868
„ 22.	0.3594	0.4216	0.4498	0.4880	0.1544
Mar. 3.	0.4628	0.6316	0.5746	0.4156	0.0254
„ 9.	0.4153	0.4708	0.4810	0.5286	0.1314
„ 16.	0.4500	0.6282	0.5032	0.4462	0.0922
„ 24.	0.4216	0.5247	0.5003	0.5412	0.1424
„ 31.	0.4148	0.5398	0.4612	0.4669	0.0973
April 7.	0.4366	0.4812	0.4434	0.2974	0.1320
„ 14.	0.4292	0.5272	0.5304	0.5800	0.1478
„ 21.	0.4472	0.6151	0.4603	0.4958	0.1138
„ 28.	0.4876	0.4840	0.4650	0.4569	0.0701
May 5.	0.4759	0.4944	0.4963	0.5073	0.1895
„ 12.	0.4701	0.5979	0.5018	0.4506	0.1054
„ 19.	0.4900	0.4989	0.5192	0.5336	0.1354
„ 26.	0.4752	0.5316	0.4524	0.4600	0.1049

Date.	I. Soil alone.	II. Soil + $MgCO_3$ + K_2HPO_4.	III. Soil + $MgSO_4$ + $CaCO_3$ + K_2HPO_4.	IV. Soil + $CaCO_3$.	V. Soil + $MgCO_3$ + K_2HPO_4 + Fungus.
June 2.	0.4586	0.5880	0.4922	0.5204	0.1685
„ 9.	0.4911	0.5065	0.4566	0.5169	0.4018
„ 12.	0.4645	0.5378	0.4809	0.5032	0.3501
„ 23.	0.4506	0.5093	0.4472	0.4302	0.0097
„ 30.	0.4541	0.4924	0.4775	0.4912	0.2966
July 7.	0.4552	0.5221	0.4671	0.4900	0.2685
„ 14.	0.4578	0.5280	0.4714	0.4611	0.0769
„ 21.	0.4678	0.5032	0.4590	0.1715	0.0723
„ 28.	0.4320	0.4522	0.4376	0.4252	0.4253
Aug. 4.	0.4320	0.4678	0.4314	0.3604	0.0400
„ 26.	0.5198	0.6074	0.3190	0.0156	0.0074
Sept. 2.	0.3598	0.4130	0.4218	0.1132	0.0237
„ 8.	0.4089	0.4798	0.4392	0.2203	0.0625
„ 15	0.4110	0.4335	0.4160	0.1138	0.0502
„ 22.	0.4879	0.2895	0.3195	0.0799	0.0399
„ 29.	0.4080	0.4549	0.4062	0.3095	0.0366
Oct. 6.	0.3983	0.3601	0.1288	0.0432	0.0271
„ 12.	0.3648	0.3998	0.4177	0.2220	0.0217
„ 20.	0.3082	0.3102	0.3966	0.3900	0.2736
Nov. 8.	0.5794	0.6735	0.6690	0.4468	0.3922
„ 22.	0.4064	0.3894	0.4302	0.4206	0.3064
Sum.	15.8912	17.7770	16.3070	13.7559	4.8998

If now the results in II., III. and IV. are compared with those in the control case it becomes evident that magnesium carbonate (II.) promoted the development of carbonic acid,[1] while calcium carbonate (IV.) retarded it. Hence also the humification process is *promoted* by *magnesium* carbonate and *retarded* by *calcium* carbonate.

In comparing III. with IV. we see that the addition of 0.5 g. potassium phosphate to the flask III. had a very esssential influence on the increase of the carbonic acid. The case IV. would confirm the experiment of Kossowitch, but in nature the case is different, since in the humification process of leaves in the soil these are in contact with soluble phosphates. Hence we can further conclude that the opinion of Hilgard corresponds more to the *natural condition* of the humification process. I have also examined the physical properties of the leaves and their microscopical aspect and became convinced that the change in color and brittleness and also the development of mycelium go parallel with the development of carbonic acid. It remains further to be mentioned that after sterilisation and introduction of the peculiar kind of Penicillium known thus far only under the name of "Schokoladenfarbener Schimmelpilz" the humification process proceeded much slower than under the original conditions. The amount of carbonic acid in flask V. was only about one third of that of control flask I. This experiment on the formation of humus will be continued so long until all the particles of leaves are transformed into real black humus. It is noticeable that eleven months have sufficed to transform the particles of leaves very considerably, the color has become very dark and the cohesion of the particles has been almost destroyed.

[1] Supposing that some acid decomposition products would act upon the carbonate added to the flask II., III and IV. and liberate carbonic acid from that source, I subtracted the carbonic acid contained in the carbonate added from the amount of carbonic acid developed during this experiment with the following result :

No. of flasks.	II. ($MgCO_3$, 5 g.)	III. ($CaCO_3$, 5 g.)	IV. ($CaCO_3$, 5 g.)
CO_2—developed.	17.7770	16.3070	13.7559.
CO_2—content of carbonates.	2.6079	2.1978	2.1978.
Difference.	15.1691	14.1092	11.5581.

Here also the amount was greatest in the case of the flask II.

A New Variety of Mycoderma Yeast as a Cause of a Sake Disease.

BY

T. Takahashi.

Saké, the Japanese rice wine or rice beer is not unfrequently altered by bacteria causing either acidity or a change of flavour and a turbidity. Several authors have reported on such sake diseases.[1]

Recently, however, a "turned" Saké was sent to me for investigation, which proved to be infested with a Mycoderma yeast.[2] The colonies of this yeast on koji-extract gelatine were white and showed concentric rings with radiations. A preliminary test, further, showed thal pasteurized saké, containing 17 vol. % of alcohol was attacked by this yeast on infection, whereby after 10 days cultivation at 20-28° C the alcoholic content was decreased to 9.37%.

This observation led me to study this yeast purified by Lindner's droplet culture. The following characteristics were then observed :

1. Form and usual size : Elliptic, filamental or sausage shape, rarely globular. Two or three fat globules are often seen in the large cells.

2. Growth.

a. On koji-extract agar : pasty white colonies ; on plate culture : yellowish white cup shaped center filled with granular masses. On streak culture : folded and semitransparent on the margin ; in

[1] Atkinson, Chemistry of Saké Brewing, 63 ; K. Ōtani Journal of Tokyo Chemical Society XXII. Vol. 8 ; G. Torii. Jōzōshikenjo hō kō ku. No. 2.

[2] It came from the Niigata prefecture and contained 8.1 Vol. % of alcohol, 0.36% acids, 1.5% of extractive matters.

stab culture : develops chiefly at the mouth forming a white granular pin head, altering to greyey after long culture.

b. On koji-extract gelatine : grayey-white and pasty coating, granular in the central part. Stab-culture : grayey white mesenteric growth chiefly on the mouth of the canal, gelatine is easily liquefied.[1] Gigantic colony (at 10–14° C) in plate culture shows a fine mesenteric structure and numerous concentric rings (see Plate IV.), which is one of the characteristics.

c. On wort-agar : the surface culture (at room temperature) gave a white somewhat pasty and mesenteric coating ornamented by radiated lines on the margin. A greyish white and moist growth, mesenteric in the central part smooth on the margin, was observed witt stab-culture (at 22° C). Gigantic colony (at 19–20° C) on plate culture was pasty, and showing coarse folds more or less feather-like in the central part.

d. Bouillon-agar culture : surface culture a pasty white growth of coarse folds on margin and fine folds in central part. Stab-culture yellowish white veins, growth restricted chiefly to the surface. Gigantic colony (at 19.5–25° C) was pasty and of irregular margin.

e. Surface culture on wort gelatine : greyish white coating with fine radiation on the margin.

f. Saké-agar culture : Surface culture (at 25–26° C) was white and its central part showed an elevation, surrounded by mesenteric folds. Stab-culture (at 26–28° C) : white, developing on the surface as well as in the canal.[2] Gigantic colony (at 24–25° C) shows white mesenteric folds thicker in the central part than on the margin.

g. A number of experiments have shown, that this yeast develops best at 25° C.

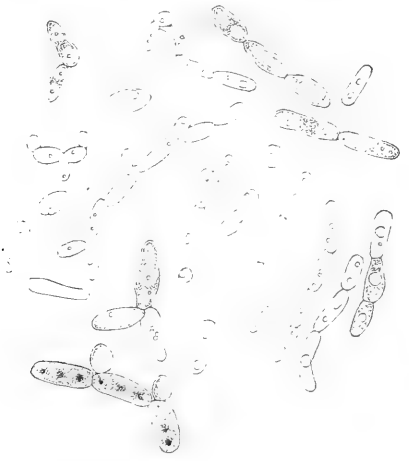
h. The faculty of the growth on Mayer's and Nägeli's solution containing 9% of alcohol, distinguishes this from Mycoderma cerevisiæ.

[1] By this character we distinguish this Mycoderma from Endoblastoderma glucomyces. I. II. III.

[2] This is one of the characteristics of this yeast which distinguishes it from Mycoderma cerevisiæ, vini. and 12 other varieties (except Mycoderma 8) described in these Bul., Vol. VI. Mycoderma 8 (Bulletin Vol. VI. No. 4) gives brownish-yellow growth and very coarse folds on saké-agar.

i. Hayduck's solution containing 2% and 4% of acetic acid was unfavorable for growth, but some growth was observed at 1%. In this regard it differs also from Mycoderma cerevisiæ.

j. Spore formation was not observed in spite of many trials, hence this yeast must be regarded as a variety of Mycoderma.



Myoderma saprogenes saké.

k. Behavior to carbohydrates.

1. It assimilates glucose, fructose, saccharose, maltose and galactose, the latter two sugars not so well as the others, however.

2. It ferments glucose and galactose very feebly but not fructose, saccharose, and maltose.[1]

l. It does assimilate neither nitrate nor nitrite.

m. On culture in Hayduck's solution at 27–32° C during 17 days a trace of volatile substance of the flavour of altered vinegar and an acid reaction are produced. On sterilised saké, poor in alcohol, the same flavour is produced, which has a certain relation to alcohol; for if we add to the culture a few cc. of alcohol we can perceive the characteristic odor after 1–2 hours at 20–30° C. After 5 months culture in

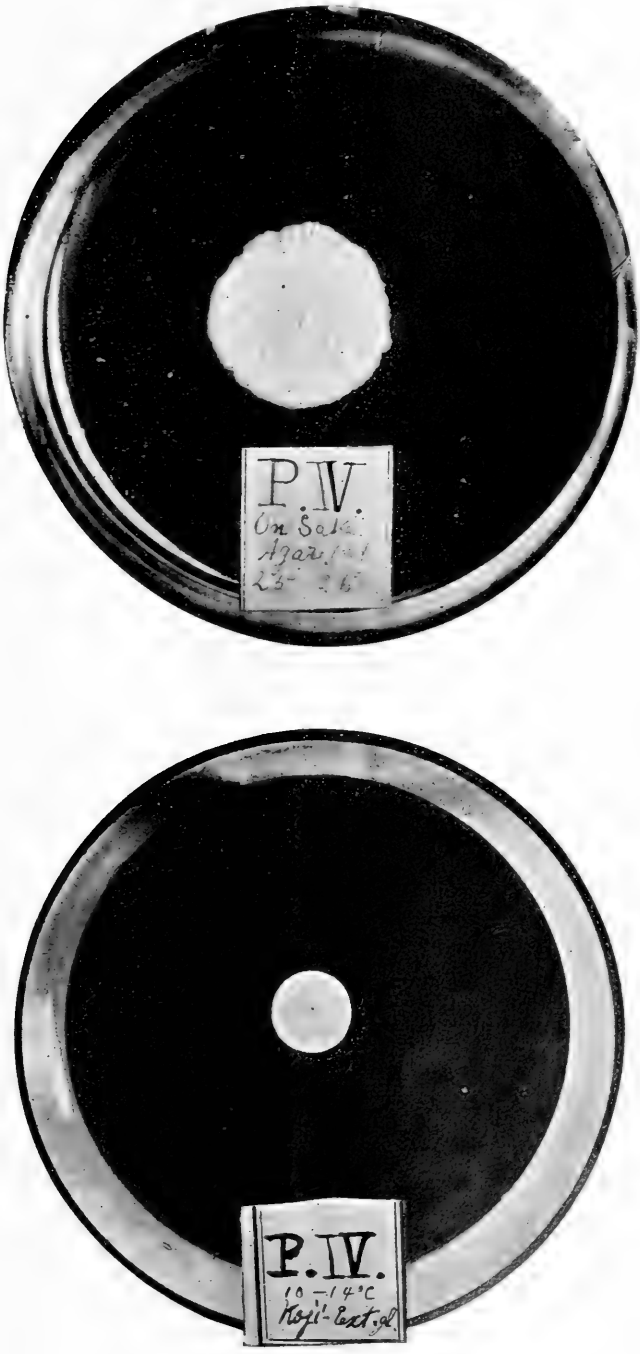
[1] By this behavior this yeast is distinguished from all other varieties of Mycoderma which I have described, in a former Bulletin (Vol. VI. No. 4).

saké with 13.85% alcohol there was only found a trace of ethyl alcohol but neither aldehyde, nor methyl-alcohol, nor acetone.

n. The resistance to alcohol diminishes after long artificial culture. Upon adding to a growth on sterilised saké 25 Vol. % of alcohol it was observed that it took five days to kill all the cells.

o. The resistance to heat. A young culture was heated to different degrees and then infection made into koji-extract containing a little tartaric acid. Thus it was observed that this yeast was killed in a moment at 70° C, or on exposure to 55° for 5 minutes.

Summing up it can be infered that this Mycoderma represents a new variety. It rapidly oxidises alcohol to CO_2 and H_2O, forming only a trace of organic acid. Since it causes a saké disease, the name: Mycoderma saprogenes saké is proposed.



Colonies of a *Mycoderma* causing a disease of saké.
To the article of Takahashi.

Note on Bacteria Pathogenic to Silk-worm.

BY

S. Sawamura.

The writer[1] had infered from his experiments that the flacherie of silk-worms can be caused by various bacteria of general occurrence. During that disease no specific bacteria can be found which would be restricted to the occurrence in that epidemic. As a further support of his view the causation of flacherie by subcutaneous infection of various bacteria, may be mentioned. The following bacteria were found by the writer to produce flacherie by multiplying in the body of the silk-worm, causing mostly vomiting and diarrbea with death following :

1. Bacillus coli.
2. Bacillus Ellenbachi.
3. Bacillus ferrugenus.
4. Bacillus fuchsinus.
5. Bacillus megaterium.
6. Bacillus megaterium bombycis.[2]
7. Bacillus mycoides.
8. Bacillus pyocyaneus.
9. Bacillus rubefaciens.
10. Bacillus viridans.
11. Various species of Proteus.
12. Micrococcus pyogenes aureus.

[1] This Bulletin Vol. V. No. 4.

[2] Ditto. Vol. VI. No. 4.

On the Micro-organisms of Natto.

BY

S. Sawamura.

Natto is a kind of vegetable cheese prepared in Japan by fermentation of boiled soy-bean wrapped in rice straw and left for one or two days in a warm place. This product contains much mucilage filled with innumerable bacteria and it is the great viscosity that is especially esteemed with this cheese.

Yabe[1] isolated from *natto* three species of micrococci which formed yellow, orange and white colonies respectively, and a becillus which was immotile, liquefied gelatine, produced a green fluorescence and formed white colonies on soy-bean. According to Yabe the micrococcus of the yellow colonies produced the peculiar aroma of *natto* when cultured on soy-bean, but thus far it was not decided which microbe gives rise to the viscous substance or mucilage. Yabe has found that a large part of the albuminoids of soy-bean is decomposed to pepton and amido-compounds by the fermentation.

The micro-organisms of *natto* consist in the beginning chiefly of bacilli, but on being kept for some time micrococci gain predominance. The writer isolated various kinds of bacilli and micrococci from *natto* and observed their behavior in cultures on sterilised soy-bean. Two kinds of bacilli grew well on soy-bean and formed a product similar to *natto* in regard to taste, aroma and viscosity. Other bacilli, however, and micrococci in developing on soy-bean did not produce a palatable product, nor did mixed cultures of them with the former two species. Also the behavior of *Bacillus mesentericus vulgatus*, *Bac. mes. fuscus*, *Bac. subtilis*, *Bacterium filiforme* and *B. tyrothrix*

[1] This Bulletin Vol. II. No. 2.

filiformis was observed by the writer, but the products formed were of disagreeable taste and odor. The writer infered that although many bacteria can grow on soy-bean, the genuine *natto* can only be produced by certain bacilli thus for isolated from the *natto*-cheese.

Those bacilli show the followiog characteristics :

Bacillus No. 1.

Form : Cells cultured on bonillon at 30° C for 24 hours are 1 μ wide and 3–4 μ long. The ends of the rod are rounded. The bacilli unite sometime to a long thread.

Motility : The bacillus of young cultures is motile.

Spore : An oval spore is formed in the middle of the cell.

Gram's method : Positive.

Oxygen : Facultative ærobic.

Bouillon culture : A light yellowish-brown scum of dry mealy appearance is formed at 30° C after 20 hours,

Pepton-water culture : A white dry scum is formed at 30° C after 20 hours.

Gelatine plate culture : Small round light brown colonies having a point in the centre and a feather-like divided periphery are formed.

Gelatine streak culture : Gelatine is liquefied along the needle track.

Gelatine stab culture : Gelatine is liquefied in the shape of a funnel.

Agar plate culture : Light brown dry lipped colonies having a peculiar mealy appearance and sometimes with a point in the centre.

Agar streak culture : A light brown dry flat colony having a peculiar mealy appearance.

Agar stab culture : A light brown dry colony having the peculiar mealy appearance extends rapidly to the wall of the test-tube.

Potato culture : Gray folded colony at 30° C in 20 hours.

Milk culture : Milk is coagulated at first, but afterwards the casein is again dissolved and the reaction becomes alkaline.

H_2S : It is not formed.

Indol reaction : Negative.

Gas : Gas is not formed by culturing on glucose-bouillon.

Natto produced by this bacillus has a good taste and aroma, but is not of so strong viscosity as that produced by Bacillus No. 2. The colonies of Bacillus No. 1 appear always in large number when plate cultures from *natto* are made. Hence it became probable that this microbe exerts the chief action in the fermentation of *natto*. It resembles somewhat Bacillus mesentericus, still it differs again considerably.[1] The writer considers it a new species which received the name of *Bacillus natto*.

Bacillus No. 2.

Form : Cells cultured in bouillon at 30° C for 24 hours are 1 μ wide and 3 μ long. The ends of the rod are rounded and two or more are often united.

Motility : It rarely moves.

Spore : An oval spore is formed in the middle of the cell.

Oxygen : Facultive aerobic.

Gram's method : Positive.

Bouillon culture : A light yellow compact scum is formed.

Pepton-water culture : A gray folded scum is formed at 30° C in 20 hours.

Gelatine plate culture : Small white round colonies which quickly liquefy gelatine.

Gelatine streak culture : White colony which liquefies quickly gelatine.

Gelatine stab culture : It liquefies gelatine in the shape of a funnel.

Agar plate culture : White moist folded irregular colonies.

Agar streak culture : A light brown folded colony. The folds are sometimes wanting.

Agar stab culture : Light brown colony,

Potato culture : A gray viscous folded colony is formed at 30° C in 24 hours. The folds are deeper than those of Bacillus No. 1.

Milk culture : Milk is coagulated at first, but afterwards the casein is dissolved again.

H_2S : It is not formed.

Indol reaction : Negative.

Gas : Gas is not formed in glucose-bouillon culture.

[1] A striking difference is the mealy appearance of the colonies of Bacillus No. 1.

Soy-beans changed by this bacillus show a stronger viscosity but a less agreeable taste and aroma than those produced by Bac. No. 1. It resembles Bac. mes. vulgatus, of which it is probably a variety. It is probable that for the formation of a good *natto*-cheese both the bacilli just described must be present.

In order to test for enzyms produced on soy-beans by Bac. No. 1 and No. 2, the precipitates obtained by alcohol with the extracts were redissolved in water and once more precipitated. The aqueous solution in presence of some thymol quickly dissolved the proteins of soy-bean at 36° C. This proteolytic action was accelerated by 0.3% Na_2CO_3 but retarded by 0.2% HCl, acetic or lactic acid ; hence the enzym was of tryptic nature.

Bac. No. 2 produced also a diastatic enzym which was confirmed by formation of reducing sugar in bouillon containing starch. From these facts we can infer that *natto* may exert some beneficial action on digestion.

The writer expresses his thanks to Mr. T. Yamasaki, assistant of the College who assisted him in these investigations.

On Wine from the Loquat Fruit.

BY

T. Takahashi.

The loquat, Eriobotrya japonica, Lindl, family of the Rosaceae is a plant of the subtropical zone. Its fruit is of yellow color and round form and has a very fragrant flavour, sweet taste and serves as food. Since this fruit grows in great quantities in many provinces of Japan especially in Kishiu and Boshiu, it was desirable, to utilise it for some technical purpose ; the production of a wine from it seemed to me the most profitable use.

The fruit and its juice was analysed with the following result :

The fruits.

Dry matter ....26.43%.

Water .....74.57%.

In the dry matter :

Seed.....13.72.

Flesh .....12.71.

The must, of reddish color, showed 12–13° B, at 17.5° C, and contained :

Glucose ..... 7.3%.

Pentosanes[1] ..... 0.395%.

Pectin ..... 3.266%.

Acids[2] { Citric acid..... 0.2842%. / Malic „ ..... 0.07034%.

Ash ..... 0.544%.

For the determination of pectin, the juice was treated with tannin, filtered, and oxalic acid was then added to remove the lime while pectin was precipitated in the filtrate from the calcium oxalate by addition of alcohol.

[1] Determined as furfurol-phenylhydrazon.

[2] Tartaric acid was absent.

For the analyses of the organic acids present served the usual methods.

For the preparation of wine this fruit was crushed and to 70 litres juice some cane sugar[1] was added.

13 kilo fruits yielded 6 litres of juice. This juice was allowed to ferment in a porcelain jar in a cellar at 16–20° C. After 4 weeks the fermented must was filtered and left to after-fermentation. The clarified wine, of a very pleasant taste and flavor, yielded the following result :

Specific gravity	1.1381
Alcohol	6.93%.
Glycerin	0.102%.
Acids	0.3619%.
a. Volatile acid (as acetic acid)	0.01194%.
b. Non-volatile acid	0.3551%.
Aldehyd (Medicus' reaction)	trace.
Ash	0.494%.

By further addition of cane-sugar, amounting to 15%, a wine resulted resembling Tokayer in taste and flavor.

Further experiments were carried out on a large scale with 108 hl. must with application of a pure culture of wine-yeast.

The wine showed the following composition :—

Specific gravity	1.0229
Alcohol	7.130%.
Glycerin	0.0873%.
Acids	0.542%.
a. Volatile acid (as acetic acid)	0.145%.
b. Non-volatile acid (as citric acid)	0.437%.
Sugar (glucose.)	0.284%.
Extractive matters	3.116%.
Ash	0.490%.

[1] Addition of sugar was necessary on account of the low percentage in the original juice.

A Condensed Vegetable Milk.

BY

T. Katayama.

When soy-beans are soaked in water then after well crushing are boiled with water, a liquid is obtained closely resembling cow's milk. The composition of this milk prepared in Japan for the purpose of obtaining Tōfu by the precipitation with calcium or magnesium salts, is in average as follows :[1]

SOY-BEAN MILK.

Water	92.53%
Protein	3.02%
Fat	2.13%
Fibre	0.03%
Non-nitrogenous extract, including carbohydrates (galactan)	1.88%
Ash	0.41%

There can be no doubt that this vegetable milk is of a certain value as an easily digestible food, although in regard to the nourishment of children it can certainly not replace cow's milk or mother's milk, even if the necessary amount of milk sugar be added.

The idea suggested itself to prepare from that vegetable milk, the soy milk, by addition of sugar and evaporation a durable preparation, resembling condensed cow's milk. Since on direct evaporation after addition of sugar the dissolved protein separated in little flocculi, I added a small dose of dipotassium phosphate to prevent this separation.

[1] These Bulletins Vol. II, No. 4.

In 4 litres of soy-milk were dissolved 4 g. dipotassium phosphate and 600 g. cane sugar and the solution concentrated in vacuo to a very thick liquid. This no doubt can replace the much more expensive condensed cow's milk for certain purposes of the cuisine as e.g. for sweetening coffee and tea, and for the preparation of chocolate.

It has an agreeable taste like cow's milk, but a very feeble odor recalling crude beans and a slight yellowish color. If this preparation should be manufactured on a large scale, it must be, of course, always sold under its proper name, and adultration of condensed cow's milk with condensed soy milk must be strictly prohibited. It was in this connection of course of value to decide by chemical investigation whether such an adulteration can be easily discovered.

My experiments in this direction answer this question in the affirmative. Above all it has to be taken in consideration that the chemical properties of casein of milk differs considerably from the globulin of the soy milk. This globulin is kept in solution by secondary sodium and potassium phosphates and it was to be expected that by the action of rennet (lab, chymosin) the casein of milk could be separated from the globulin (glycinin) of the soy milk and the filtrate from the coagulated mass would then give a precipitate with calcium or magnesium salts. I added therefore rennet to a cow's milk which had been mixed with $\frac{1}{20}$ of its volume soy milk, and after 2 hours digestion added to the filtrate of the coagulated mass some calcium nitrate. Indeed a protein precipitate was formed, while none in the control case.[1]

I have observed further: Addition of sodium carbonate to a decided alkaline reaction causes the soy milk to become deeply yellow (probably from the change of a tannin like body), while the cow's milk remains colorless. 10% of soy milk in cow's milk can thus be recognized.

Since the Tōfu milk contains a very small amount of fibres from the original soybeans, I have tried also the microscopical test to discover the

[1] Upon the direct addition of calcium salts without previous coagulation of casein the soy-globulin is also precipitated, but in the presence of much cow's milk a small amount of a precipitate cannot well be recognized. However this test would well show the presence of more than 10% of soy milk.

presence of soy milk by this means. I digested at first the proteins in the mixed milk by trypsin and examined the sediment after long standing but the results were not satisfactory.

Finally I tried whether the following qualilative test could be easily applied. I have mentioned above that the soy milk has a very peculiar odor far different from that of cow's milk and it was to be expected that this smelling principle might be obtained in higher concentration by distillation. In my test I mixed 100 cc. cow's milk with 5 cc. soy milk, and in a second case 50 cc. of both kind were mixed. The mixtures were diluted with 200 cc. of distilled water and after addition of a few drops of dilute sulphuric acid to produce a slight acid reaction about 30 cc. were distilled off. This distillates had a very characteristic odor of considerable intensity, while cow's milk alone thus treated showed no odor.

Summary.

1. The condensed milk from soybean, the so-called soy milk, can be easily transformed into a durable product analogous to the condensed cow's milk.
2. This condensed soy milk has no doubt a considerable nutritive value and may be used with great advantage for preparing various articles of food, but it can never replace cow's milk or mother's milk in the nourishment of children.
3. In order to discover the adulteration of condensed cow's milk with condensed soy milk, it is proposed to add some sodium carbonate, yellow coloration indicates the presence of soy milk. Further a portion of the suspected milk is mixed with double of its volume of water and $\frac{1}{10}$ distilled off after the addition of few drops of diluted sulphuric acid; hereby the characteristic odor of raw beans will develop.

A further test consists in separating the casein of cow's milk with rennet and adding some calcium nitrate to the filtrate; a precipitate indicates the presence of the glyobulin of soy milk, the so-called glycinin.

On the Preparation of a Vegetable Cheese from the Protein of the Soy Bean.

BY

T. Katayama.

The soybean which serves in Japan not only for the preparation of Miso[1] and of Shōyu sauce,[2] but also for the preparation of Tōfu,[3] contains according to *Osborne* and *Campbell*[4] as the chief proteid constituent[5] glycinin, a globulin similar in properties to legumin but of somewhat different composition, containing nearly twice as much sulphur, four tenths per cent more carbon, and a half per cent less nitrogen. The composition was found by these authors to be :

Carbon	52.12
Hydrogen	6.93
Nitrogen	17.53
Sulphur	0.79
Oxygen	22.63
	100.00

This protein can be extracted from the soybean by boiling water on account of the presence of phosphate of soda and potassa. The liquid thus obtained resembles cow's milk in appearance, and yields a precipitate with

[1] These Bulletins, Vol. I. No. 6.

[2] „ „ Vol. III. No. 3.

[3] „ „ Vol. II. No. 4.

[4] Journal of American Chemical Society, Vol. XX. No. 6, 1898.

[5] The small quantity of other proteins consists of legumelin, contained also in pea, vetch, horse bean and lentil ; a protein resembling phaseolin, and a proteose.

calcium and magnesium salts, and thus a product is obtained which is in commerce in Japan under the name of Tōfu. It closely resembles freshly precipitated casein from milk which suggested the experiment to try whether it could not be transformed into a cheese similar to the well known *Swiss Cheese*.

Some tabular pieces of freshly prepared Tōfu were pressed in order to remove a portion of the water. 450 g. of such pressed Tōfu were mixed with 60 g. of common salt and with 50 g. of pressed casein obtained from fresh milk by precipitation with acetic acid, and further 2 g. of finely grounded swiss cheese. These addition of casein and cheese were made in order to introduce the characteristic microbes. This mass was wrapped in a linnen cloth saturated with a solution of common salt, and left for 5 months in a room with an average temperature of 15° C, moistening the cloth from time to time. After this time the mass had acquired a very compact consistency ; the color however was not white but grey, perhaps due to a trace of changed tannin. Further the numerous pores produced by gas development in the swiss cheese were absent in this tōfu cheese, although some milk sugar (2 g.) had been added in the beginning to produce a fermentation similar to that of the swiss cheese. A crust was gradually formed on the surface, but its odor differed from that of swiss cheese. The taste of the vegetable cheese thus obtained was quite agreeable however and in no way recalling any putrefaction. A small portion was crushed in a mortar, extracted with water and filtered.

The filtrate behaved as follows :

On boiling some albumin was coagulated ; the filtrate was slightly yellowish and neutral, but yielded a weak ammoniacal reaction by Nessler's test; Biuret reaction was also very decisive. By saturation with ammonium sulphate, some albumoses were precipitated. The colorless filtrate gave with nitric acid on boiling a yellowish color, and phosphotungstic acid a voluminous precipitate in the presence of some sulphuric acid, indicating pepton and basic compounds produced by the bacterial enzyms from protein.

Second Experiment.

Here the addition of fresh casein was omitted and the milksugar increased. Fresh Tōfu was pressed in a linnen sac and divided into three parts and mixed with the following amounts of salt, cheese and milksugar :

	A	B	C
Pressed Tōfu.	700 g.	700 g.	550.0 g.
NaCl.	105 g.	84 g.	82.5 g.
Swiss Cheese.	30 g.	10 g.	3.0 g.
Milk-Sugar.	35 g.	35 g.	20.0 g.

These masses were wrapped in linnen cloth moistened with common salt and kept in a warm room as in the former experiments.

The rough surface of these mixtures became after few weeks smooth and the original yellowish white color changed to greyish ; also the characteristic smell of soybean entirely disappeared. It was considered ripe when a complete compactness, density and uniformity was reached. It was somewhat unexpected that here, notwithstanding the increased amount of milksugar, no formation of holes due to gasbubbles by fermentation, was observed.

After removal of the crust the taste of this cheese was quite agreeable although different from that of Swiss Cheese.

In consideration that *Tōfu* is an exceedingly cheep material, and further that it has to be freshly prepared every day on account of easily undergoing putrefaction, the preparation of this cheese seems to me of some importance.

On the Composition of the Fibrous Part of the Japanese Orange.

BY

Rana Bahadur.

The juice of the orange has been an object of chemical investigation,[1] but not the fibrous part which remains behind when the juice has been pressed out and all the soluble parts completely removed. This insoluble part, the fibrous pulp and the flesh-sac walls, resembling thin films, deserved however some chemical examination. It was above all of interest to see whether the polysaccharids of this material would also correspond to the sugars in the juice. Sometimes there exists not the expected relationship between these carbohydrates. Thus, e. g., with the Kaki fruit the juice contains invert sugar and canesugar, but no mannose, while the seeds are rich in mannan.

Five Japanese oranges yielded 4.5 g. of this insoluble matter. After becoming air dry, a careful analysis was made, according to the usual methods. For the estimation of galactan, 3.704 g. of the substance was boiled with HNO_3 of sp. gr. 1.15 until the mixture was reduced to one-third of its original volume. The mucic acid formed was after some time collected on a filter and further purified. It weighed 0.584 g. From the quantity of mucic acid thus obtained, the amount of galactan was calculated. For the estimation of pentosan, 4.496 g. of the substance was distilled with HCl of

[1] The juice of the European orange contains among others :—

1.93% Free acid (citric acid).
5.31% Cane Sugar.
5.43% Dextrose.
0.50% Mannitol and Pectin.
85.04% Water.

sp. gr. 1.06. The furfurol thus obtained in the distillate was precipitated with phloroglucin and from this precipitate amounting to 1.447 g., the amounts of furfurol and pentosan were calculated. Starch was not found by microscopical test with iodine. Mannan was also found absent, when after hydrolysis with sulphuric acid of 3%, neutralization with baryta, evaporation to a syrup, phenyl-hydrazin acetate was added and the mixture stirred; mannose hydrazon was thus not obtained. 2.254 g. of the substance extracted with ether gave 0.029 g. ether extract, and 1 g. yielded after Kjehldahl 0.010245 g. NH_3 equal to 0.008439 g. Nitrogen.

The final result of the analysis was :—

Hygroscopic water	12.16%.
Protein	5.27 ,,
Ether Extract	1.28 ,,
Ash	2.15 ,,
Galactan	18.91 ,,
Pentosan	27.72 ,,
Cellulose	32.51 ,,

We notice here again the interesting fact that this insoluble part of the orange contains polyanhydrids of sugars that are not found in the juice.

Fresh Water Algae as an Article of Human Food.

BY

S. Namikawa.

Among the vegetable articles, of human food in Japan are not only sea water algae but also two kinds of fresh water algae. The former have repeatedly been the object of chemical investigation[1] but not so the latter, hence some chemical data suitable as a measure for the nutritive value of these eatable algae[2] may be welcome.

The one kind is *Nostoc Phylloderma* of the group of the Shizophyceae, with the Japanese names: Suizenji-nori, kotobuki-nori. It is chiefly collected in the mountainous regions of the provinces of Higo and Chikuzen in Kiu-shu, and is extensively sent to Central Japan, where it forms a somewhat expensive delicacy, especially as an addition to soup. It is collected by small nets, all the year, mostly however in June and July, and cleaned from adhering other algae. One man can collect only 1 litre a day. The mass is cut into small pieces, spread on briks and dried in the sun; it forms thus thin cohering sheets. About 2 litres fresh mass give one sheet of 2 sq. feet. 5 sheets weigh 90 g. and cost 2½ *yen*.

The second kind of eatable algae is *Prasiola japonica* of the group of the *Chlorophyceae* with the Japanese names: Daiyagawa-nori, Nikko-nori. It is chiefly collected at Nikko in Central Japan.

[1] These marine algae are frequently cultivated in shallow places on the coast. There are 6 kinds: Porphyra vulgaria (nori), Enteromorpha compressa (ao-nori), Cystophyllum Fusiforme (hijiki), Capea elongata (arame), Ulothrix pinnatifida (wakame), and Laminaria japonica (kobu). Many species of seaweed serve as food also in the Hawaii Islands.

[2] Fresh water algae in general have but rarely been the subject of chemical tests. *O. Loew* and *Th. Bokorny* (Journ. prakt. Chem. 36, 272) have analyzed a species of Zygnema and found fat, lecithin, cholesterin, starch, tannin and succinic acid, but not asparagin. In the dry matter was 28-32% protein, 6–9% fat and 60–66% cellulose and starch.

Nostoc Phylloderma.

The dried product of commerce swells up considerably in warm water, making the presence of certain hemicelluloses probable. Cold water extracts a special coloring matter; after soaking for two or three days, the liquid shows dichromatic property: a pink red by transmitted ray, and a reddish violet by reflected ray. Mineral acids and also acetic acid turn the color to violet while alkalies decolorize it; on addition of acids the color is regenerated. Ether, benzene, chlorform, carbon disulfide do not dissolve it. Some galactan, starch and lecithin were present while mannan, sugar and tannin were absent.

The following analytical data were obtained after the usual methods. In regard to determine the galactan 20 g. of the air dry matter were boiled for seven hours with 400 cc. dilute sulfuric acid of 5%, replacing the water lost by evaporation. The filtrate was neutralized with baryta and this filtrate evaporated nearly to dryness, then boiled with nitric acid. Mucic acid soon separated as a crystalline powder.

For determination of the total N, 3 g. air dry substance (=2.4576 g. dry matter) yielded after Kjeldahl=0.0975 N.

3 g. air dry substance yielded by extraction with ether=0.0168 g. fat.

5 g. (=4.096 g. dry matter) were for the determination of pentosans boiled with hydrochloric acid of 1.06 sp. gr.; the distillate yielded with phloroglucin=0.394 g. precipitate (=0.204 g. furfurol).

The final result was as follows:

Hygroscopic moisture 18.07%

In 100 parts of dry matter:

Crude protein	24.75%
Crude fat	0.93 ,,
Crude fibre	3.64 ,,
Pentosans	4.50 ,,
Galactan	1.86 ,,
Ash	12.28 ,,
Difference, chiefly starch	58.40 ,,

Contributions to the Study of Silk-Worms.

II. On the polygamous habit of the silk-worm.

BY

K. Toyama.

It is a general belief amongst our silk-worm breeders that when a male moth mates with more than one female, the health of the offspring thus produced is much affected, that is to say, the offspring of later copulations will be weak and unhealthy, and that the longer the copulation the better will be the health of the offspring. Moreover, it is said that if the copulation is not of sufficient duration, less eggs will be laid.

From these considerations, silk-worms are kept in a monogamous state among Japanese raisers.

It is, consequently, often the case that breeders can not get good pairings when the moths come out, and considerable pecuniary loss is the consequence, since in our races it is exceedingly difficult to distinguish the sex of the cocoons.

The object of our experiments was to see if there exists any such relation between the habit of the parent and the health of the offspring.

Series I.

First of all, we will see what the effect of polygamous habit is on the eggs laid.

Experiment a.

One male was made to pair with nine females in three days. The moths used for the experiment belonged to the Siamese multivoltine yellow race.

Number of copulation.	Date of emergence. ♀	Date of emergence. ♂	Date of pairing.	Duration of copulation.	Number of eggs laid. Hatched.	Unfertilized.	Dead.	Total.	Weight of newly hatched worms. per 100 heads.
First copulation.	Oct. 7th.	Oct. 7th.	Oct. 7th.	2 hours.					
a.	,,	,,	,,	,,	173*	2	2	—	0.0325 gr.
b.	,,	,,	,,	,,	329	0	2	331	
Second.									
a.	,,	,,	,,	,,	325	2	6	333	0.0322 gr.
b.	,,	,,	,,	,,	281	2	1	284	
Third.									
a.	,,	,,	,,	,,	30*	1	11	—	0.0297 gr.
b.	,,	,,	,,	,,	363	4	0	367	
Fourth.									
a.	,,	,,	,,	,,	—	28	—	—	0.0310 gr.
b.	,,	,,	,,	,,	226	2	0	228	
Fifth.									
a.	,,	,,	,,	,,	104*	1	0	—	0.0312 gr.
b.	,,	,,	,,	,,	322	8	4	334	
Sixth.									
a.	,,	,,	8th.	4 hours.	348	7	2	357	0.0309 gr.
b.	,,	,,	,,	,,	157*	10	6	—	
Seventh.									
a.	,,	,,	,,	,,	316	4	1	321	0.0332 gr.
b.	,,	,,	,,	,,	296	73	0	369	
Eighth.									
a.	,,	,,	,,	,,	184	125	0	309	0.03066 gr.
b.	,,	,,	,,	,,	18	57	0	—	
Ninth.	Oct.								
a.	9th.	,,	9th.	6 hours.	396	3	1	400	0.0327 gr.
b.	,,	,,	,,	,,	0	54	0	—	

* Those marked with an asterisk laid some of their eggs in the cells and the figures do not represent the total member of the eggs laid.

Experiment b.

In this series, eight copulations were gone through by a single male. The moths were of the same race as before.

Number of copulation.	Date of emergence. ♀	Date of emergence. ♂	Date of mating.	Duration of copulation.	Number of eggs. Hatched.	Unfertilized.	Dead.	Total.
First copulation.	June 18th.	June 18th.	June 18th.	30 minutes.	292	2	3	297
Second copulation.	,,	,,	,,	one hour 30 mt.	0	52	0	52
Third.	,,	,,	,,	3 hours.	346	2	9	357
Fourth.	,,	,,	,,	7 hours.	350	0	3	353
Fifth.	,,	,,	18–19th.	10 hours.	404	12	15	431
Sixth.	,,	,,	19th.	½ whole day.	389	3	3	395
Seventh.	19th.	,,	19–20th.	½ whole night.	337	108	16	461
Eighth.	,,	,,	20th.	½ whole day.	368	3	20	391

Experiment c.

In this series also, one male was made to pair with eight females, the moths being of the same race as before.

Number of mating.		Date of emergence. ♀	Date of emergence. ♂	Date of mating.	Duration of mating.	Number of eggs. Hatched.	Unfertilized.	Dead.	Total.	Weight of newly hatched worms.
First mating.	1	July 3rd.	July 3rd.	July 3rd.	30 minutes.	304	17	1	322	0.033 gr.
	2	,,	,,	,,	,,	364	17	4	385	
	3	,,	,,	,,	,,	297	10	3	310	
Average.						355	14.6	2.6	339	
Second.	1	,,	,,	,,	one hour 10 minutes.	0	56	0	56	0. gr.
	2	,,	,,	,,	,,	0	38	0	38	
	3	,,	,,	,,	,,	0	9	2	11	
Average.						0	34.3	0.66	35	

Number of mating.		Date of emergence. ♀	Date of emergence. ♂	Date of mating.	Duration of mating.	Number of eggs. Hatched.	Unfertilized.	Dead.	Total.	Weight of newly hatched worms.
Third.	1	July 3rd.	July 3rd.	July 3rd.	2 hours.	358	9	5	372	0.0335 gr.
	2	,,	,,	,,	,,	359	17	4	380	
	3	,,	,,	,,	,,	332	28	4	364	
Average.						349.6	18	4.3	371.9	
Fourth.	1	,,	,,	,,	3 hours.	376	4	3	383	0.034 gr.
	2	,,	,,	,,	,,	195	6	69	270	
	3	,,	,,	,,	,,	299	41	4	344	
Average.						290	17	25.3	332.3	
Fifth.	1	,,	,,	,,	4 hours.	394	7	8	409	0.0315 gr.
	2	,,	,,	,,	,,	116	26	2	144	
	3	,,	,,	,,	,,	304	11	4	319	
Average.						271.3	14.6	4.6	290	
Six.	1	4th.	3rd.	4th.	4 hours.	321	7	9	337	0.031 gr.
	2	,,	,,	,,	,,	330	16	20	366	
	3	,,	,,	,,	,,	0	6	0	6	
Average.						217	9.6	9.6	236	
Seventh.	1	,,	,,	,,	,,	351	8	8	367	0.035 gr.
	2	,,	,,	,,	,,	0	5	0	5	
	3	,,	,,	,,	,,	318	10	7	335	
Average.						223	7.6	5	235.6	
Eighth.	1	,,	,,	,,	4 hours. 10 minutes.	0	32	0	32	0.035 gr.
	2	,,	,,	,,	,,	102	9	46	157	
	3	,,	,,	,,	,,	0	31	0	31	
Average.						34	24	15.3	73.3	

From these experiments, we see that there is no considerable difference between the first and subsequent copulations either in the total number of eggs laid, or in the relative number of unfertilized or dead eggs. No difference can be detected also in the newly hatched worms. If we pick out particular cases, the best result is found in the ninth (*a*) and fourth (*b*, *c*) copulations, not in the first or second.

As a general result, however, we may say that after fifth or sixth copulation, the number of unfertilized and dead eggs gradually increased, which is not a good omen for breeding purposes.

Series II.

Let us now consider the effect of the age of the moths on fertilization. The worms used were of the Siamese cross-bred multivoltine yellow breed.

Experiment a.

WITH OLD FEMALES.

Number of Pairing.		Date of emergence. ♀	Date of emergence. ♂	Date of pairing.	Duration of pairing.	Number of eggs. Hatched.	Unfertilized.	Dead.	Total.	Weight of newly hatched worms. per 100.
First copulation.	1	July 4th.	July 5th.	July 5th.	30 minutes.	0	215	0	215	0. gr.
	2	,,	,,	,,	,,	0	78	0	78	
	3	,,	,,	,,	,,	0	39	0	39	
	4	,,	,,	,,	,,	0	103	0	103	
	5	,,	,,	,,	,,	0	31	0	31	
Average.						0	93.2	0	93.2	

	Number of pairing.	Date of emergence. ♀	Date of emergence. ♂	Date of pairing.	Duration of pairing.	Number of eggs. Hatched.	Unfertilized.	Dead.	Total.	Weight of newly hatched worms. per 100.
Second copulation.	1	July 4th.	July 5th.	July 5th.	1 hour.	380	0	11	400	
	2	,,	,,	,,	,,	286	5	11	302	
	3	,,	,,	,,	,,	348	15	82	445	0.032 gr.
	4	,,	,,	,,	,,	380	20	19	419	
	5	,,	,,	,,	,,	0	43	0	43	
Average.						278.8	18.4	24.6	321.8	
Third copulation.	1	,,	,,	,,	3 hours.	357	10	25	392	
	2	,,	,,	,,	,,	0	97	0	97	
	3	,,	,,	,,	,,	397	3	20	420	0.033 gr.
	4	,,	,,	,,	,,	361	3	27	391	
	5	,,	,,	,,	,,	139	13	6	158	
Average.						250.8	25.2	15.6	291.6	
Fourth copulation.	1	,,	,,	,,	4 hours.	359	13	19	391	
	2	,,	,,	,,	,,	340	6	19	365	
	3	,,	,,	,,	,,	302	17	9	328	0.032 gr.
	4	,,	,,	,,	,,	360	13	7	380	
	5	,,	,,	,,	,,	342	24	34	400	
Average.						340.6	14.6	17.6	372.8	
fifth copulation.	1	July 6th.	July 5th.	July 6th.	,,	378	10	37	425	
	2	,,	,,	,,	,,	396	5	51	452	
	3	,,	,,	,,	,,	386	13	8	407	0.033 gr.
	4	,,	,,	,,	,,	10	280	0	290	
	5	,,	,,	,,	,,	331	31	27	389	
Average.						300	67.8	24.6	392.6	

Experiment b.

WITH OLD MALES.

The worms used for this experiment belonged to a cros-bred race between Japanese divoltine white and French univoltine "Var."

	Number of pairing.	Date of emergence. ♀	Date of emergence. ♂	Date of pairing.	Duration of pairing.	Number of eggs. Hatched.	Unfertilized.	Dead.	Total.	Weight of newly hatched worms.
First copulation.	1	June 28th.	June 26th.	June 28th.	6 hours.	413	4	6	423	——
	2	,,	,,	,,	,,	358	1	4	363	——
	3	,,	,,	,,	,,	415	3	2	420	——
	4	,,	,,	,,	,,	429	0	2	431	——
	5	,,	,,	,,	,,	84	4	0	88	——
	6	,,	,,	,,	,,	397	0	3	400	——
	7	,,	,,	,,	,,	409	0	3	412	——
	8	,,	,,	,,	,,	496	3	1	500	——
	9	,,	,,	,,	,,	436	1	3	440	——
	10	,,	,,	,,	,,	427	8	3	438	——
	11	,,	,,	,,	,,	213	0	2	215	——
	12	,,	,,	,,	,,	412	0	13	425	——
Average.						374	2	3·5	379·5	——
Second copulation.	1	June 28th.	June 27th.	June 28th.	6 hours.	396	2	1	399	——
	2	,,	,,	,,	,,	406	2	0	408	——
	3	,,	,,	,,	,,	415	0	4	419	——
	4	,,	,,	,,	,,	323	16	8	347	——
	5	,,	,,	,,	,,	417	1	2	420	——
	6	,,	,,	,,	,,	460	3	1	464	——
	7	,,	,,	,,	,,	399	1	2	402	——
	8	,,	,,	,,	,,	351	20	2	373	——
	9	,,	,,	,,	,,	382	1	5	388	——
	10	,,	,,	,,	,,	278	0	0	278	——
	11	,,	,,	,,	,,	442	13	4	459	——
Average.						388	5·3	2·9	396	——

	Number of pairing.	Date of emergence. ♀	Date of emergence. ♂	Date of pairing.	Duration of pairing.	Number of eggs. Hatched.	Unfertilized.	Dead.	Total.	Weight of newly hatched worms.
Third copulation.	1	June 29th.	June 26th.	June 29th.	6 hours.	198	241	13	452	——
	2	"	"	"	"	419	4	4	427	——
	3	"	"	"	"	0	36	0	36	——
	4	"	"	"	"	366	73	23	462	——
	5	"	"	"	"	358	58	15	431	——
	6	"	"	"	"	340	16	10	366	——
	7	"	"	"	"	473	9	5	487	——
Average.						307	62.4	10	380	——

From experiment *a*, with old females, we see that the number of unfertilized eggs and especially of dead ones is much greater, when compared with the result obtained with new female and old male. (See series I and series II *b*).

Some analogous facts have been observed in Lepidoptera by Standfuss,[1] who says that "female germs seem to be much more sensible to influences than the male."

We may say, therefore, that the process of fertilisation is affected by the condition of the female much more than by that of the male. Even with males which have been kept during three days, we can get a good result when paired with new healthy females; but those which had been kept during four days did not give as good a result, as the table shows.

Series III.

In this series of experiments we have two cases; in the one case, the moths were paired during 30 minutes, and in the other, during four to six hours, which is a common Japanese custom.

[1] Standfuss Synopsis etc. 1900.

The result will show the effect of the duration of copulation upon fertilisation.

The breed used for the experiment was a cross between Japanese and Siamese breeds.

(A)

1.

Number of moth.	Duration of copulatoin.	Total number of eggs.	Number of hatched worms.	Number of unfertilised eggs.	Number of dead eggs.
1	30 minutes.	373	371	1	1
2	,,	345	331	7	7
3	,,	340	328	6	6
4	,,	388	380	2	6
5	,,	303	293	3	7
6	,,	313	311	2	0
7	,,	372	366	2	4
8	,,	385	378	5	2
9	,,	387	382	2	3
10	,,	218	211	2	5
11	,,	360	354	2	4
12	,,	315	310	4	1
13	,,	325	319	2	4
14	,,	344	339	2	3
15	,,	352	350	1	1
Average.		341.3	334.8	2.8	3.6

(A)

2.

Number of moth.	Duration of copulation.	Total number of eggs.	Number of hatched worms.	Number of unfertilised eggs.	Number of dead eggs.
1	4 hours.	349	342	2	5
2	,,	363	356	2	5
3 *	,,	128	127	1	0
4	,,	358	349	4	5
5	,,	369	361	5	3
6	,,	351	344	4	3
7	,,	370	370	0	0
8	,,	365	354	5	6
9	,,	355	339	8	8
10	,,	353	345	1	7
11	,,	315	307	3	5
12	,,	359	344	10	5
13	,,	338	331	2	5
14	,,	307	293	13	1
15	,,	249	247	1	1
Average.		328,6	310,6	4	3.9

* This does not represent the total number of the eggs laid by a moth, since some of the eggs were laid on the cell.

(B)

I.

Number of moth.	Duration of copulation.	Total number of eggs.	Number of hatched worms.	Number of unfertilised eggs.	Number of dead eggs.
1	30 minutes.	291	229	31	31
2	,,	246	190	29	27
3	,,	294	285	3	6
4	,,	390	350	3	37
5	,,	400	380	1	13
6	,,	433	416	6	11
7	,,	0	0	0	0
8	,,	414	382	2	30
9	,,	373	342	3	28
10	,,	337	310	4	23
11	,,	297	270	4	14
12	,,	427	422	1	4
13	,,	337	301	4	32
14	,,	393	386	1	6
15	,,	358	351	0	7
16	,,	67	57	7	3
17	,,	344	262	58	24
18	,,	333	299	1	33
19	,,	319	253	5	61
20	,,	294	250	5	29
21	,,	332	285	10	37
22	,,	124	107	10	7
23	,,	348	332	3	13
24	,,	277	221	25	31
25	,,	381	341	29	10
26	,,	348	343	3	2

(B)

I.

Number of moth.	Duration of copulation.	Total number of eggs.	Number of hatched worms.	Number of unfertilised eggs.	Number of dead eggs.
27	30 minutes.	474	456	2	16
28	,,	395	351	25	19
29	,,	376	375	0	1
30	,,	391	390	0	1
31	,,	417	390	6	21
32	,,	344	322	5	17
33	,,	246	218	3	25
34	,,	356	338	7	11
35	,,	324	298	7	19
36	,,	0	0	0	0
37	,,	346	338	2	6
38	,,	333	315	2	17
39	,,	263	194	44	25
40	,,	388	341	2	45
41	,,	246	171	15	60
42	,,	419	409	1	9
43	,,	338	319	2	17
44	,,	308	279	12	17
45	,,	105	75	22	8
46	,,	286	230	31	25
47	,,	366	275	24	67
48	,,	405	396	3	60
49	,,	377	363	5	9
50	,,	379	355	7	17
51	,,	286	209	66	11
52	,,	351	261	12	78
Average.		320	300	10.6	20

(B)

2.

Number of moth.	Duration of copulation.	Total number of eggs.	Number of hatched worms.	Number of unfertilised eggs.	Number of dead eggs.
1	6 hours.	0	0	0	0
2	,,	326	319	2	5
3	,,	360	357	1	2
4	,,	311	263	16	32
5	,,	241	215	10	16
6	,,	105	81	21	3
7	,,	50	0	50	0
8	,,	358	298	15	45
9	,,	0	0	0	0
10	,,	342	311	10	21
11	,,	251	221	16	14
12	,,	0	0	0	0
13	,,	370	354	7	9
14	,,	354	323	9	12
15	,,	351	348	3	0
16	,,	346	335	3	9
17	,,	0	0	0	0
18	,,	413	391	10	12
19	,,	323	0	323	0
20	,,	373	357	3	13
21	,,	391	319	57	15
22	,,	342	335	0	7
23	,,	107	0	107	0
24	,,	378	372	0	6
25	,,	168	148	17	4
26	,,	371	337	0	34

(B)

2.

Number of moth.	Duration of copulation.	Total number of eggs.	Number of hatched worms.	Number of unfertilised eggs.	Number of dead eggs.
27	6 hours.	0	0	0	0
28	,,	0	0	0	0
29	,,	0	0	0	0
30	,,	360	344	7	9
31	,,	152	46	88	18
32	,,	275	227	26	22
33	,,	371	351	3	17
34	,,	469	456	2	11
35	,,	21	0	21	0
36	,,	315	273	19	23
37	,,	398	358	3	37
38	,,	0	0	0	0
39	,,	367	333	6	28
40	,,	42	0	42	0
41	,,	409	396	1	12
42	,,	379	352	3	24
43	,,	230	212	13	5
44	,,	355	283	41	31
45	,,	256	225	17	14
46	,,	416	397	1	18
47	,,	332	324	4	4
48	,,	379	363	1	15
49	,,	291	276	13	2
50	,,	320	297	1	22
51	,,	196	190	2	4
52	,,	369	342	10	17
Average.		256	225	19.3	11.3

Both series gave nearly identical results. The results of the preceding series of experiments also support this fact. It seems that the significance of the duration of copulation has been overrated.

It is, however, to be borne in mind that if the copulation is too short, it sometimes occurs that all the eggs laid are unfertilised, most probably on purely mechanical grounds.

Then the question naturally arises: What is the proper duration? The next series of experiment will give an answer to it.

Series IV.

In this series of experiment, a single white female was made to pair with two males, one white and the other yellow, the duration of the copulation being different in each case.

As the white eggs fertilised by the white male will produce white worms while those fertilised by the yellow, yellow worms, the proportion of these two kinds of worms in the same batch will determine the proper duration of the pairing.

The worms used for the experiments were a cross between the Japanese and Siamese yellow races.

	Number of white female.	Kind of ♂ and duration of copulation.		Number of worms resulted.	
		1st pairing.	Second pairing.	White.	Yellow.
First group.	1	30 minutes by yellow.	five hours by white.	167	129
	2	do.	do.	359	0
	3	do.	do.	146	50
	4	do.	do.	230	60
	5	do.	do.	296	43
Average.				239.6	56.4

	Number of white female.	Kind of ♂ and duration of copulation.		Number of worms resulted.	
		1st pairing.	Second pairing.	White.	Yellow.
Second group.	6	one hour by yellow.	four hours by white.	213	161
	7	do.	do.	91	231
	8	do.	do.	222	110
	9	do.	do.	217	47
	10	do.	do.	200	120
Average.				188.6	133.8
Third group.	11	two and half hours by yellow.	two and half hours by white.	227	102
	12	do.	do.	76	285
	13	do.	do.	220	120
	14	do.	do.	248	65
	15	do.	do.	207	62
Average.				195.6	126.8
Fourth group.	16	three and half hours by yellow.	two hours by white.	151	228
	17	do.	do.	317	21
Average.				234	124.5

In group I, the white worms were more numerous than the yellow, but in group II, there was no considerable difference between them, in spite of a great difference in the relative duration of the copulation in the two cases. The third proup in which the relative duration of pairing with the two males was equal gave a similar result to that of group II. In group IV we again see much difference between the numbers of these two kinds of worms produced.

Thus we may say that a longer pairing than two hours produces by no means a better effect on the fertilisation of the eggs.

Series V.

Now we will compare the growth of the worms raised from the various copulations.

Before going further, it must be mentioned that as the growth of the worms, the production of the cocoons, etc. are much influenced by the management, climatological conditions, infectious diseases, the quality of the leaves given, and other various causes, it is very difficult to arrive at a definite result. I have made several series of experiments since 1898. Sometimes the first copulation gave better results than other copulations, while in others quite contrary results were obtained. Again, there were cases where no difference could be observed among the offspring raised from the various copulations.

The following which is one of the most reliable cases many be cited.

(I.)

	1st copulation.	2nd copulation.	3rd copulation.	4th copulation.	5th copulation.
Duration of pairing.	One hour.	Two hours.	Three and half hours.	Four and half hours.	Twelve hours.
Body weight of the worms.					
1st age. Full grown. (50 per heads).	0.161 gr.	0.163 gr.	0.158 gr.	0.162 gr.	0.155 gr.
Second age. do. (50 per heads).	0.606 „	0.647 „	0.610 „	0.640 „	0.571 „
Third age. do. (50 per heads).	2.54 „	2.53 „	2.53 „	2.555 „	2.40 „
Fourth age. do. (50 per heads).	11.15 „	11.20 „	11.27 „	17.18 „	11.19 „
Fifth age. do. (50 per heads).	67.80 „	68.21 „	68.98 „	68.88 „	70.35 „
Matured worms.	56.82 „	57.08 „	55.89 „	59.58 „	56.36 „
Number of fresh cocoons in one hundred grams.	148 „	147 „	152 „	145 „	139 „

(II.)

No. 1. first copulation. 2 hours.
„ 2. second „ 3 „

Temperature during

Average temperature at 6 A.M. 21. 1° C.
„ „ „ 11 A.M. 25. 2° C.
„ „ „ 3 P.M. 27. 5° C.
„ „ „ 8 P.M. 25° C.

Every management is quite the same in

Number.	Date of hatching.	Weight of newly hatched worms.	Date of mounting.	Total weight of cocoons.			Number of diseased worms thrown away.
				Good.	Dupions.	Spoiled.	
				gram.		grain.	heads.
1	Nov. 30th.	1.066 gr.	January 8–10.	1,140	40	8	492
2	„	„	„	855	9	9	761
3	„	„	„	875	10	10	782
4	„	„	„	1,160	20	20	552
Normal.	„	„	„	1,116	12	12	600

The worms used for the experiment were of the Siame

No. 3. third ,, 3 ,,
,, 4. fourth ,, 4 ,,

the rearing.

	Difference between the dry and wet bulbs.
.....	1°. 7 C.
.....	3°. 5 C.
.....	4°. 6 C.
.....	2°. 9 C.

every group.

Weight of fresh cocoons per 50.	Weight of worms just after,					Weight of newly hatched worms per 100.
	First moulting.	Second moulting.	Third moulting.	Fourth moulting.	Matured ones.	
gram.	gram.	gram.	gram.	gram.	gram.	gram.
30.32	0.108	0.400	1.80	8.60	46.24	0.032
29.59	0.107	0.419	1.82	8.41	45.43	0.0315
30.60	0.107	0.416	1.85	8.03	45.87	0.0325
29.56	0.102	0.419	1.84	8.05	45.23	—
29.71	—	—	—	—	—	—

se multivoltine yellow race.

As far as our experiments went, we could not find any deffinite difference between the offspring of the first copulation and later copulations.

Let us next see the habit of some wild silk-worms.

Theophila mandarina, m. which is considered to be one of the nearest allies[1] of the domesticated silk-worm is quite polygamous. The eggs laid, however, are well fertilised.

A similar case is found in *Antheraea Yamamai*, but in this species copulation lasts longer than in *Theophila*.

When reared *Cricura trifenestrata* of Burmah in 1903, we observed that during the night a male visited many females; yet all the eggs laid by them are well fertilised.

Thus we may say that polygamy is a normal habit in some wild silk-worms.

There is an opinion that if the copulation is not sufficiently long the eggs will be imperfectly fertilised. This is the cause of the custom of preventing the polygamous habit of the domesticated silk-worm. On this subject, there is nothing better than to quote Prof. Weismann's statement[2] which will give a final decision to the matter. He says: "nowadays we are no longer justified in using such an expression as imperfect fertilisation. Whenever a living spermatozoon enters an egg, the latter becomes fertilised; and an imperfect fertilisation could only be supposed to occur if the spermatozoon is abnormal—if, for instance, it contains too few idants."

Henking[3] observed cases in the silk-worm where several spermatozoa entered the egg, yet only one of them united with the egg-nuclens while the others degenerated.

Thus we may safely determine the duration of copulation by the number of unfertilised eggs. When there are no unfertilised eggs or very few of them, we may conclude that the duration has been snfficient.

[1] Concerning this point, Prof. Sasaki has published valuable observations. Annotatiôns zoologicae Jap. Vol. II. Pars II.

[2] Weismann's Germ-plasm. 1893.

[3] Henking—Untersuchungen über die ersten Entwicklungsvorgänge in den Eiern der Insekten. 1892.

Summary and Conclusion.

Taking into account all the facts and considerations above referred to, it seems that there is no escape from the following conclusion, with which we will bring our discussion to a close.

1. Polygamy is a normal habit of the silk-worm; even when a male pairs with eight or more females in two or three days fertilisation is complete.

2. Fertilization is much more influenced by the condition of the females.

3. For healthy males and females, copulation for thirty minutes may be considered sufficient. For practical purposes, however, it is advisable to allow two or three hours to avoid mechanical disturbances.

4. Where good pairs of moths can not be obtained, a male may be allowed to copulate twice or more according to the circumstances. If the males are more numerous than the females the former may be keep over until the next day when new females may emerge.

To keep males in a healthy state, they should be removed from the cocoon baskets or trays before they have smelted the scent of the alluring glands. When once stimulated, they become much excited, fluttering their wings and walking about until they become nearly exhausted. Even a distance of two or three meters on the leeward side is not sufficient to prevent the odor from reaching the males. To keep the males in good order, therefore, strict care should be taken to avoid the odor of the alluring glands. If the above precaution is carefully attended to, the males can be kept quietly lying in a dark cool place without injury to their health.

Contributions to the Study of Silk-worms.

III. On the parasitic fly of the domesticated silk-worms of Siam.

BY

K. Toyama.

(With plate V.)

During my stay in Siam, I had an opportunity of studying that pernicious tachina-fly, the larvae of which are parasitic in the body of the silk-worm, and make terrible havocs among them. In the following pages I shall give an account of the investigations made in Siam.

The egg. (Figs. I, II, III).

The egg is laid on the skin of the silk-worm. It is milky white and is long and slender, cylindrical, and slightly tapering anteriorly.

The ventral side with which it attaches itself to the skin of the worm is flat and membranous, while the dorsal surface is convex, and the chorion is marked out into characteristic polygonal areas.

Its average size is 0.5 to 0.57 mm. by 0.2 to 0.22 mm.

From its first deposition until hatching no change of color takes place.

The larva. (Figs. IV, V, VI, VII, XI).

The larva or young maggot emerges from the egg through a hole (Figs. IV, V, o′) made in the chorion on the anterior portion of the ventral side of the egg, and makes its way into the body of the worm on which the egg was laid. At the same time, a round pore (Figs. IV, V, o) is formed on the anterior portion of the dorsal side of the egg. It probably serves as a breathing pore for the developing maggot.

Fig. IV represents a surface view of an egg and its maggot, the latter lying under the skin of a worm. In the anterior portion of the egg we see a round hole (o), under which we again see another depressed hole (o'). The latter is the opening on the ventral side of the egg, through which the young larva has passed into the body of the worm. A little in front of the egg is seen the larva through the skin of the worm. In a living specimen, it is seen as a translucent spot on the skin of the silk-worm. At this stage, the size of the young larva or maggot is $\frac{6}{10}$ mm. long.

A longitudinal section of an egg and maggot in a similar stage is shown in Fig. V. E. is the egg attached to the skin. On the right side of the figure we see clearly the dorsal (o) and the ventral (o') holes already described in the surface view. The ventral hole is continued into a sac made partly of the cuticle and partly of the hypodermal epithelium of the host, in which the young maggot (m) lies. The wall of the sac near the opening is colored dark. This color gradually becomes more intense, and at last a black patch is produced in the skin of the host (Fig. II), which may be depended on as a certain guide to the presence of this parasite in the body of the silk-worm.

The shape, size and position of this spot or patch are not constant. Some of them are nearly round, some pear-shaped while still others are irregular in form. It is a common fact, however, that in its middle or periphery there is a small round pore with rough edge, a little elevated above its surroundings. Rarely we meet with a vermiform patch, with a hole at either end. Such a one is probably produced by two eggs laid side by side.

The size of the patch is generally 1–1.5 mm. in diameter, but sometimes larger. A vermiform one may be 3 mm. long and 0.36 to 0.4 mm. broad.

When the maggot gets within the body of the host, numerous large migrating cells may be observed near the hypodermal epithelium constituting a portion of the sac in which the maggot lies. They gradually arrange themselves around the wall of the sac and contribute to its enlargement, which is necessary for the growing maggot.

Fig. VI represents a maggot 3 mm. long and 1 mm. or a little more broad. It is enclosed within a fibrous sac (in the figure a portion of the sac is broken to show the maggot) with a black posterior end. With this

black end, it is firmly attached to the dermal cuticle and hangs down in the body cavity of the host, supported by some adipose tissue, tracheae etc.

When the maggot has nearly attained its maturity, it gets free from the sac and passes into the body cavity of the worm. Then it makes a hole through the skin of the host, and keeps its posterior end always close to the hole, evidently in order to have a free access to the air.

In the mean time, the infested portion of the body of the host gradually undergoes disintegration, while the other parts still remain alive. Such half disintegrating worms are often meet with in a culture.

The number of maggots which may become full grown whithin the body of a worm is not constant, sometimes one, sometimes two or three or more.

When the parasite becomes quite mature, it pierces through the skin of the host and leaves it in the form commonly called maggot.

The full-grown larva or maggot. (Fig. XI).

The adult maggot is yellowish white in color and cylindrical in form. It tapers anteriorly while posteriorly it is somewhat broad and abruptly truncated.

Its size is 9–10 mm. long and 3.5–4 mm. broad.

The body is composed of twelve segments, each of which is provided in its anterior portion with several transverse rows of minute brown setae.

At the anterior end of the first segment (Fig. VII A.) a little ventrally there are two pairs of processes, and from the ventral side a pair of black mandibles project, near the base of which we again notice a small tubular process on either side.

On the lateral line of the posterior edge of the second segment (Fig. VII C.) we meet with a process, on the apex of which open three round spiracles (Fig. IX A.). Each spiracles (Fig. IX B.) is surrounded by a thin chitinous ring of a brown color. Its diameter is 0.02 to 0.024 mm.

On the posterior edge of the fifth segment on the same lateral line on which the spiracles of the second segment are situated, there is a round hole or spiracle (Fig. VIII). It is larger than those of the second segment and

has the average diameter of 0.09 mm. Around this hole there are arranged many rows of setae.

The anus (Fig. VII B.) opens on the ventral median line of the eleventh segment, and is surrounded by regular rows of setae.

On the truncated end of the last segment we see two large spiracles as black spots. Under the microscope, they are not simple spots, but have a complicated structure. Fig. X represents the central portion of the truncated end of the last segment in which the two spiracles are situated. Each spiracle, as the figure shows, consists of a black chitinous disc, on which three vermiform thin portions having a complicated structure are present. In the ventral portion of the disc we find a round membranous space with a pore.

As the maggot makes its exit from the body of the host or from the cocoon, it immediately seeks a dark place, and especially a moist ground by getting down through some cracks or fissures in the floor of the house where it comes out, and in about 10 hours it begins to pupate, regardless of whether it has found a good sheltered place or not. Thus after some hours, we find it as a cylindrical puparium tinged with dark brown.

The puparium. (Fig. XII).

The puparium is of cylindrical form rounded at both ends, and is dark brown to nearly dark in color. Its average size is 7.17 mm. by 3. mm.

The same number of body-segments as in the larval stage may be observed, the first being much contracted.

The stigmata on the second and fifth segments may be seen as black spots. On the ventral median line of the 11th segment the anus can be seen as a slightly elevated and pigmented area.

On the end of the last segment we see those conspicuous spiracles as in the larva.

The Imago or fly. (Figs. XII, XIV).

A bristly greyish fly of a moderate size.[1] The average length of the body and the expansion of the wings are : in the female 10. mm. and 18 mm. and in the male 11.5 mm. and 20. mm. respectively.

[1] The size of larva, puparium and imago differ according to the size of the host.

The head is somewhat triangular and bears large compound eyes which are dark reddish brown in color. In some specimens we may observe some short hairs on the surface of the eyes while in others they are wholly absent. The space situated between the compound eyes and the mouth parts is provided with some black bristles and is silver white in color. Dorsally the space gradually becomes of a light brownish shade.

Three simple eyes of a reddish brown color are situated on the dorsal part of the head, making a triangle with one of the angles directed toward the front.

The antenna (Fig. XVI) is club-shaped and greyish black in color. It consists of three segments, the terminal one, which is the largest and a little depressed, bears the dorsal bristle, about twice as long as the segment itself. It is two-jointed ; the proximal is very short and the distal is long, tapering to a whip-like shape. The segment and the dorsal bristle are covered with very fine hairs. In the second segment of the antenna we notice some short bristles on its dorsal portion.

The maxillary palpus is club-shaped, reddish brown in color, and covered with some black bristles.

The dorsal surface of the thorax is brownish-grey and is marked with black stripes. On the prœscutum we notice four stripes which extend to the scutum, the middle two terminating at the middle of the scutum, while the other two nearly reach the posterior edge of the scutum. Besides these stripes we may observe a median black stripe running through the scumtum from its anterior to the posterior edge. This marking is wanting on the prœscutum. The spaces situated between those stripes are covered each with a single row of black bristles.

The scutellum is nearly hemispherical. Its anterior portion has the same color as that of the preceding segment, while posteriorly it gradually assumes a brown tint, and is also provided with some bristles.

Under the magnifier we can observe very fine black hairs on the whole surface of the thorax, besides those enumerated above.

The wing (Fig. XV) is light greyish and transparent. The shape and the venation are represented in the figure. The costal vein bears short, black hairs on its frontal edge. Near the basal portion of the third longitudinal

vein, we again observe a row of some short black hairs on the vein. The first hind-marginal cell becomes narrowed at the margin near the apex. In the hind angle of the first marginal cell, there is an elongation of the fourth longitudinal vein, but it is not well developed. The fifth longitudinal vein does not reach the margin; near the hind transverse vein it stops and the remaining portion of the vein is represented in an imperfect way. The 6th and 7th longitudinal veins also do not attain their full development except in the basal portion. The posterior lobe is well developed.

The allulae are greyish white.

The legs are of moderate size, and are provided with black bristles; the femur is dark-grey, and the rest black. The pulvilli are membranous and are tinged with brown.

The abdomen.[1] The abdomen is long oval and is slightly shorter in the female than in the male. Four segments can be seen from above or below. The first is dark colored, while each of the rest is whitish-grey in its anterior portion and black in the posterior half, thus producing transverse striped markings on the abdomen. The entire surface of the abdomen is covered with small hairs. Those on the dorsal median line and on the posterior portion of the third and the last segments are long and stout.

The ventral side of the abdomen is greyish-black and is provided with black hairs.

Habit of fly and its life history.

The fly is very active in the magnanerie. When it frequents the worm-baskets, it does not stay in one place, but always flies from one worm or place to another, and cleverly finds some shade, such as is afforded by the leaves or the worms. Especially, when it is disturbed it soon hides itself in a shady corner. Even when a basket-frame was covered with a mosquito-net to prevent the flies, some were found after some time to have cleverly got in through some slit that may have been left.

When it visits a worm, it bends its abdomen and stretches out the ovipositor, and no sooner than the end of the ovipositor touches the body

[1] In some specimens we observe a brownish patch extending between the second and the third segments, on both sides of the body.

of the worm, than a white egg is laid there, and the fly then goes away to an other place or worm. Sometimes it stays on a worm and lays many eggs.

It freely lays eggs in confinement, doing so even when it is put together with some worms in a large reagent bottle.

If we put a fly together with two sets worms, one beginning to moult and the other just after moulting in a bottle, the latter is invariably first attacked by the fly. After visiting most of the second set of worms and laying eggs on them, it haunts the first set of worms, the skin of which is much more extended and harder than that of the former. In these worms, the eggs laid will be cast off before hatching together with the old skin. It must also be mentioned that in the former cases, the fly lays eggs without any hesitation while in the latter case it takes a long time to lay an egg, moving its body many times. Hence it seems that it prefers softer skinned worms to lay eggs on.

In the following, we give a note of the rearing of the parasitic fly :—

Sept. 11th, 1.30 P.M. Oviposited on six worms.

„ 12th, 1.30 P.M. No change has occurred.

„ 13th, 10 A.M. All eggs hatched. We can see on each worm one or two small translucent spots on the skin, near the anterior end of the egg. (This section is represented in Fig. V).

„ 16th, 4 P.M. Those translucent spots have become black. Average size 1 mm. by 0.5 mm. Some of the spots are round, some elliptical, most of them long pear-shaped. (Fig. II).

„ 17th. Of the six worms, two are nearly dead. On one of these there is in the ninth segment a hole 1 mm. in diameter, through which the posterior end of the maggot may be seen, all the segments from the 7th to the last have become rotten, while the anterior segments are quite healthy. Three other worms have nearly lost its apetite and become very sluggish. Sometimes they lift up their heads and bend the body considerably, as if suffering from pain.

Sept. 18th, 1 P.M. All the worms nearly dead. From some of them maggots are coming out.

" " 3.30 P.M. Maggots have begun to pupate. Toward evening they have gradually become light brown.

" 19th. In the early morning, we got two maggots.

" " 10.30 A.M. Their color has begun to change.

" " 5. P.M. Become dark brown.

Oct. 29th. Since a few days, the color of the puparium has become considerably darker, and some flies emerged during the preceding night, and all the rest emerged by the next morning.

Hence we may say that

1. The egg stage lasts about 30 to 40 hours ;
2. The larval stage lasts { parasitic life about 7 days. / free life about 10 hours.
3. The pupal stage " about 10 days.

Total duration of a generation about 18 to 19 days.

In the coldest season, viz. December or January, the pupal stage lasts longer, that is to say, two to three weeks according to circumstances, and consequently the total duration of a generation is increased to about 25–30 days.

The fly is polyvoltine. According to our experience, at least 8 or 9 generations are gone through in a year.

Oviposition takes place not only on the silk-worms, but on many other wild worms. Hence, when we first reared some silk-worms in Bangkok, where for some 50 or 60 miles around people have no custom to rear the worms we suffered from the attack of this fly.

The puparium may be transported together with the cocoons or some implements to considerable distances. We have experienced such cases during our collection of cocoons from various districts of Siam.

It is also an interesting fact that in the magnanerie belonging to the Royal Siamese Sericultural Department and situated at a locality surrounded on all sides by paddy fields, and at least 300–400 meters removed from any jungles or bushes, where the flies may be expected to haunt, no attack of the

fly has been experienced during two years of our experiments, except once when the infection was supposed to have been due to a large importation of cocoons from Korat. This may serve to show that the flies do not wander to distant places.

Distribution of the fly.

This fly is widely distributed throughout the whole Indo-Chinese peninsula.

In Siam, we find it in all districts where sericulture is practised. Thus, on the east we find it in great abundance in Korat plateau which is the principal silk-producing district in Siam, extending between about 14° 5 and 18° N. L. The worm-raisers of the district have suffered greatly from the injury of the flies. During my inspection there, I often met with cases where some 70 or 80% of a whole lot of worms reared by the natives were injured by the parasite. On the Cambodian frontier and also in Cambodia, we have been told by some Chinese residents there that a similar disease producing black spots on the body of the worm prevails there. In Muntong Rachaburi, the district situated along the mountain range separating Siam from Burmah we have observed the flies haunting the native huts where silk-worms were being reared. Even in the northern mountainous districts extending between 17° and 20° N. L., such as Muntong Pitsanulok, worms can not escape the attack of this fly.

We are told that in Annam, a similar disease occurs among the worms reared by the natives.

In Canton and Southern China, Chinese books[1] often refer to some flies doing injury to the worms. We have not yet in our hands specimens of these flies, yet we have good reason to believe that they certainly belong to the same species as that of Siam, since it is also found in northern China, where it frequents the magnanerie and deposits eggs on the silk-worms. Prof. Sasaki[2] identifies it as *Tachina rustica L.*, to which he also refers the flies we have obtained in Siam.

[1] Chifushinsho.

[2] On the parasitic fly on silk-worms in China. 1899, and Text-book of sericulture. 1905.

In 1903, November, I received, through the kindness of H.R.H. Prince Devawongse, Minister of Foreign Affairs in Siam and H. E. Phya Surisanthorn, Undersecretary for the Ministry of Agriculture in Siam, samples of living cocoons produced in Burmah, from which we obtained some parasitic flies belonging to the above species.

Now we may arrive at the conclusion that the distribution of this tachina-fly is very wide, extending from northern China to Burmah, including the whole Indo-Chinese peninsula. I am much inclined to believe that in India the same species exists besides that well-known parasite, *Oestracea Bengalensis*.

Some methods for the prevention.

The Siamese generally wrap some cotton cloth around each worm-basket. The Chinese, on the other hand, use mosquito-netted windows or doors to prevent the coming in of the fly to the room where worms are reared. The latter is one of the best methods, yet to do this we must take particular care to close up the slits in the floors, windows, doors etc. as carefully as possible. As already described, they cleverly come in through small fissures or slits to the room where worms are reared, so it is highly necessary to pay special attention to this habit. Even when we put a fine wire-gauzed cover on a basket, they cleverly creep in through some small slits between the cover and the basket.

An indirect means of prevention is to kill the maggots, puparium and if possible the flies when we meet with them anywhere.

December, 1905.

Zoological Institute,
College of Agriculture,
Tokyo Imperial University.

Explanation of plate V.

Fig. I. Silk-worm laid with eggs of the fly ; on the third, six and ninth segments we see white eggs. 2×.

Fig. II. Infected silk-worm. Dark patch on the 5th segment. Nat. size.

Fig. III. Eggs of the fly. × 1/aa zeiss.

Fig. IV. Surface view of an egg and a hatched larva or maggot.

L., larva ; E., egg ; O., dorsal opening and O′, ventral opening of the egg. × 1/aa zeiss. Left two figures × 1/B zeiss.

Fig. V. Longitudinal section through an egg and a hatched larva together with the skin of the worm. × 3/B zeiss.

E., egg ; ch., chorion ; V., vitelline membrane ; O., dorsal pore ; O′, ventral pore ; G., gummy substance with which the egg is attached to the skin ; C., cuticle ; H., hypodermal epithelium ; M., young maggot.

Fig. VI. Young maggot enclosed within a sac, a portion of the sac is broken to show the maggot.

Fig. VII. Portion of a maggot. Much magnified.

A., first segment bearing four pairs of appendages ; B., anus ; C., stigmata on the second segment.

Fig. VIII. Stigma on the fifth segment. × 3/B zeiss.

Fig. IX. Stigmata on the second segment.

A., entire view. × 3/B zeiss. B. two stigmata. × 3/D zeiss.

Fig. X. Truncated end of the last segment of a maggot. Two stigma are represented. × 3/aa zeiss.

Fig. XI. Matured maggot. Ventral view Nat. size.

Fig. XII. Puparium, dorsal view. Nat. size.

Fig. XIII. Imago ♀. 2×.

Fig. XIV. Frontal view of the head of female fly. 2×.

Fig. XV. Left wing of Imago.

Fig. XVI. Antenna of imago or fly. × 1/aa zeiss.

Fig. I.

Fig. II.

Fig. IX.

Fig. XI.

Fig. XII.

Fig. III.

Fig. XIII.

Fig. XIV.

Fig. XV.

Fig. IV.

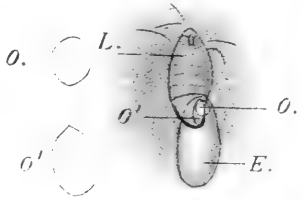


Fig. V.

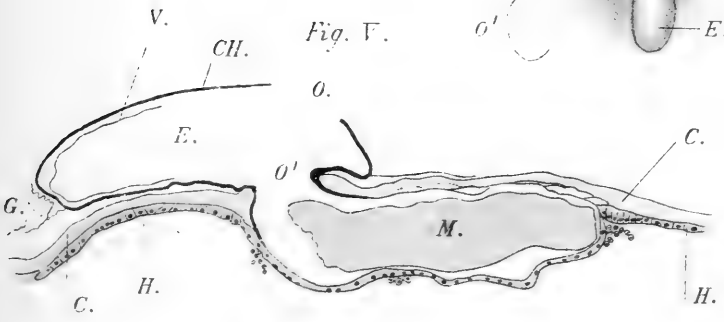


Fig. X.

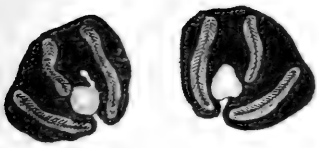
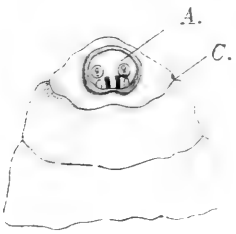


Fig. VI.

Fig. VII.



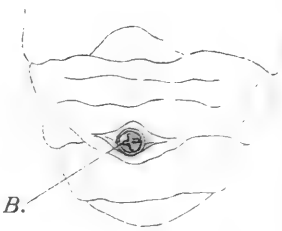


Fig. VIII.

Studies on the Hybridology of Insects.

I. On some silkworm crosses, with special reference to Mendel's law of heredity.

BY

K. Toyama.

(With plate VI—XI.)

CONTENTS.

INTRODUCTORY REMARKS.

The present subject was started in the spring of 1900 when I reared various breeds of silk-worms for a comparative study in the Zoological Institute, College of Agriculture, Tokyo Imperial University.

Since then I have continued this work in the Laboratory of the Royal Siamese Sericulture Department in Bangkok where I stayed from 1902 to 1905.

A part of my work is now finished and the results arrived at seem to be not without interest. In the following pages I will try to give an account of them.

In the spring of 1900, we made reciprocal crosses between a Japanese divoltine white race and a French univoltine yellow race called "Var", both of which races have bred true since I first got them in 1885. The crosses thus raised, amounting to 2,300 heads in the case of "white ♀ + yellow ♂" and 968 in the case of "white ♂ + yellow ♀," spun yellow cocoons without any exception.

In the next generation paired *inter se*, however, they displayed the white character in the following proportion :—

Total number of worms reared from one parent.	Number of yellow cocoons.	Number of white cocoons.
118	89 (75.3%)	29 (24.6%)

On the contrary, when we crossed the female of the first cross generation or mongrel-yellow form with the male of the pure white breed, one of their parent-races, there were produced two kinds of worms, yellow and white cocoon-spinners, their respective proportion being as follows :—

No. of parentage.	Number of white worms.	Number of yellow worms.	Total.
1	(48.97%) 143	(51.03%) 149	291
2	(46.47%) 216	(53.54%) 249	465
Average.	(47.42%) 179.5	(52.57%) 199	378.5

It is a proved fact that the colour of the abdominal legs of the caterpillar always corresponds to that of the cocoon it will spin; so that, by observing these, we can exactly tell of what colour the cocoons will be. To avoid the loss of worms incidental to rearing the countings were mostly made during the larval stage, but sometimes also by the cocoons.

Thus we see that on crossing a white and a yellow race, the offspring raised in the first generation exhibit only the yellow character, which in the next generation appears to split up into their parent-characters, the white and the yellow, according to a definite law.

The following series of experiments will prove whether there is such a general law or not.

A. NEW OBSERVATIONS.

CASE I.

Crosses between Siamese white and yellow races.

SECTION A.

The first generation.

The silk-worm generally reared by the Laos in Siam is one of the multivoltine races and may be divided into two breeds, one spinning white cocoons,[1] the other yellow cocoons.

We have reared these two breeds for five generations without obtaining even a single different form. Then, we crossed the yellow females with the white males. The worms derived from each parent being reared in a separate basket gave the following results :—

Parentage.[2]	Date of laying eggs.	Date of hatching.	Date of 'mounting.'	Number of cocoons.	Color of cocoons.
1	May 25–26	May 16	June 17–18	72	all yellow.
2	,,	,,	,,	69	do.
3	,,	,,	17–19	65	do.
4	,,	,,	,,	75	do.
5	,,	,,	17–18	79	do.
6	,,	,,	,,	70	do.
Total.				400	do.

Among the four hundred cocoons spun by the worms reared from these six parents, there was not a single white cocoon ; that is to say, the yellow character predominates over the white.

SECTION B.

The second generation.

The moths of the first generation emerged on the 26th and 27th June,

[1] These are not pure white, but rather greenish white.

[2] Throughout this paper each number represents all the eggs laid by a single parent.

and the mating was made to take place between the offspring of the same parent. Each moth was made to deposit her eggs in a separate cell, as in the last case.

We selected the eggs of four moths each from the offspring of those six parents or groups in the first generation, and reared them in the same way as in the last generation.

The following table gives the results obtained in this generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting	Cocoons.
				White.	Yellow.	Total.		
1	1	June 26th	July 5th	(27.77%) 90	239	329	July 28–29th	white and yellow.
	2	,,	,,	(30.60%) 71	161	232	,,	do.
	3	,,	,,	(26.13%) 75	212	287	,,	do.
	4	,,	,,	(25.52%) 85	248	333	,,	do.
Total.				(27.29%) 321	(72.70%) 855	1,176		do.
2	5	,,	,,	(22.22%) 64	224	288	July 28–30th	do.
	6	,,	,,	(27.45%) 28	74	102	,,	do.
	7	,,	,,	(21.53%) 56	204	260	,,	do.
	8	,,	,,	(23.07%) 51	170	221	,,	do.
Total.				(22.84%) 199	(77.15%) 672	871		do.
3	9	,,	,,	(22.25%) 77	269	346	,,	do.
	10	,,	,,	(27.45%) 84	222	306	,,	do.
	11	,,	,,	(26.45%) 77	214	291	,,	do.
	12	,,	,,	(24.05%) 57	180	237	,,	do.
Total.				(25.%) 295	(75.%) 885	1,180		do.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
4	13	June 26th	July 5th	(28.51%) 67	168	235	July 28–30th	white and yellow.
	14	,,	,,	(22.09%) 57	201	258	,,	do.
	15	,,	,,	(23.16%) 63	209	272	,,	do.
	16	,,	,,	(26.87%) 93	253	346	,,	do.
Total.				(25.20%) 280	(74.79%) 831	1,111		do.
5	17	,,	,,	(25.45%) 84	246	330	,,	do.
	18	,,	,,	(22.56%) 65	223	288	,,	do.
	19	,,	,,	(26.54%) 90	249	339	,,	do.
	20	,,	,,	(21.64%) 50	181	231	,,	do.
Total.				(24.32%) 289	(75.67%) 899	1,188		do.
6	21	,,	,,	(27.68%) 67	175	242	,,	do.
	22	,,	,,	(27.07%) 75	202	277	,,	do.
	23	,,	,,	(25.50%) 63	184	247	,,	do.
	24	,,	,,	(23.29%) 65	214	279	,,	do.
Total.				(25.83%) 270	(74.16%) 775	1,045		do.
Grand total.				(25.17%) 1,654	(74.83%) 4,917	6,571		do.

The total number of worms derived from these twenty-four parents, as the table shows, was 6,571 of which 4,917 (74.82%) were yellow worms and 1,654 (25.17%) white worms, the former spinning yellow cocoons and the latter white cocoons.

In single cases, however, the proportion of white and yellow worms varied from 30 : 70% to 21 : 79%.

Thus, we clearly see that each parent produced offspring, some (about 25%) with white or grandfather's character and others (about 75%) with yellow or character grandmother's. And, therefore we may say that in this case a simple reversion to their grandparents took place.

Section C.

The third generation.

The moths of the second generation came forth on the 6th and 8th August and those with similar characters were allowed to couple amongst themselves. Thus we have procured two kinds of eggs, one being laid by white cocoon-spinners and the other by yellow cocoon-spinners, and reared them by the same method as in the preceding generations.

The hereditary phenomena displayed by these worms can be seen from the following table:—

(A)

Offspring of the white cocoon-spinners.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
1	1	Aug. 7th	Aug. 17th	301	0	301	Sept. 10-11th	all white.
3	2	,,	,,	302	0	302	,,	do.
5	3	,,	,,	296	0	296	,,	do.
7	4	,,	,,	232	0	232	,,	do.
9	5*	,,	,,	271	0	271	,,	do.
11	6	,,	,,	195	0	195	,,	do.
13	7	,,	,,	348	0	348	,,	do.
15	8	,,	,,	280	0	280	,,	do.
17	9	,,	,,	293	0	293	,,	do.
19	10	,,	,,	259	0	259	,,	do.
21	11	,,	,,	310	0	310	,,	do.
23	12	,,	,,	205	0	205	,,	do.
Total.				3,292	0	3,292		do.

* Throughout this paper those marked with an asterisk show that some of the offspring are used in the next experiment.

(B)

Offspring of the yellow cocoon-spinners.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
2	13	Aug. 7th	Aug. 17th	0	189	189	Sept. 10–11th	yellow.
4	14	„	„	36 (18.27%)	161	197	„	white and yellow.
6	15*	„	„	87 (28.61%)	217	304	„	do.
8	16	„	„	0	348	348	„	yellow.
10	17	„	„	0	317	317	„	do.
12	18	„	„	0	212	212	„	do.
14	19	„	„	0	333	333	„	do.
16	20*	„	„	0	291	291	„	do.
18	21	„	„	64 (24.9%)	193	257	„	white and yellow.
20	22	„	„	0	322	322	„	yellow.
22	23	„	„	0	228	228	„	do.
24	24	„	„	69 (27.38%)	183	252	„	white and yellow.

Now we see that the white form produced broods, all coming true to the parents, while the yellow ones split up into two kinds of broods, one entirely like the parents, and the other containing both white and yellow worms approximately in the proportion of one white to three yellows.

Thus, we have now 3 kinds of parentages.

A. White form derived from the yellow parents in the second generation (Class I).

B. Yellow form.

1. Those from mixed offspring (yellow 75% + white 25%). (Class II).
2. Those from uniform offspring (Class III).

Each of these forms was kept separate for experiment on the next generation.

Section D.

The fourth generation.

In this generation, we have, as already stated, three classes of different parentage.

The results of rearing can be seen from the following table:—

Class I.

Offspring from the white cocoon-spinners.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
5	1	Sept. 20th	Sept. 29th	245	0	245	Oct. 27–29th	all white.
	2	,,	,,	337	0	337	26–28th	do.
	3	,,	,,	241	0	241	,,	do.
	4	,,	,,	305	0	305	,,	do.
	5	,,	,,	209	0	209	26–29th	do.
Total.				1,337	0	1,337		do.

Of the one thousand three hundred and thirty-seven worms born from five parents, none spun yellow cocoons, that is to say, the offspring raised all came true to the parents.

Class II.

Offspring from the parent which produced two kinds of worms, white and yellow, in the last generation.

(A)

Offspring raised from the yellow cocoon-spinners.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.	
				White.	Yellow.	Total.		White.	Yellow.
15	2	Sept. 20th	Sept. 29th	0	178	178	Oct. 26–27th	0	130
	3	,,	,,	(26.2%) 56	160	216	27–28th	52	141
	4	,,	,,	0	174	174	,,	0	158
	5	,,	,,	0	202	202	,,	0	187
	6	,,	,,	(26.86%) 54	147	201	26–27th	30	134
	9	,,	,,	(23.23%) 69	228	297	27–28th	62	198
	10	,,	,,	(25.4%) 63	185	248	,,	59	179
	16	,,	,,	0	311	311	,,	0	297
	17	,,	,,	0	274	274	,,	0	265
	18	,,	,,	(23.8%) 45	144	189	,,	32	135

(B)

Offspring from the white cocoon-spinners.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.	
				White.	Yellow.	Total.		White.	Yellow.
15	1	Sept. 20th	Sept. 29th	199	0	199	Oct. 26–27th	181	0
	2	,,	,,	203	0	203	27–28th	199	0
	3	,,	,,	172	0	172	,,	166	0
	4	,,	,,	149	0	149	26–27th	138	0
	5	,,	,,	208	0	208	27–28th	199	0
Total.				931	0	931		883	0

With the yellow form, we again find two kinds of offspring, one (Nos. 3, 6, 9, 10 and 18) producing white (about 25%) and yellow (about 75%) worms, the other (Nos. 2, 4, 5, 16 and 17) only yellow ones. The offspring of the white form, on the contrary, remain constant and uniform.

In this group of worms, therefore, we see the repetition of the same phenomenon observed in the last generation.

Class III.

Offspring from the parent which produced only yellow cocoon-spinners in the last generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.	
				White.	Yellow.	Total.		White.	Yellow.
20	1	Sept. 20th	Sept. 29th	0	308	308	Oct. 27–28th	0	295
	2	,,	,,	0	295	295	26–27th	0	273
	3	,,	,,	0	188	188	,,	0	174
	4	,,	,,	0	253	253	,,	0	236
	5	,,	,,	0	221	221	27–28th	0	181
Total.				0	1,265	1,265		0	1,154

Contrary to the yellow form of class II, they produce only yellow offspring.

The results above described show that the white character after being crossed with the yellow, can easily be separated again as a pure strain. On the other hand, the yellow character is difficult to separate from the white, when once intermingled with it, and some of offspring always retain the white character lying dormant in them, which will reappear in succeeding generations.

As to the question, whether this is to be looked upon as a constant rule or not, further series of experiments will show.

Section E.

The fifth generation.

In this generation, we made further breeding from the three classes of the last generation by the usual method.

Class I.

Offspring from the white form.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.	
				White.	Yellow.	Total.		White.	Yellow.
1	1	Nov. 6th	Nov. 16th	951	0	951	—	850	0
2	2	,,	,,						
3	3	,,	,,						

Again, they produce uniform white offspring.

Class II.

Offspring from the yellow parent which produced yellow and white worms in the preceding generations.

(A)

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
					White.	Yellow.	Total.		
No. 3.	yellow.	1	Nov. 6th	Nov. 16th	0	359	359	Dec. 23–24th	yellow only.
		2	,,	17th	0 (23.98%)	323	323	,,	do.
		3	,,	,,	77	244	321	,,	white and yellow.
		4	,,	16th	0	116	116	,,	yellow only.
		5	,,	17th	0	342	342	,,	do.
	white.	6	,,	16th	885	0	0	,,	white only.
		7	,,	,,					
		8	,,	,,					

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. White.	Yellow.	Total.	Date of mounting.	Cocoons.
No. 4	only yellow.	9	Nov. 6th	Nov. 16th	(26.0%) 65	185	250	Dec. 23–24th	white and yellow.
		10	,,	,,	(25.66%) 77	223	300	,,	do.
		11	,,	,,	0	175	175	,,	yellow only.
		12	,,	,,	0	205	205	,,	do.
		13	,,	,,	0	220	220	,,	do.
No. 9	yellow.	14	,,	17th	(24.87%) 50	151	201	,,	white and yellow.
		15	,,	16th	(26.38%) 95	265	360	,,	do.
		16	7th	17th	0	330	330	,,	yellow only.
		17	,,	,,	0	256	256	,,	do.
	white.	18	6th	16th	1,008	0	1,008	,,	white only.
		19	,,	,,					
		20	,,	,,					
No. 10	yellow.	21	7th	17th	0	399	399	,,	only yellow.
		22	,,	,,	(23.06%) 54	180	234	,,	white and yellow.
		23	,,	,,	0	263	263	,,	yellow only.
		24	,,	,,	(19.24%) 71	298	369	,,	white and yellow.
		25	,,	,,	(26.45%) 82	228	310	,,	do.
	white.	26	,,	,,	677	0	677	,,	white only.
		27	,,	,,					
		28	,,	,,					

The phenomenon of segregation of parental characters is just the same as in the last generation. It will be remembered, however, that some of the eggs (Egg Nos. 9—13) were derived from the parents which produced only yellow offspring in the last generation, whilst others were derived from those

which produced mixed offspring (white 25%+yellow 75%). The former is, therefore, an analogous case with those of class III and the latter with those of class II in the preceding generation. Both of them, however, give identical results, in this generation.

(B)

Offspring from the white cocoon-spinners.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
2	1	Nov. 6th	Nov. 16th	380	0	380	—	all white.
3	2	7th	17th	608	0	608	—	do.
4	3	,,	,,					
5	4	,,	,,					
Total.				988	0	988		do.

No coloured offspring was produced.

CLASS III.

Offspring from the yellow parents which produced uniform offspring in the preceding generations.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
1	1	Nov. 6th	Nov. 16th	0	330	330	—	all yellow.
2	2	,,	,,	0	400	400	—	do.
3	3	,,	,,	0	342	342	—	do.
4	4	,,	,,	0	288	288	—	do.
5	5	,,	,,	0	294	294	—	do.
Total.				0	1,654	1,654		do.

The result is just the same as in the last generation.

Section F.

The sixth generation.

In this generation, we kept only two kinds of worms: one, the white cocoon spinners of class I, and the other, the yellow cocoon-spinners derived from the parents which produced mixed offspring in the last generation. (Class II).

Class I.

Offspring of the white cocoon-spinners.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
	5	Jan. 5th	Jan. 18th	146	0	146	Feb. 26–28th	all white.
1, 2, 3.	6	,,	,,	276	0	276	,,	do.
	7	,,	,,	141	0	141	,,	do.
Total.				563	0	563		do.

They were again uniform and constant.

Class II.

Offspring of the yellow cocoon-spinners.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
	1	Jan. 6th	Jan. 19th	(25.86%) 30	86	116	Feb. 29th	white and yellow.
	2	,,	,,	0	89	89	,,	only yellow.
3	3	,,	,,	0	99	99	,,	do.
	4*	7th	,,	(29.74%) 58	137	195	,,	white and yellow.
	5	,,	,,	(21.6%) 31	112	143	,,	do.

The result is just the same as in the last generation.

Section G.

The seventh generation.

In this generation, we reared only the yellow form, which gave rise to two kinds of offspring, white and yellow, in the last generation.

Class II.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.	
				White.	Yellow.	Total.		White.	Yellow.
4	1	March 10th	March 20th	0	241	241	April 20–21st	0	158
	2*	,,	,,	(26.15%) 68	192	260	,,	32	156
	3	,,	,,	(28.17%) 71	181	252	,,	42	166
	4	,,	,,	0	283	283	,,	0	259
	5	,,	,,	0	250	250	,,	0	227

The result is similar to that obtained in the third generation.

Section H.

The eighth generation.

Class II.

Yellow form derived from the parent which produced two kinds of worms.

Parent: age.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. White.	Yellow.	Total.	Date of mounting.	Cocoons.
2	1*	April 29th	May 10th	0	263	263	June 7–8th	only yellow.
	2*	,,	,,	0	319	319	,,	do.
	3*	,,	,,	(25.21%) 30	89	119	,,	white and yellow.
	4*	,,	,,	(24.67%) 76	232	308	,,	do.
	5	30th	,,	(25.67%) 85	226	331	8-9th	do.
	6	,,	,,	(24.54%) 67	206	273	,,	do.

The result is again the same as in the preceding generations.

Section I.

The ninth and further generations.

As a conclusion to these series of experiments we endeavoured to rear all the yellow offspring derived from a single parent, for which purpose we chose the worms Nos. 1, 2, 3 and 4. The former two produced exclusively yellow offspring in the last generation, while the latter two mixed offspring, white and yellow in the usual proportion.

From No. 1 were obtained fifty-five batches of eggs, each representing the offspring from a single parent. Each batch of eggs was, as usual, reared separately in a single basket, and the result confirmed the fact that there was no hybrid, all being pure yellow cocoon spinners.

Those derived from this group, as far as our experiments went, no longer produced any white worms in the succeeding generations. So we may say that they have become a constant breed.

Those of No. 2, on the contrary, produce two kinds of offspring, one giving rise to uniform offspring and the other to mixed offspring (white 25% +yellow 75%). Among the 81 batches of eggs laid—each batch represent-

ing the offspring from a single parent—those[1] laid by sixteen mother-moths (19.75%) produced white and yellow worms in the usual proportion, while the rest (80.25%) gave rise to yellow worms only.

As seen above the parents of these two kinds of worms, Nos. 1 and 2, produced only yellow worms in the last generation; yet the offspring from No. 2 produced two kinds of worms in this generation, while those from No. 1 produced only yellow worms remaining constant through subsequent generations.

Nos. 3 and 4. Both produced mixed offspring in the last generation and we may expect from the facts already obtained that further breeding will only give similar results.

No. 3. The yellow form bred *inter se* laid 16 batches of eggs, of which seven (43.74%) produced mixed offspring in the usual proportion, while the others (56.26%) produced only yellow offspring.

No. 4 gave an analogous result to the preceding. Amongst the fifty-three batches of eggs derived from the yellow form we found twenty-four (45.28%) to give rise to mixed, and the others (54.71%) to uniform, offspring.

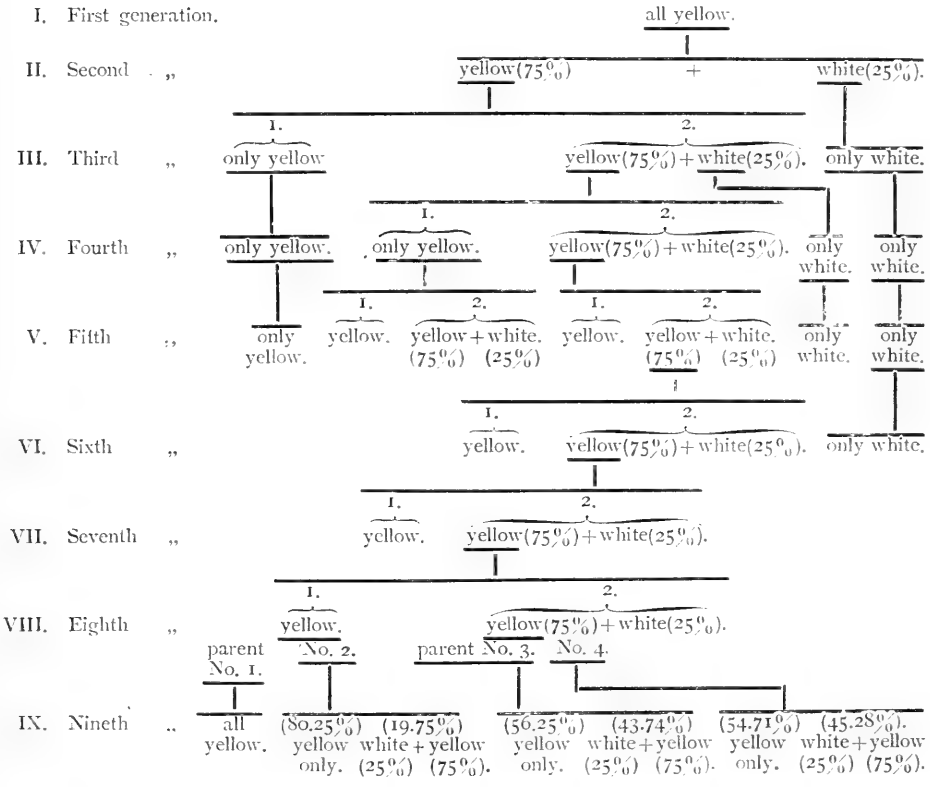
Now we see clearly that in the yellow form once separated as uniform offspring from the hybrid parent, there may be found some apparently yellow or hybrid ones in which the white character lies latent. The proportion of such hybrid forms in the offspring from a single parent is represented, in the former case (No. 2) to be about 20% (hybrid one) and 80% (uniform one), and in the latter (Nos. 3 and 4) about 50% : 50%.

Résumé of the results so far obtained about the hereditary phenomena regarding the two colours of cocoons, the white and the yellow :—

[1] The total number of worms raised from these 16 parents was 3,945, of which 881 (23.3%) were white cocoon-spinners and 3,064 (77.6%) yellow cocoon-spinners.

A PEDIGREE REPRESENTING THE INHERITANCE OF COLOUR (WHITE AND YELLOW) CHARACTERS IN THE HYBRID.

♂ (white) + ♀ (yellow).



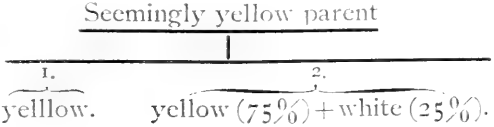
1. Thus in the first cross generation between the yellow and white breeds, the yellow character predominates over the white, and the offspring raised, without any exception, spin yellow-cocoons.

In the second generation paired *inter se*, however, the yellow form breaks up into two forms, each with one of the parental characters, in the proportion of one to three.

As far as our experiments went, the white character, when once separated from the yellow, persists, the yellow form never being produced from it.

With the yellow form it was different. Although they were bred *inter se*, they never produced uniform offspring: Some of them produced only

yellow offspring which remained constant, as in the case of the white ; others produced yellow offspring which, however, split up into the two parental characters in subsequent generations, according to the following scheme :



The proportion of the uniform and the mixed offspring was not constant : in one case we got approximately the ratio 80% : 20%, in another about 55% : 45%.

2. When the white and the yellow characters are brought together by crossing, they are transmitted to the offspring without fusion, but the white may be transmitted in the latent state for a generation or more in the yellow ones without losing its independent quality.

The former may be called recessive and the latter dominant character, after Mendel.

CASE II.

Crosses between Japanese univoltine white and Siamese multivoltine yellow breeds.

SECTION 1.

The first cross generation.

Japanese white (♀) by Siamese yellow (♂).

We obtained six batches of eggs by this cross, each batch representing all the eggs laid by a moth, as in the last case. Most of the eggs laid, however, turned out to be univoltine and did not hatch.

In May, between the 25th and the 28th, 1903, twenty-nine worms hatched out from one of these batches. They spun twelve cocoons, all of which were pure yellow in colour, and spindle shaped.

Thus we may say that in the first cross generation, as in the last series of experiments, the yellow character predominates over the white.

Moths emerged on the 23rd to 26th, June, and laid eggs. Most of them were again univoltine and did not hatch, except those laid by moth No. 3, which furnished the material for experiment on the next generation.

Section 2.

The second generation.

The total number of eggs laid by moth No. 3 was four hundred and twelve, among which there were 218 darkly pigmented eggs (univoltine eggs), 120 light yellow ones (multivoltine eggs) and 74 lightly pigmented ones (some univoltine, some multivoltine).

From these multivoltine worms, the following result was obtained :—

Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.			
		White.	Yellow.	Total.		Yellow.	P. P.* yellow.	Greenish white.	White.
June 25th	July 3–7th	(25.3%) 35	(74.7%) 103	138	July 24–30th	(55.11%) 70	(16.53%) 21	(18.89%) 24	(9.44%) 12

Now we see that the proportion of yellow and white worms well corresponds with the result of the second cross generation of the experiment, case I, and that each kind of worms could be divided into two ; namely in the yellow worms there were yellow and pale-pinkish-yellow cocoon-spinners and among the white worms, white and greenish white cocoon-spinners.

In this case, however, the worms raised were not the whole of the offspring derived from a single parent and consequently we are unable to draw an exact conclusion as regards the mutual relation between those various kinds of worms, yet it is one of the interesting phenomena of heredity, since both parent races hitherto have never spun any pale-pinkish-yellow or greenish white cocoons, as far as we have experimented.

We may, therefore, conclude that as a result of the crossing new characters—which perhaps have lain hidden for ages—appeared in this case.

Section 3.

The third cross generation.

In this generation, we have four kinds of worms derived from the last generation :—

Group A. Those derived from the yellow cocoon spinners.

* P. P. yellow = pale pinkish yellow.

Group B. Those derived from the pale-pinkish yellow cocoon spinners.
Group C. Those derived from the pure white cocoon spinners.
Group D. Those derived from the greenish white cocoon spinners.

Group A.

Offspring of the worms which spun yellow cocoons in the preceding generation.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.			Total.
			White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.	
1	Aug. 3rd	Aug. 12th	58	229	287	Sept. 1–2nd	(22.83%) 50	(56.62%) 124	(20.54%) 45	219
3	4th	13th	0	375	375	2–5th	0	(74.16%) 221	(25.83%) 77	298
4	..	..	98	297	395	1–2nd	(23.07%) 69	(76.92%) 230	0	299
5	..	..	2*	399	401	1–5th	2**	239	51	292
6	5th	..	61	162	223	..	(23.8%) 45	(76.1%) 144	0	189
7	7th	16–17th	50	171	225	4–7th	(23.52%) 44	(76.47%) 143	0	187
8*	..	..	0	319	319	..	0	(74.33%) 223	(25.66%) 77	300
10	9th	18–19th	89	283	372	6–8th	(23.26%) 77	(76.73%) 254	0	331
11	..	..	0	213	213	..	0	181	0	181
12*	7th	15–17th	86	287	373	4–7th	(24.7%) 86	(56.3%) 196	(18.9%) 66	348

In this group, we obtained four different kinds of offspring: the first, those producing only yellow cocoon-spinners; the second, those producing yellow and white cocoon-spinners; the third, yellow and pale-pinkish-yellow cocoon-spinners and the last, those producing yellow, pale-pinkish-yellow and white cocoon-spinners. The respective proportion of the various worms in the offspring is represented in the following :—

** Perhaps mixed from other groups.

The first kind ..... Only yellow cocoon-spinners.

The second kind ...	White cocoon-spinners	235 (23.35%).
	Yellow cocoon-spinners	771 (76.64%).
	Total	1,006 heads.
The third kind	Pale-pinkish-yellow cocoon-spinners	154 (25.75%).
	Yellow cocoon-spinners	444 (74.24%).
	Total	598 heads.
The fourth kind ...	* White cocoon-spinners	136 (23.98%).
	Yellow cocoon-spinners	320 (56.43%).
	Pale-pinkish-yellow cocoon-spinners	111 (19.57%).
	Total	567 heads.

This confirms that in the yellow form the various characters lay dormant.

Group B.

This group of worms was derived from the worms which spun pale-pinkish-yellow cocoons in the last generation.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		White.	P. P. yellow.	Total.
1	Aug. 7th	Aug. 17–18th	29	99	128	Sept. 6–11th	25	85	110
2*	8	,,	33	85	118	6–10th	30	79	108
Total.			(25.2%) 62	(74.7%) 184	246		(25.2%) 55	(74.7%) 164	218

We obtained only pale-pinkish-yellow cocoons and white cocoons, no yellow ones. The respective proportion among the offspring from a single

* Among these, we may distinguish the pure white and the greenish white cocoons.

parent was about 75% of the former and 25% of the latter. It is analogous to a case which we met with in the cross between the white and yellow breeds.

Group C.

These are the descendants of the pure white cocoon-spinners of the last generation.

Eggs.	Date of hatching.	Date of laying eggs.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		White.	Yellow.	Total.
1*	Aug. 7th	Aug. 16th	296	0	296	Sept. 5–8th	268	0	268

All the offspring spun white cocoons.

Group D.

Offspring of worms which spun greenish white cocoons in the preceding generation :—

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.	
			White.	Yellow.	Total.		White and greenish white.	Total.
1*	Aug. 7th	Aug. 15–16th	280	0	280	Sept. 5–12th	198	198
2*	8th	16–17th	450	0	450	8–12th	388	388
3*	,,	17–18th	437	0	437	7–10th	322	322

As the table shows, there are no deep coloured cocoons such as yellow or pale pinkish yellow. On a closer examination, however, we could observe that some of the cocoons were pure white, while the others were light greenish white, and the latter were always much more numerous than the former, but we failed to count them accurately because it was very difficult to distinguish exactly light greenish ones from some spoiled white ones.

SECTION 4.

The fourth generation.

In this generation, we have four kinds of silk-worms as in the last generation :—

Group A. Offspring of group A of the last generation, which may be divided, as already described, into four kinds :—

Kind 1. Worms which spun only yellow cocoons.

Kind 2. Worms which spun yellow and white cocoons.

Kind 3. Worms which spun yellow and pale-pinkish-yellow cocoons.

Kind 4. Worms which spun yellow, pale-pinkish-yellow and white cocoons.

Among these four kinds of worms, the offspring of the third and fourth kinds were kept for experiment on this generation.

Group B. Offspring of group B of the preceding generation, that is, those which spun only pale-pinkish-yellow and white cocoons.

Group C. Offspring of group C of the third generation, that is, those derived from pure white cocoon spinners.

Group D. Offspring of group D of the last generation which spun some white and some greenish white cocoons.

GROUP A.

KIND 3.

Parents : No. 8 of the third generation.

a. Offspring of the yellow form.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.			Total.
			White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.	
1	Sept. 17th	Sept. 26th	0	277	277	Oct. 21–22th	0	(74.8%) Dupion 2 183	(25.1%) 63	250
2	..	..	0	330	330	21–23th	0	(73.35%) D. 6 178	(26.64%) D. 2 65	259
3*	..	..	0	281	281	21–24th	0	212	0	212
4	..	..	0	253	253	20–23th	0	D. 4 223	0	231
5*	..	..	0	290	290	20–22th	0	(77.81%) D. 2 210	(22.18%) 61	275
6	..	..	0	357	357	21–24th	0	(70.4%) D. 5 197	(29.59%) D. 6 75	294
7	..	..	0	298	298	20–22th	0	(74.47%) D. 4 205	(25.52%) D. 1 71	286
8*	..	..	0	407	407	21–23th	0	(74 93%) D. 10 261	(25.06%) D. 4 86	375
9*	..	..	0	366	366	20–23th	0	D. 7 310	0	324
10*	..	..	0	368	368	21–23th	0	(74.16%) D. 13 195	(25.83%) 77	298

Of the offspring from ten parents, those raised from parents No. 3, 4 and 9 spun uniform yellow cocoons, while the remainder, Nos. 1, 2, 5, 6, 7, 8 and 10, two kinds of cocoons, yellow and pale-pinkish-yellow, but no white.

The total number of cocoons spun by these worms was 2,037 in which there are 1,513 (74.27%) of yellow and 524 (25.72%) of pale-pinkish-yellow cocoons.

In the offspring from each parent, nearly the same proportion of yellow and pale-pinkish-yellow cocoons was obtained, as the table shows.

b. Offspring of the pale-pinkish-yellow form.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		White.	P. P. yellow.	Total.
1	Sept. 18th	Sept. 27th	0	316	316	Oct. 22–23rd	0	*D. 1 291	293
2	,,	,,	0	301	301	22–24th	0	D. 2 287	291
3	,,	,,	0	179	179	23rd	0	D. 8 139	155
4	,,	,,	0	206	206	22–23rd	0	D. 2 199	203
5	,,	,,	0	318	318	23–25th	0	D. 5 259	269
6	,,	,,	0	185	185	22–23rd	0	D. 7 163	177
7	,,	,,	0	300	300	,,	0	D. 13 257	283
8	19th	28th	0	334	334	24–26th	0	D. 13 175	201
9	,,	,,	0	312	312	24–27th	0	D. 8 239	255
Total.			0	2,451	2,451		0	D. 59 2,009	2,127

Other batches of eggs laid by nine moths gave similar results, only pale-pinkish-yellow cocoons being obtained.

We see clearly that the hereditary relation between the yellow and the pale-pinkish-yellow character is quite the same as that between the white and the yellow breeds, before mentioned.

Group A.

Kind 4.

Parents: No. 12 of the last generation which spun three kinds of cocoons, yellow, pale-pinkish-yellow and white.

* D. means dupion.

a.

Offspring of the yellow form.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.			
			White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.	Total.
1	Sept. 17th	Sept. 26th	0	323	323	Oct. 20–22nd	0	*D. 9 249	0	263
2*	,,	,,	71	224	295	,,	(24.16%) 58	(58.33%) 140	(17.5%) 42	240
3	,,	,,	0	307	307	21–22nd	0	D. 14 187	0	215
4	,,	,,	0	308	308	20–22nd	0	(76.30%) D. 12 195	(23.69%) D. 4 60	287
5	,,	,,	0	277	277	,,	0	(79.54%) D. 4 202	(20.45%) D. 3 48	264
6	,,	,,	77	264	341	,,	(20.59%) D. 1 60	(62.12%) D. 10 167	(17.27%) D. 2 48	301
7	,,	,,	0	365	365	21–22nd	0	(74.76%) D. 20 203	(25.23%) D. 14 54	325
8	,,	,,	0	328	328	21–23rd	0	D. 32 151	(27.11%) D. 9 58	291
9	18th	27th	2	289	291	22–24th	2	D. 3 185	(22.49%) D. 2 52	249
10	,,	,,	0	426	426	,,	0	D. 5 258	(24.5%) D. 5 77	355
11*	17th	26th	83	229	312	20–22nd	(25.%) D. 2 66	(75.%) D. 11 188	0	280
12*	,,	,,	0	299	299	21–22nd	0	D. 10 158	0	178
13	,,	,,	75	221	296	20–22nd	(18.75%) D. 1 46	(60.93%) D. 28 100	(20.31%) D. 6 40	256

* D. means dupion.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.			
			White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.	Total.
14*	Sept. 17th	Sept. 26th	78	260	338	Oct. 20–22nd	(21.29%) D. 2 52	(78.70%) D. 15 177	0	263
15	,,	,,	0	315	315	,,	0	D. 16 185	D. 5 59	286
16	,,	,,	88	264	352	,,	(25.11%) 58	(62.33%) 144	(12.55%) 29	231
17*	18th	27th	0	384	384	23–24th	0	D. 13 231	0	257
18	,,	,,	0	394	394	22–23rd	0	D. 20 236	(26.20%) D. 2 94	374
19	,,	,,	0	396	396	23–25th	0	D. 22 281	0	325
20	,,	,,	70	241	311	22–23rd	(20.25%) D. 1 56	(79.74%) D. 15 198	0	286

We again meet with the same phenomenon of the separation of the parental characters as that displayed by the worms, group A in the third generation, that is to say,

(1) Some (Nos. 1, 3, 12, 17 and 19) of the parents producing only the worms which spin yellow cocoons only ;

(2) Some (Nos. 11, 14 and 20) producing the worms spinning yellow and white cocoons, the total number of cocoons spun being 829, in which

the white	184 (22.19%)
the yellow	645 (77.80%) ;

(3) Some (Nos. 4, 5, 7, 8, 9, 10, 15 and 18) producing two kinds of worms, one spinning yellow cocoons and another spinning pale-pinkish-yellow cocoons, the total number of cocoons being 2,429, of which we find

the pale-pinkish-yellow	590 (24.28%)
the yellow	1,839 (75.71%)

and (4) the remaining (Nos. 2, 6, 13 and 16) spinning three kinds of cocoons, white, pale-pinkish-yellow and yellow. The total number was 1,028, in which there are

* the white	226 (21.98%)
the yellow	627 (60.99%)
the pale-pinkish-yellow	175 (17.02%).

β.

Offspring of the pale-pinkish-yellow form.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		White.	P. P. yellow.	Total.
1*	Sept. 16th	Sept. 25–26th	(23.63%) 82	265	347	Oct. 18–20th	D. 3 61	D. 13 214	307
2*	,,	,,	(20.87%) 43	163	206	18–19th	D. 2 39	D. 12 125	192
3*	,,	,,	(26.19%) 66	186	252	19–21st	35	D. 17 161	230
4*	,,	,,	(22.07%) 74	261	335	18–19th	D. 3 57	D. 22 207	314
5*	17th	26–27th	0	294	294	20–22nd	0	D. 12 221	245
6*	,,	,,	(25.%) 65	195	260	,,	D. 1 50	D. 16 146	230
7*	,,	,,	0	290	290	,,	0	D. 21 229	271
8*	19th	28–29th	(23.37%) 78	257	385	24–26th	53	D. 32 188	305
9*	,,	,,	0	265	265	23–25th	0	D. 9 187	205
10*	,,	,,	(20.76%) 81	309	390	23–26th	D. 4 62	D. 23 238	354

* It must be remembered, however, that among the white, there are some having light greenish shade, as in the case of the last generation.

Of the ten parents, Nos. 1—4, 6, 8 and 10 produced mixed offspring (white 25%+p. p. yellow 75%), while the others produced uniform pale-pinkish-yellow offspring.

The total number of the worms raised from the former group is 2,125, of which 489 (23.%) belonged to the white cocoon-spinners and the remaining 1,636 (76.%) to the pale-pinkish-yellow cocoon-spinners.

This phenomenon of heredity is quite the same as that observed in the case of the cross between the white and the yellow.

γ.

Offspring of the white form.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		white.	Yellow.	Total.
1	Sept. 16th	Sept. 25–26th	319	0	319	Oct. 18–20th	*D. 19 253	0	291
2	,,	,,	270	0	270	18–19th	D. 10 233	0	253
3	,,	,,	281	0	281	,,	D. 6 203	0	215
4	18th	27th	384	0	384	22–23rd			
5	,,	,,	275	0	275	,,	D. 40 773	0	853
6	,,	,,	338	0	338	,,			

No colored cocoons were produced.

GROUP B.

This is the third generation of the pale-pinkish-yellow form which diverged from the yellow form of the first generation.

Parents : No. 2, group B of the preceding generation.

* D=dupions.

1. Offspring of the pale-pinkish-yellow form.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		White.	P. P. yellow.	Total.
1*	Sept. 17th	Sept. 26th	0	277	277	Oct. 20–22nd	0	D. 9 237	
2*	18th	27th	(24.19%) 90	282	372	20–23rd	68	D. 13 236	
3*	,,	,,	(26.22%) 96	270	366	,,	D. 2 76	D. 13 219	
4*	19th	28th	0	411	411	23–25th	0	D. 11 232	
5	,,	,,	0	397	397	,,	0	D. 33 296	
6	,,	,,	0	250	250	,,	0	D. 3 198	
7	,,	,,	(27.82%) 69	179	248	,,	62	D. 6 161	
8	,,	,,	(24.77%) 111	337	448	,,	D. 2 91	D. 7 224	
9	20th	29th	0	406	406	25–28th	0	D. 17 283	

The offspring of some parents (Nos. 1, 4, 5, 6 and 9) spun only one kind of cocoons while the others (Nos. 2, 3, 7 and 8) two kinds, the white and the pale-pinkish-yellow, but no pure yellow cocoons. The total number of worms belonging to the latter kind was 1,434, of which 366 (25.52%) belonged to the white cocoon spinners, while the remaining 1,068 (74.47%) were pale-pinkish-yellow cocoon-spinners.

Thus we again meet with the same hereditary phenomena as those displayed by the crossing between the white and the yellow breeds.

2. Offspring of the white form.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		White.	Yellow.	Total.
1*	Sept. 19th	Sept. 28th	372	0	372	Oct. 22–26th	D. 8 234	0	250
2*	,,	,,	400	0	400	,,	D. 14 297	0	325
3	,,	,,	258	0	258	22–24th	—	—	—
4	,,	,,	347	0	347	23–26th	D. 23 231	0	277
5	,,	,,	396	0	396	,,	D. 32 246	0	310
Total.			1,773	0	1,773		D. 77 1,008	0	1,162

No colored cocoon, the result being a repetition of that of the last generation.

Group C.

Offspring of the white form, the group C of the last generation.

Parent: No. 1.

We reared the worms derived from the eggs laid by thirty-two parent-moths and obtained 4,511 cocoons, which, without any exception were pure white in color.

Group D.

Offspring of the light greenish-white form.

Parents: Nos. 1, 2 and 3 of the last generation.

As in the case of group C, we reared worms derived from 43 parent-moths in common baskets without discriminating the worms from different

parents, and obtained 7,501 cocoons. There were no yellow or pale-pinkish-yellow cocoons. On a closer examination, however, we could distinguish, as in the case of the last generation, some pure white cocoons among the light greenish-white ones. Since, an exact discrimination between these cocoons is connected with some difficulty as already mentioned, we have discontinued this series of experiments.

Section 5.

The fifth generation.

In this generation, we kept only offspring derived from group A, kind 3 and 4 and from group B for experiment. Thus the eggs kept for experiment on this generation were follows :—

Group A.

Kind 3.

a. Offspring of the yellow form which produced the yellow and the pale-pinkish-yellow cocoon-spinners in the preceding generations.

b. Offspring of the pale-pinkish-yellow form originally derived from the yellow form.

Kind 4.

a. Offspring of the breed which had four kinds of offspring in the preceding generation.

β. Offspring of the pale-pinkish-yellow form which spun two kinds (the pinkish-yellow and the white cocoons) of cocoons in the preceding generations.

γ. Offspring of the white form.

Group B.

Offspring of the pale-pinkish-yellow form which separated themselves from the yellow form in the second generation. Since then, they spun only the white and the pale-pinkish-yellow cocoons but no pure yellow cocoon.

1. Offspring of the pale-pinkish-yellow form.
2. Offspring of the white form.

GROUP A.

KIND 3.

(*a*).

1. The parents: No. 3 of the fourth generation which spun only yellow cocoons.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.
1*	Oct. 31st	Nov. 9th	0	289	289	Dec. 13–14th	0	D. 10 244	0
2	Nov. 1st	11th	0	257	257	15–16th	0	all yellow.	0
3	2nd	12th	0	265	265	15–17th	0	,,	0
4	,,	13th	0	311	311	19–23rd	0	D. 3 146	0
5	,,	,,	0	341	341	19–21st	0	D. 1 163	0
Total.			0	1,463	1,463		0		0

Among one thousand four hundred and sixty-three worms raised from the eggs laid by five moths there were no pale-pinkish-yellow cocoons that is to say, they exhibited perfectly uniform yellow character.

2. The parents: No. 9 of the fourth generation which also spun only yellow cocoons in the last generation.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
			White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.
1	Oct. 1st	Nov. 11th	0	270	270	Dec. 15–16th	0	D. 5 152	D. 6 60
2	,,	,,	0	308	308	,,	0	D. 18 228	0
3	2nd	12th	0	248	248	16–17th	0	all yellow.	0
4	,,	,,	0	245	245	,,	0	,,	0
5	,,	,,	0	217	217	,,	0	,,	0

Among the offspring raised from the five parent-moths, those from four parents spun yellow cocoons, while the rest (those from No. 1) spun two kinds of cocoons, yellow and pale-pinkish-yellow, in the proportion of 70 to 30% respectively.

3. The parents : No. 5 of the preceding generation which spun both yellow and pale-pinkish-yellow cocoons.

	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
				White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.
Offspring of the yellow.	1	Oct. 31st	Nov. 10th	0	297	297	Dec. 13–14th	0	D. 7 165	0
	2	Nov. 1st	11th	9	249	249	14–16th	0	some yellow, some pale-pinkish-yellow, number missed.	
	3	"	"	0	288	288	"	0	D. 14 214	0
Offspring of the P. P. yellow.	1	"	"	0	301	301	"	0	0	D. 6 225
	2	"	"	0	136	136	"	0	0	D. 4 123

4. The parents : No. 8 of the preceding generation which, as in those of No. 9, produced two kinds of worms, the yellow and the pale-pinkish-yellow cocoon-spinners.

	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
				White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.
P. P. yellow.	3	Oct. 31st	Nov. 9th	0	353	353	Dec. 12–13th	0	0	D. 22 274
	5	Nov. 1st	11th	0	282	282	15–17th	0	0	D. 7 223
Yellow.	9*	2nd	12th	0	371	371	18–21st	0	D. 8 176	D. 3 59
	10	"	"	0	257	257	"	0	D. 6 99	D. 1 26

5. The parents: No. 10 of the last generation, which as in the case of the preceding two series, produced two kinds of worms, the yellow and the pale-pinkish-yellow cocoon-spinners.

Eggs.		Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
				White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.
Pale-pinkish-yellow form.	1	Oct. 1st	Oct. 11th	0	317	317	Dec. 14–16th	0	0	D. 4 262
	2	2nd	12th	0	242	242	15–17th	0	0	D. 3 202
	3	,,	,,	0	279	279	,,	0	0	D. 1 254
	4	,,	13th	0	307	307	19–21st	0	0	D. 8 209
Yellow form.	1	2nd	11th	0	250	250	—	0	yellow only, number missed.	0
	2*	,,	12th	0	274	274	—	0	D. 6 146	0
	3	,,	,,	0	271	271	—	0	D. 6 130	0
	4	,,	,,	0	273	273	—	0	some yellow, some pale-pinkish-yellow, number missed.	

As we see, the first two series of worms (Nos. 1 and 2) were descended from the parents which produced uniform yellow offspring in the last generation and the other three series (Nos. 3, 4 and 5) came from the parents which produced mixed offspring (yellow 75% + pale-pinkish-yellow 25%), yet the worms raised from one of the former series spun mixed cocoons, while those from another spun uniform yellow cocoons. All those of the latter series spun mixed cocoons (yellow 75% and pale-pinkish-yellow 25%), as their yellow parents did in the last generation.

Quite contrary to the yellow worms those reared from the pale-pinkish-yellow forms exhibited uniform parent character.

(*b*).

Offspring of the pale-pinkish-yellow form, which diverged from the yellow form in the last generation.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
			White.	Yellow.	Total.	
1*	Nov. 3rd	Nov. 13th	0	335	335	all pale-pinkish-yellow.
2*	2nd	12th	0	289	289	do.
3*	3rd	13th	0	253	253	do.
4*	,,	,,	0	269	269	do.
5*	,,	,,	0	255	255	do.
Total.			0	1,401	1,401	do.

Uniform offspring. No other parent-characters appeared.

GROUP A.

KIND 4.

a.

(*a*).

Parents: No. 2 of the last generation, which spun three kinds of cocoons, yellow, pale-pinkish-yellow and white.

	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
				White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.
White parents.	1	Oct. 31st	Nov. 10th	1,185	0	1,185	Dec. 13–14th	white without any exception.		
	2	,,	,,							
	3	,,	,,							
	4	,,	,,							
	5	,,	,,							

	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
				White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.
P. P. yellow parents.	1	Oct. 31st	Nov. 10th	(27.31%) 62	165	227	Dec. 12–13th	D. 1 45	0	D. 7 122
	2	,,	,,	(24.71%) 64	195	259	13–14th	D. 1 54	0	D. 15 163
	3	,,	,,	(23.79%) 69	221	290	,,	D. 3 49	0	D. 14 171
	4	,,	,,	(23.07%) 60	200	260	,,	D. 1 41	0	D. 16 162
	5	,,	,,	0	226	226	,,	0	0	D. 4 154
Yellow parents.	1	,,	9th	0	322	322	12–13th	0	D. 9 202	(19.11%) D. 1 50
	2*	,,	,,	(20.52%) 71	275	346	,,	(15.92%) D. 2 43	D. 18 187	0
	3	,,	10th	0	217	217	,,	0	D. 9 128	(22.91%) D. 1 44
	4*	Nov. 1st	11th	0	253	253	14–15th	0	D. 7 99	0
	5	,,	,,	0	285	285	,,	0	D. 11 197	0

1. The worms reared from the white parents again spun no colored cocoons, as in the case of the other white breeds derived from the colored forms.

2. Those derived from the pale-pinkish-yellow parents produced two forms, one, spinning two kinds of cocoons, the white and the pinkish-yellow, their respective proportion being 25% and 75% and the other, spinning only pale-pinkish-yellow cocoons.

3. The third or those derived from the yellow parents produced three kinds of offspring :—

a. Those (Nos. 1 and 3) producing two kinds of worms, one spinning yellow and the other pale-pinkish-yellow cocoons.

b. Those (No. 2) producing white and yellow forms.

c. Those (Nos. 4 and 5) producing only yellow forms.

In this generation there were none of that kind which produced three kinds of worms, the yellow, the pale-pinkish-yellow and the white cocoon-spinners in the offspring from the same parent.

(*b*).

Offspring of the parents which spun two kinds of cocoons, the yellow and the white, in the preceding generation.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
					White.	Yellow.	Total.		
No. 14.	Yellow.	1	Oct. 31st	Nov. 9th	0 (26.44%)	284	284	Dec. 12–13th	all yellow.
		2	Nov. 2nd	11th	73	203	276	15–16th	white and yellow, number missed.
	White.	1	Oct. 31st	9th	472	0	472	12–13th	all white.
		2	,,	,,					
No. 11.	Yellow.	1	,,	,,	0	264	264	,,	all yellow.
		2	,,	,,	0	302	302	,,	do.
		3	,,	,,	0	346	346	,,	do.
		4	,,	,,	0	315	315	,,	do.
		5	,,	,,	0	326	326	,,	do.
	White.	1	,,	,,	274	0	274	,,	all white.
		2	Nov. 1st	10th	1,313	0	1,313	13–14th	do.
		3	,,	,,					
		4	,,	,,					
		5	,,	,,					

In this case, the offspring from the yellow parent No. 14 produced two kinds of worms, as is usual, while those from No. 11 produced only one kind, spinning yellow cocoons only.

Those descended from the white ones spin again white cocoons in both cases.

(*c*).

Offspring from the worms which spun only yellow cocoons in the last generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
No. 12. Yellow only.	1	Oct. 31st	Nov. 9th	66	1,230	1,296	Dec. 12–13th	white and yellow, number missed
	2	,,	,,					
	3	,,	,,					
	4	,,	,,					
	5	,,	,,					
No. 12. Yellow.	1	Nov. 2nd	11th	82	442	524	15–16th	do.
	2	,,	,,					
	3	3rd	12th	0	645	645	16–18th	yellow only, number missed.
	4	,,	,,					

They exhibited only the yellow character in the last generation, yet some of their offspring in this generation, as we see, again split up into two forms, the white and the yellow.

In this series of experiments, however, we reared the worms descended from different mother-moths indiscriminately in one or two baskets, so we could not exactly determine which mother-moth produced mixed offspring, but from the experience hitherto obtained, we have good reason to believe that, in both cases one of the mother-moths produced mixed offspring, and consequently the respective percentage of the two forms in the offspring from one parent being about 25% and 75%, thus exactly corresponding with the results obtained in the series of the experiment, Case I and also of the preceding series.

We shall, therefore, discontinue this and the preceding series of experiments in the next generation.

β.

Offspring derived from the pale-pinkish-yellow form.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
					White.	Yellow.	Total.		
No. 1.	White.	1	Oct. 28th	Nov. 6th	361	0	361	Dec. 5–7th	all white.
	Pale-pinkish-yellow.	2*	,,	,,	(25.25%) 54	159	213	,,	some white, some pale-pinkish-yellow.
No. 2.	W.	3	,,	,,	310	0	310	,,	all white.
	P.P.Y.	4	,,	,,	(23.35%) 78	256	334	,,	white and pale-pinkish-yellow.
No. 3.	W.	5	30th	9th	349	0	349	8–10th	white only.
	P.P.Y.	6	,,	,,	(30.27%) 99	228	327	,,	white and P. P. Y., number missed.
No. 4.	W.	7	28th	6th	390	0	390	5–7th	white only.
	P.P.Y.	8	,,	,,	0	274	274	,,	P. P. Y. only.
		9	29th	7th	0	247	247	6–8th	do.
No. 6.	W.	13	31st	10th	1,171	0	1,171	9–11th	white only.
		14							
		15							
		16							
	P.P.Y.	17*	,,	9th	(24.13%) 70	220	290	12–13th	white and P. P. Y.
		18	,,	10th	0	225	225	,,	P. P. Y. only.
		19	,,	,,	0	283	283	,,	do.
		20	,,	,,	0	252	252	,,	do.
No. 8.	W.	26	Nov. 5th	13th	424	0	424	15–16th	white only.
		27	,,	,,					
	P.P.Y.	24	,,	15th	0	337	337	18–20th	all P. P. Y.
		25	,,	,,	0	363	363	,,	do.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. White.	Yellow.	Total.	Date of mounting.	Cocoons.
No. 10.	W.	33	Nov. 3rd	Nov. 13th	391	0	391	Dec. 15–17th	all white.
		34	..	..					
	P.P.Y.	30	..	12th	0	295	295	12–15th	all P. P. Y.
		31	..	13th	0	257	257	13–15th	do.
		32	4th	14th	0	325	325	14–16th	do.
No. 7.*	P. P. Y.	21	Oct. 31st	10th	0	754	754	13–14th	all P. P. Y.
		22	..	..					
		23	..	..					
No. 9.	P. P. Y.	28	Nov. 3rd	13th	0	584	584	15–16th	do.
		29	..	..					

We have become so familiar with these phenomena of heredity in the preceding series of experiments that any further explanation about them seems to be superfluous.

7.

Offspring of the white form.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. White.	Yellow.	Total.	Date of mounting.	Cocoons.
1	1	Oct. 29th	Nov. 8th	335	0	335	Dec. 5–7th	all white.
2	3	28th	7th	438	0	438	7–9th	do.
	4							
3	2	..	6th	865	0	865	5–8th	do.
	5							
	6							
Total.			..	1,638	0	1,638		do.

Again white offspring.

Group B.

Offspring of the pale-pinkish-yellow form which diverged from the yellow parents in the second crossed generation.

(1)

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
					White.	Yellow.	Total.		
3	White.	1	Nov. 1st	Nov. 11th	1,094*	0	1,094	Dec. 15–16th	all white.
		2	,,	,,					
		3	,,	,,					
		4	,,	,,					
	Pale-pinkish-yellow.	5	2nd	12th	0	287	287	16–17th	all P. P. yellow.
		6	,,	,,	0 (25.87%)	308	308	,,	do.
		7*	,,	,,	74 (25%)	212	286	,,	white and P. P. Y.
		8*	,,	,,	75	225	300	,,	do.
		9	,,	,,	0	376	376	,,	all P. P. yellow.
2	White.	10	,,	,,	603	0	603	17–18th	all white.
		11	,,	,,					
		12	3rd	13th	330	0	330	18–20th	do.
	P. P. yellow.	13	2nd	12th	(26.96%) 72 (25.64%)	195	267	17–18th	white and P. P. Y.
		14	,,	,,	80	232	312	,,	do.
		15	3rd	13th	0	331	331	18–19th	P. P. yellow only.
4*	Pale-pinkish-yellow.	16	,,	,,	0	786	786	19–23rd	all P. P. yellow.
		17	,,	,,					
		18	,,	,,					
		19	,,	14th	0	584	584	,,	do.
		20	4th	,,					

* Compare Group A, Kind 4, *a. c.* last generation.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. White.	Yellow.	Total.	Date of mounting.	Cocoons.
I	Pale-pinkish-yellow.	21	Oct. 31st	Oct. 10th	72*	435	507	Dec. 14–16th	white and P. P. yellow, number missed.
		22	,,	,,					
		23	Nov. 1st	11th	64*	518	582	15–16th	do.
		24	,,	,,					

We see clearly now that the hereditary phenomena displayed by this group correspond very well to those shown by the cross between the white and the yellow breeds in the fourth or further generations (see Case I).

(2)

Offspring from the white form which diverged from the pale-pinkish-yellow parent in the third generation.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. White.	Yellow.	Total.	Date of mounting.	Cocoons.
I.	♂	2	Nov. 2nd	Nov. 12th	953	0	953	Dec. 18–19th	white only.
		4	,,	,,					
		5	,,	,,					
II.*	♂	2	3rd	13th	2,375	0	2,375		all white.
		3	,,	,,					
		4	,,	,,					
		5	,,	,,					
		6	,,	,,					
		7	,,	,,					
		8	4th	,,					

Quite familiar phenomenon observed in other white forms.

Section 6.

The sixth generation.

In this generation, we kept the following kinds of worms for experiment :—

1. Group A.

Kind 3.

a.

1. Offspring from the yellow form which spun only one kind of cocoons, that is to say, the yellow.

2. Offspring of the yellow form which spun two kinds of cocoons, the yellow and the pale-pinkish-yellow.

b. Offspring of the pale-pinkish-yellow form which spun only pale-pinkish-yellow cocoons.

Kind 4.

a.

a. Offspring of the yellow form which produced three kinds of worms in the fourth and fifth gererations. We kept now only the yellow form which produced (1) both the white and the yellow forms and (2) the yellow form only in the offspring from a parent.

b. Was no longer reared.

c. „ „ „ „

β. In this group there were two kinds, one spinning only (1) pale-pinkish-yellow cocoons and the other (2) two kinds of cocoons, the pale-pinkish-yellow and the white.

γ. Offspring of the white form.

2. Group B.

Offspring of the pale-pinkish-yellow form which diverged from the yellow parent in the second generation.

GROUP A.

KIND 3.

(*a*).

1. Offspring of the yellow form which spun only one kind of cocoons, the yellow, in the last generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
				White.	Yellow.	Total.		White.	Yellow.	Total.
	1	Dec. 25th	Jan. 8th	0	157	157	Feb. 13–14th	0	D. 1 112	114
1	2	27th	10th	0	123	123	16–17th	0	D. 1 78	80
	3	28th	,,	0	146	146	,,	0	94	94

Again produced uniform offspring.

2. Offspring of the yellow form which spun yellow and pale-pinkish-yellow cocoons in the last generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.		
				White.	Yellow.	Total.		White.	Yellow.	P. P. yellow.
S. 9.	1	Jan. 5th	Jan. 17th	0	221	221	Feb. 25–28th	0	77	24
	2	3rd	16th	0	188	188	21–22nd	0	D. 3 81	D. 1 24
	3	,,	15th	0	203	203	20–22nd	0	D. 1 120	0
10. 2.	4	4th	17th	0	143	143	25–28th	0	57	19
	5	,,	,,	0	83	83	26–28th	0	32	8
	6	,,	,,	0	220	220	,,	0	94	0

A repetition of the same phenomenon as observed in the preceding generations.

b).

Offspring of the pale-pinkish-yellow form.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of monnting.	Cocoons.
				White.	Yellow.	Total.		
	..	Jan. 1st	Jan. 14th	0	162	162	Feb. 20–23rd	all P. P. yellow.
1. 2. 3.	..	..	,,	0	198	198	..	do.
	..	..	..	0	224	224	..	do.

Quite the same as in the preceding generations.

KIND 4.

a.

a.

1. Offspring of the yellow form which spun yellow and white cocoons in the last generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.	
				White.	Yellow.	Total.		White.	Yellow.
2.	1	Dec. 29th	Jan. 11th	(25.75%) 60	173	233	Feb. 18–19th	D. 1 21	D. 3 62
	2	..	..	0	66	66	..	all yellow.	

2. Offspring of the yellow form which spun only yellow cocoons in the last generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
2.	1	Dec. 26th	Jan. 8th	0	236	236	Feb. 13–16th	all yellow.
	2	..	..	0	213	213	..	do.
	3	..	,,	0	255	255	..	do.

Quite the same phenomenon as observed in the case of the crossing between the white and the yellow breeds.

β.

Offspring of the pale-pinkish-yellow form.

1. Those derived from the parent which produced two kinds of worms.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
					White.	Yellow.	Total.		
1. 2.	P. P. yellow.	1	Dec. 18th	Jan. 2nd	0	230	230	Feb. 5–6th	P. P. yellow only.
		2	..	..	0	230	230	..	do.
		3	..	..	69 (27.6%)	181	250	..	white and P. P. yellow.
	White.	4	..	..	352	0	352	..	white only.
		5	..	..					
6. 17.	P. P. yellow.	1	25th	8th	0	197	197	13–14th	all P. P. yellow.
		2	26th	..	0	278	278	..	do.
		3	..	..	0	271	271	..	do.

2. Those derived from the pale-pinkish-yellow form which spun only one kind of cocoons.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
No. 7. 21.	1	Dec. 27th	Jan. 9th	0	207	207	Feb. 13–14th	all P. P. yellow.
	2	..	..	0	241	241	..	do.
	3	..	..	0	194	194	..	do.

The same phenomenon as repeatedly observed in the preceding generations.

7.

Offspring of the white-form.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
1 and 2	1	Dec. 17th	Dec. 31st	539	0	539	Janu. 28–30th	all white.
	2	,,	,,					

They have retained uniform character from their first production.

Group B.

Offspring from the pale-pinkish-yellow form descended from the yellow parent in the second generation.

(1).

a. These spun two kinds of cocoons, pale-pinkish-yellow and white, in the last generation. We reared only the former in this generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
3	1	Jan. 1st	Jan. 14th	0 (24.65%)	127	127	Feb. 19–21st	all P. P. yellow.
	2	,,	,,	36	110	146	,,	white and P. P. yellow.

b. These are descended from the parent which produced one kind of worms in the last generation, that is to say, the pale-pinkish-yellow.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
4	1	Jan. 5th	Jan. 18th	0	274	274	Feb. 24–25th	all P. P. yellow.
	2	,,	17th	0	201	201	,,	do.
	3	,,	18th	0	250	250	,,	do.

Such instances have been met with many times in the preceding generations.

(2).

Offspring of the white form.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
			White.	Yellow.	Total.		
1	Jan. 1st	Jan. 14th					
2	..	..	783	0	783	Feb. 19–21st	all white.
3	..	..					

Again uniform offspring.

Summary.

Now we will sum up the hereditary phenomena of the various colours of the cocoons observed in this series of experiments, as follows :—

When we crossed a female moth of a Japanese univoltine white breed, called *Aobiki* with a male moth of the Siamese multivoltine yellow breed, the offspring of the first generation spun yellow cocoons without any exception, that is to say, the yellow character predominates over the white.

In the next generation paired *inter se* (the second cross generation), the yellow form splits up into four different characters, the yellow, the white, the pale-pinkish-yellow and the light greenish-white, the latter two characters being quite new.

Next we will describe separately the hereditary phenomena displayed by each of those forms produced in the second cross generation in the following order :—

I. The yellow form.
II. The pale-pinkish-yellow form.
III. The greenish white form.
IV. The white form.

I. The yellow form.

The yellow form paired among themselves produces four different kinds of offspring, that is to say, the first kind spinning only yellow cocoons, the second kind, the yellow (75%) and the white (25%), the third, the yellow (75%) and the pale-pinkish-yellow (25%), and lastly the fourth, the yellow (56.2%), the white[1] (24.65%) and the pale-pinkish-yellow (19.13%).

a. With the first and the second kind, we have every reason to believe that they will follow the same law which we repeatedly observed in the case of crossing between the white and the yellow breeds. So we have discontinued further experiments.

b. In the third kind, the yellow form produces some uniform (the yellow only) and some mixed [the yellow (75%) and the pale-pinkish-yellow (25%) in the offspring from a parent] offspring in the next generation; the yellow offspring separated from both yellow forms again exhibit the same cycle of hereditary phenomena in subsequent generations.

The pale-pinkish-yellow form, on the contrary, when once separated from the yellow form remains constant throughout.

Thus the relation between the yellow and the pale-pinkish-yellow forms is the same as that between the yellow and the white characters.

c. In the fourth kind, (1) the yellow form again produces four kinds of offspring, but in the next generation, we have missed that kind which produces three kinds of worms, the yellow, the pale-pinkish-yellow and the white, probably owing to the scantiness of the worms reared for experiment.

(2) The pale-pinkish-yellow form splits up into the white and the pale-pinkish-yellow, displaying quite the same phenomena of heredity as those exhibited by the white and the yellow.

(3) The white form, on the contrary, produces always uniform offspring through subsequent generations.

II. The pale-pinkish-yellow form.

This form yields two kinds (the white 25% and the pale-pinkish-yellow 75%) of offspring, in the next generation.

[1] In this, as already described, some greenish-white ones are contained.

The white form thus raised remains constant in colour generation after generation, while the pale-pinkish-yellow form produces some uniform (producing only the pale-pinkish-yellow) and some mixed (the white 25% and the pale-pinkish-yellow 75%) offspring in each succeeding generation.

III. The greenish-white form.

In this form, as in the preceding, we have two kinds of worms, one spinning pure white cocoons and other light-greenish-white cocoons, in the next generation. Although we failed to count the number of both forms, yet we have good reason to believe that they will follow the same law governing the hereditary phenomena of the other colours mentioned above.

IV. The white form.

Not only this, but every white form since from its first production remains true to itself, as far as our experiments have gone.

Thus we come to the conclusion that as a result of crossing, the yellow form splits up into four different forms, the pure yellow, the pale-pinkish-yellow, the white and the greenish-white.

Among the four characters thus enumerated, the yellow is predominant over the others; next comes in succession, the pale-pinkish-yellow, the greenish-white and the white, that is to say, the white is absolutely recessive in this case. When these four characters are combined by crossing, the yellow appears as an active character in the offspring, while the others become latent. On the contrary, when the other three characters are combined, the pale-pinkish-yellow one becomes active character and the other two latent, and finally in the case of the greenish-white and the white ones, the former is active and the latter latent. The latent character, however, becomes active in the next generation, occupying one-fourth of the whole number of the offspring from a single parent.

The relation of these four characters to one another, therefore, is quite constant, displaying always the same phenomenon in successive generations, which may be summarised as follows :—

A = dominant character.

B = recessive character.

The first cross generation (A + B) Offspring A only.

The second cross generation Offspring A (75%) + B* (25%).

The third cross generation
- From A
  - 1. Offspring A only. = A^1.
  - 2. Offspring A (75%) = A^2 + B* (25%).
- From B Offspring B* only.

The fourth cross generation
- From A^1
  - 1. Offspring A only.
  - 2. Offspring A (75%) + B* (25%).
- From A^2
  - 1. Offspring A only.
  - 2. Offspring A (75%) + B* (25%).
- From B Offspring B* only.

If we mingle together four different characters,

A, B, C, and D, we have

in the first cross generation, A only = Dominant.

in the second cross generation,
- A. (56.43%).
- B. (19.57%).
- C + D† (23.98%).

in the third cross generation,
- From A.
  - A. (60.99%).
  - B. (17.02%).
  - C + D† (21.98%).
- From B.
  - B. (75%).
  - D. (25%).
- From C.
  - C. (75%).
  - D. (25%).
- From D. D. only.

and so on, in this way.

Lastly, it must be mentioned here that in each of these four forms mentioned above, there may be found some intermediate forms, or better, some varieties. For instance, among the yellow form, some are deep yellow,

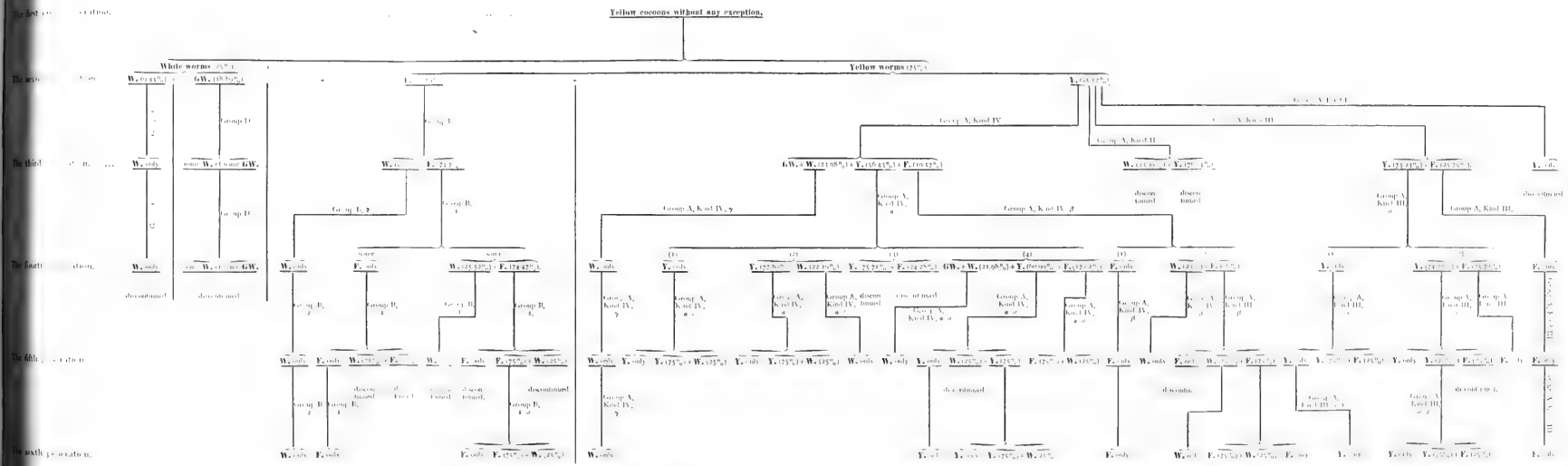
* These remain true to itself.

† We failed to separate them, as already described.

A [illegible] representing the inheritance of the various colours of cocoons during [illegible] of six generations:—

CROSSING BETWEEN JAPANESE UNIVOLTINE WHITE (♀) AND SIAMESE MULTIVOLTINE YELLOW (♂) BREEDS.

W_1 = white cocoon; Y_1 = yellow cocoon; GW_1 = greenish white cocoon; F_1 = pale-pinkish-yellow or flesh coloured cocoon



while others light yellow and between these two extreme forms plenty intermediate forms which can not be exactly distinguished from one another may be found. Any one of them, however, will follow the same law mentioned before. And consequently, if we select the lightest colored from more deeply coloured ones, we can get a light coloured new form which again display the same hereditary phenomena observed in other forms. We have observed plenty of such instances during our experiments.

CASE III.

We will give another instance of crossing between the Japanse white and the Siamese yellow breeds.

SECTION I.

The first generation.

SERIES 1.

a. { Japanese divoltine white breed, '*Igata*' ♀.
{ Siamese multivoltine yellow breed ♂.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
			White.	Yellow.	Total.		
1	Feb. 8th	Feb. 17th	0	71	71	March 9–12th	all yellow.
2	..	..	0	98	98	..	do.
Total.			0	169	169		do.

b. { Japanese divoltine white, '*Igata*' ♂.
{ Siamese yellow ♀.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
			White.	Yellow.	Total.		
1	Feb. 6th	Feb. 15th	0	291	291	March 8–13th	all yellow.
2	7th	16th	0	259	259	12–15th	do.
3	,,	,,	0	232	232	10–12th	do.
Total.			0	782	782		do.

SERIES 2.

a. {Japanese tetravoltine white, '*Tsunomata*' ♀.
{Siamese multivoltine yellow ♂.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
			White.	Yellow.	Total.		
1	Feb. 6th	Feb. 15th	0	66	66	March 6–10th	all yellow.
2	,,	,,	0	86	86	,,	do.
Total.			0	152	152		do.

b. {Japanese tetravoltine white, '*Tsunomata*' ♂.
{Siamese multivoltine yellow ♀.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
			White.	Yellow.	Total.		
1	Feb. 6th	Feb. 15th	0	171	171	March 7–11th	all yellow.
2	,,	,,	0	189	189	10–15th	do.
Total.			0	360	360		do.

We see now that the result of reciprocal crossing between the white and the yellow forms is quite the same and that the yellow character predominates over the white one as is the case in the preceding series of experiments. Of the 1,463 worms derived from this reciprocal crossing there was no white cocoon.

SECTION II.

The second generation.

SERIES 1. *a*.

Offspring from the yellow form derived from crossing Japanese white female and Siamese yellow male.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
			White.	Yellow.	Total.		
1	—	—	56	131	187	—	white and yellow.
2	—	—	36	119	155	—	do.
3	—	—	52	177	229	—	do.
Total.	—	—	(25.21%) 144	(74.78%) 427	571	—	do.

Each group derived from the same parent produced two kinds of worms, the white (25%) and the yellow (75%).

SERIES 2. *a*.

Offspring from the yellow form, series 2. *a*. of the last generation.

Parentage.	Eggs.	Number of worms.			Cocoons.			
		White.	Yellow.	Total.	Yellow.	P. P. yellow.	White.	Total.
1	1	48	148	196	90	0	28	118
	2	31	111	142	68	0	21	89
	3	19	41	60	20	0	12	32
	4	24	72	96	31	6	3	40
2	5	16	61	77	28	0	11	39
	6	26	71	97	22	6	7	35
	7	48	144	192	105	0	39	144
	8	16	77	93	35	19	11	65
Total.		(23.92%) 228	(76.0%) 725	953				562

Some produced three forms, the yellow, the pale-pinkish-yellow, and the white; some two, the yellow and the white. Owing to the ravage of pebrine in this generation, very few cocoons were obtained. So we could not draw any accurate conclusion about the relation of the three forms produced in the former case. In the latter case, we obtained six hundred and sixty seven worms of which one hundred and sixty two (24.28%) spun white cocoons and the remaining five hundred and five worms (75.71%) yellow cocoons.

Section III.

The third generation.

In this generation, we kept some offspring from No. 1, series 1. *a*, of the last generation.

Eggs.		Date of laying eggs.	Date of hatching.	Number of worms.			Date of mounting.	Cocoons.
				White.	Yellow.	Total.		
Yellow parent.	1	June 1st	June 10th	0	141	141	July 2–3rd	yellow only.
	2	,,	,,	(22.22%) 46	161	207	,,	white and yellow.
White parent.	3	,,	,,	189	0	189	,,	white only.
	4	2nd	11th	130	0	130	,,	do.

The yellow parents produced two kinds of offspring, and the white uniform offspring.

As regards the hereditary phenomena of the colour of the cocoons, we again meet with similar facts as observed in the preceding series of experiments, thus

SERIES 1.

The first generation : only yellow off-spring.

The second ,, white(25%) yellow(75%).

The third ,, white only. some yellow only. some white(25%)+yellow(75%).

SERIES 2.

The first generation only yellow off-spring.

The second ,, white+yellow (25%). (75%). white+yellow+pale-pinkish-yellow.

The result of the experiments above referred to are exactly like those obtained in the preceding series of experiments, Case I and II.

CASE IV.

From the foregoing series of experiments, we have obtained some knowledge with regard to the hereditary phenomena of the various colours of cocoons when crossed with each other.

Next we will consider what offspring will result from the back-crossing of the cross-bred form with one of the pure parent breeds. The following series of experiments will give us some notion about it.

SECTION I.

The first generation.

SERIES 1.

Back-crossing the cross-bred yellow form with the white parent-breed.

(*a*).

♀ The yellow form of No. 3, Class II of the fourth generation, Experiment Case I ; namely, that which produced two kinds of worms in the offspring from a parent.

♂ Pure Siamese white.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
			White.	Yellow.	Total.	
1♂	Nov. 6th	Nov. 17th	0	269	269	yellow only.
2♂	..	..	0	198	198	do.
4	..	16th	0	361	361	do.
5	..	17th	175	151	326	white and yellow.
9♂	7th	18th	156	156	312	do.

b.

♂ The yellow form of No. 3, Class II of the fourth generation.

♀ Pure Siamese white.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
			White.	Yellow.	Total.	
1	Nov. 6th	Nov. 17th	158	159	317	white and yellow.
2	7th	..	0	367	367	yellow only.
3	..	18th	134	138	272	white and yellow.
4	..	17th	198	221	419	do.

The hereditary phenomenon of the colours of the cocoons observed in this reciprocal crossing was quite different from that obtained when pure races were crossed, that is to say, in the latter case, as we know well, the offspring of the first cross generation exhibit only the dominant character, or the yellow. In this case, on the contrary, we obtained two kinds of offspring as the result of the crossing, one spinning only yellow cocoons; the other mixed offspring, spinning white and yellow cocoons, the respective percentage in the offspring of each parent being about 50% for each. This

agrees very well with the result which we obtained in 1900, and already mentioned at the beginning of this work (see page 263).[1]

Series 2.

(*a*).

♀ The yellow form of No. 9, Class II of the fourth generation, Case I which produced two kinds of worms, the white (25%) and the yellow (75%).

♂ Pure Siamese yellow breed.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
			White.	Yellow.	Total.	
1	Nov. 6th	Nov. 16th	0	375	375	yellow only.
2	,,	,,	0	382	382	do.
3	,,	17th	0	302	302	do.
4*	,,	,,	0	381	381	do.
5*	,,	,,	0	359	359	do.
6*	,,	,,	0	390	390	do.
7	8th	18th	0	350	350	do.
Total.			0	2,539	2,539	do.

(*b*).

♀ Pure Siamese yellow.

♂ The yellow form of No. 9, Class II, of the fourth generation.

[1] A similar case has been observed with Japanese races: when we crossed divoltine striped worms which sometimes produce common marked worms with common marked breed, we got the following figures:—

	I.	II.
common marked worms.	130 heads.	122.
striped worms.	145 heads.	145.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
			White.	Yellow.	Total.	
1	Nov. 8th	Nov. 18th	0	412	412	yellow only.
2	..	..	0	410	410	do.
3	..	..	0	415	415	do.
4	..	..	0	424	424	do.
5	..	..	0	426	426	do.
Total.			0	2,087	2,087	do.

In these reciprocal crosses, only yellow offspring were obtained.

Section II.

The second generation.

Series 1.

(a).

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
				White.	Yellow.	Total.	
No. 1. Yellow form.	1	Jan. 5th	Jan. 18th	62	123	185	white and yellow.
	2	6th	19th	47	181	228	do.
	4	..	..	69	224	293	do.
	5	..	..	50	142	192	do.
Total.				(25.38%) 228	(74.61%) 670	898	do.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. White.	Yellow.	Total.	Cocoons.
No. 2.	Yellow form.	1	Jan. 6th	Jan. 19th	48	149	197	white and yellow.
		2	,,	,,	38	72	110	do.
		3	,,	,,	51	195	246	do.
Total.					(24.77%) 137	(75.22%) 416	553	do.

Now we see that in the offspring from each parent which spun only yellow cocoons in the last generation, there may be found two kinds of worms, one spinning yellow and the other white cocoons, the respective percentage being 75% (the yellow) and 25% (the white).

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. White.	Yellow.	Total.	Cocoons.
No. 9 of the 1st generation.	Yellow form.	1	Jan. 6th	Jan. 19th	32	78	110	white and yellow.
		2	,,	,,	47	158	205	do.
		3	,,	,,	61	192	253	do.
		4	,,	,,	37	139	176	do.
		5	,,	,,	58	185	243	do.
		6	,,	,,	47	153	200	do.
	Total.				(23.76%) 282	(76.23%) 905	1,187	do.
	White form.	7	,,	,,	206	0	206	white only.
		8	,,	,,	119	0	119	do.
	Total.				325	0	325	do.

As in the case of the last generation, the yellow form again produces two kinds of worms, white and yellow, yet their proportion in the offspring of a parent is quite different from that observed in the last generation, that

is to say, in the former generation one-half of the offspring from a parent consists of the white and the orher half of the yellow form, while in this generation three-fourths is yellow and one-fourth white.

Series 2.

(*a*).

The offspring of the worms which spun only yellow cocoons in the last generation.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
				White.	Yellow.	Total.	
No. 4.	1	Jan. 6th	Jan. 19th	0	212	212	all yellow.
	2	,,	,,	0	265	265	do.
	3	,,	,,	0	242	242	do.
	4	,,	,,	0	193	193	do.
	5	,,	,,	0	254	254	do.
No. 5.	6	,,	,,	0	257	257	do.
	7	,,	,,	0	131	131	do.
	8	,,	,,	0	263	263	do.
	9	,,	,,	0	152	152	do.
	10	,,	,,	0	231	231	do.
No. 6.	11	5th	18th	0	205	205	do.
	12	,,	,,	0	162	162	do.
	13	6th	19th	0	275	275	do.
	14	,,	,,	0	189	189	do.
	15	,,	,,	0	146	146	do.
Total.				0	3,177	3,177	do.

In this series, no white cocoons were found.

In consequence of the scantiness of the leaves, we discontinued

this series of experiments, so we will give, as before, a short résumé as follows :—

SERIES 1.

Back-crossing of a cross-bred yellow form with a pure parental white breed. The reciprocal crosses gave a quite parallel result, as follows :—

1. The first back-crossed generation :	some parents produced only yellow form.			some yellow (50%).	+	white (50%).
2. The second ,, ,,	yellow (75%).	+	white (25%).	yellow (75%).	+	white (25%).	white only.

SERIES 2.

Back-crossing of a cross-bred yellow form with a pure parental yellow form. The reciprocal crosses gave a quite parallel result as follows :—

2. The first back-crossed generation :	only yellow.
2. The second ,, ,,	again only yellow.

Thus we see that when a cross-bred dominant form is back-crossed with the parental recessive breed, it produces some uniform and some mixed offspring ,of the dominant and the recessive forms, their proportion in the offspring from each parent being about 50% for each.

In the succeeding generations, however, the two dominant forms which appeared in the first generation will split up into their parental characters according to the law already observed, when a yellow breed was crossed with a white breed, that is to say, 75% of the dominant character and 25% of the recessive.

The white form produced in each generation will no more produce any coloured offspring.

On the contrary, when we back-crossed a cross-bred dominant form with the pure parental dominant breed, the offspring showed only the dominant character. In the second generation, they again produced only dominant form.

In consequence of the inability to continue this experiment for further generations, we could not draw any definite conclusion with regard to their further fate, yet we have every reason to believe that they will split up into their parental characters in the succeeding generations. The account will be dealt with subsequently in the chapter on general considerations.

CASE V.

In the preceding series of experiments, the inheritance of the various colours of cocoons was discussed. Now we shall pass on to the consideration of the various markings on the larval body.

For this purpose, we selected two breeds, one (Pl. X. Fig. *b*, Pl. XI, I) having no markings on the body, except on the dorsal portion of the first segment where some very faint dark markings could be observed ; the other (Pl. X, Fig. *a*, Pl. XI, II) having a black stripe on each intersegmental region. The former breed will be called hereafter under the name " pale breed ", and the latter " striped breed ".

The pale breed remained constant for six generations, while the striped breed sometimes produced a few pale worms, in spite of our efforts to exclude all the pale forms in each generation. We can not say, therefore, that it is a pure race, but rather a cross-bred form between the striped and the pale ones. Both these breeds, however, always spun white cocoons, never yellow ones.

The result of the reciprocal crossing between these two breeds will be given in the following pages.

SECTION I.

The first generation.

SERIES 1.

♀ The pale breed. (Pl. XI, I).
♂ The striped breed. (Pl. XI, II).

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
			Pale.	Striped.	Total.	
2*	Sept. 21st	Sept. 30th	0	370	370	all white.
3*	..	..	0	376	376	do.
4	..	..	186	198	384	do.
5	..	..	180	165	345	do.
6*	..	..	159	165	324	do.
Total.			(49.9%) 525	(50.0%) 528	1,053	do.

Some parents (Nos. 2 and 3) produced only striped worms, while others (Nos. 4, 5 and 6) both pale (about 50%) and striped ones (about 50%).

SERIES 2.

♀ The striped breed.

♂ The pale breed.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
			Pale.	Striped.	Total.	
3	Sept. 22nd	Oct. 2nd	193	180	373	all white.
4	..	..	0	366	366	do.
5	..	..	0	342	342	do.
9	..	..	0	287	287	do.
10	..	..	0	356	356	do.

Quite the same as in the last series.

Now the result of this reciprocal crossing reminds us of that of the back-crossing mentioned in the preceding series of experiments, Case IV.

Section II.

The second generation.

Series 1.

a. Offspring of the parents which produced only striped-worms.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
				Pale.	Striped.	Total.	
No. 2.	2*	Nov. 6th	Nov. 17th	(23.11%) 49	163	212	white only.
	3	,,	,,	(25%) 42	126	168	do.
	4	,,	,,	(19.04%) 64	272	336	do.
	5	,,	,,	(22.42%) 50	173	223	do.
	7	,,	,,	(23.9%) 60	191	251	do.
No. 3.	8	,,	16th	(24.03%) 56	177	233	do.
	9	,,	,,	(26.4%) 47	131	178	do.
	10	,,	,,	(25.25%) 49	145	194	do.
Total.				(23.2%) 417	(76.76%) 1,378	1,795	do.

b. Offspring of the parents which produced two kinds of worms, striped and pale.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
					Pale.	Striped.	Total.	
No. 6.	Pale.	11	Nov. 8th	Nov. 18th	248	0	248	white only.
		12	,,	,,	291	0	291	do.
		13	,,	19th	275	0	275	do.
		14	,,	,,	319	0	319	do.
	Striped.	15	,,	,,	86 (26.22%)	292	328	do.
		16	,,	,,	53 (17.34%)	244	297	do.
		17	,,	,,	92 (25.55%)	268	360	do.
		18	,,	18th	90 (26.67%)	247	337	do.
		19	,,	19th	0	254	254	do.
		20	,,	18th	85 (25.14%)	253	338	do.
		21	,,	,,	60 (24.47%)	185	245	do.
		22	,,	,,	0	287	287	do.

SERIES 2.

a. Offspring of the parents which produced striped worms only.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
				Pale.	Striped.	Total.	
No. 4.	1	Nov. 8th	Nov. 18th	74	244	318	white only.
	2	,,	19th	69	189	258	do.
	3	,,	18th	74	264	338	do.
	4	,,	19th	106	280	386	do.
	5	,,	,,	103	307	410	do.
	6	,,	,,	56	223	279	do.
	7	,,	18th	82	257	339	do.
	8	,,	,,	80	304	384	do.

Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
				Pale.	Striped.	Total.	
No. 5.	9*	Nov. 8th	Nov. 19th	94	312	406	white only.
	10	..	..	70	280	368	do.
	11	..	..	59	197	256	do.
	12	..	..	81	206	287	do.
No. 10.	13	..	..	100	280	380	do.
	14	..	..	72	202	274	do.
	15	..	..	71	200	271	do.
Total.				(24.22%) 1,200	(75.77%) 3.754	4.954	do.

b. Offspring of the parents which produced two kinds of worms.

Parentage.		Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
					Pale.	Striped.	Total.	
No. 8.	Pale.	1	Nov. 8th	Nov. 18th	286	0	286	all white.
		2	..	..	297	0	297	do.
		3	..	..	401	0	401	do.
		4	..	..	255	0	255	do.
		5	..	..	301	0	301	do.
		Total.			1,540	0	1,540	do.
	Striped.	7	..	19th	(26.41%) 70	195	265	do.
		8	..	..	(24.06%) 90	284	374	do.
		9	..	..	(25.13%) 94	280	374	do.
		10	..	..	0	227	227	do.
		11	..	..	0	316	316	do.

Now we learn that all the striped worms descended from the parents which produced only striped worms in the last generation split up into their parental forms, the striped and the pale ones. The proportion of these two kinds of worms appearing in the offspring from a parent is about 75% of the former and 25% of the latter.

The striped form derived from the parent which produced the two kinds of worms, striped and pale, in the last generation, on the contrary, again produce two kinds of offspring, one uniform (striped only), the other mixed [the striped (75%) and the pale ones (25%)].

The former case may be seen in the second cross generation of pure races and the latter in the third cross generation.

Section III.

The third generation.

Series 1.

Offspring of the striped form which produced two kinds of worms in the last generation.

	Parentage.	Eggs.	Date of laying eggs.	Date of hatching.	Number of worms. Pale.	Striped.	Total.	Cocoons.
No. 2.	Striped.	1	Jan. 6th	Jan. 19th	(24.3%) 52	162	214	white only.
		2	..	..	0	146	146	do.
		3	..	..	0	184	184	do.
		4	..	..	(22.6%) 39	133	172	do.
		5	..	..	(30.5%) 59	134	193	do.
	Pale.	12	..	..	198	0	198	do.
		13	..	..	165	0	165	do.
		14	..	..	198	0	198	do.

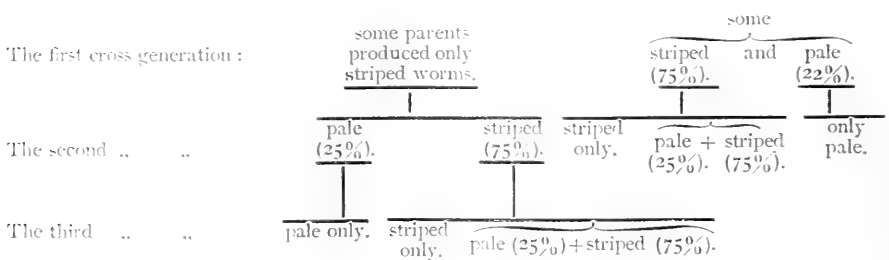
SERIES 2.

Offspring of the striped form which produced two kinds of worms in the last generation.

Eggs.	Date of laying eggs.	Date of hatching.	Number of worms.			Cocoons.
			Pale.	Striped.	Total.	
1	Jan. 7th	Jan. 20th	0 (28.82%)	229	229	white only.
2	..	..	49	121	170	do.
3	8th	..	0	219	219	do.
4	..	..	0	262	262	do.
5	..	..	0	77	77	do.
7	7th	..	151	0	151	do.
8	..	..	146	0	146	do.
9	..	..	85	0	85	do.
10	..	..	232	0	232	do.
11	..	..	137	0	137	do.

Now we have arrived at a familiar phenomenon repeatedly observed in the preceding series of experiments and may conclude with safety that this is a case of back-crossing between a cross-bred race and a pure parental race and also that various larval markings of the silk-worm will follow the same law as that governing the heredity of various colours of cocoons.

The following is the summary :—



Hence the striped character is dominant and the 'pale' recessive.

CASE VI.

In this series of experiments, we will try to consider, what will be the result when two races having many different characters are crossed.

For this purpose, we crossed a pale breed spinning yellow cocoons with a striped breed spinning white cocoons. These may respectively be called "pale-yellows" and "striped whites." Thus four different characters were brought together as the result of this crossing :—

From one breed { 1. The character producing pale larvae.
2. The character spinning yellow cocoons.

From the other breed { 1. The character producing striped larvae.
2. The character spinning white cocoons.

SECTION I.

The first generation.

SERIES 1.

♀ Siamese pale race spinning yellow cocoons or 'pale yellows'.

♂ Siamese striped race spinning white cocoons or 'striped whites'.

Eggs.	Number of worms.					Cocoons.
	Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
1	0	0	207	0	207	yellow only.
2	0	0	327	0	327	do.
3	0	0	308	0	308	do.
4	0	0	281	0	281	do.
Total.	0	0	1,123	0	1,123	do.

Days.	Number of worms.					Cocoons.
	Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
5	0	0	150	131	281	yellow only.
6	0	0	143	163	306	do.
7	0	0	288	0	288	do.
8	0	0	284	0	284	do.
9	0	0	351	0	351	do.
10	0	0	365	0	365	do.
11	0	0	405	0	405	do.
12	0	0	164	202	366	do.
13	0	0	360	0	360	do.
14	0	0	307	0	307	do.
15	0	0	389	0	389	do.
16	0	0	352	0	352	do.
17	0	0	393	0	393	do.
18	0	0	203	207	410	do.
19	0	0	170	154	324	do.
20	0	0	202	194	396	do.
Total.	0	0				do.

SERIES 2.

♀ Siamese striped race spinning white cocoons.

♂ Siamese pale race spinning yellow cocoons.

Eggs.	Number of worms.					Cocoons.
	Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
1	0	0	90	85	175	yellow only.
2	0	0	212	0	212	do.
3	0	0	120	96	216	do.
4	0	0	122	107	229	do.
5	0	0	94	96	190	do.
6	0	0	339	0	339	do.
7	0	0	381	0	381	do.
8	0	0	332	0	332	do.
9	0	0	340	0	340	do.
10	0	0	356	0	356	do.
11	0	0	115	113	228	do.
12	0	0	299	0	299	do.
13	0	0	163	132	295	do.
14	0	0	182	187	369	do.

From the result of this reciprocal crossing between 'pale yellows' and 'striped whites', we learn that in the first generation some parents produce only striped worms, spinning yellow cocoons or "striped yellows", while others 'striped yellows' (about 50%) and "pale-yellows" (about 50%), or pale worms spinning yellow cocoons but no white one, that is to say, in one case two dominant characters become active and in the other one dominant (the yellow) and one recessive (the pale) characters become visible. In general, the result arrived at in this generation agrees very well to that obtained in the two preceding series of experiments, Case IV and V.

Section II.

The second generation.

Series 1.

a. Offspring of the parent which produced only the striped yellow forms in the last generation.

Parentage.	Eggs.	Number of worms.					Cocoons.
		Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
No. 2.	1*	(25.4%) 84	(7.2%) 24	(52.7%) 174	(11.5%) 38	320	white and yellow.
	2	(18.8%) 45	(6.6%) 16	(53.1%) 127	(21.3%) 51	239	do.
	3	(18.8%) 57	(6.6%) 20	(58.%) 176	(16.5%) 50	303	do.
	Total.	(21.57%) 186	(6.96%) 60	(55.33%) 477	(16.12%) 139	862	do.
No. 7.	4	60	20	154	50	284	do.
	5	48	23	139	61	271	do.
	6*	83	22	256	76	437	do
	7	55	22	164	47	288	do.
	8	65	23	227	80	395	do.
	Total.	(18.56%) 311	(6.56%) 110	(56.11%) 940	(18.74%) 314	1,675	do.
No. 8.	9	55	28	204	56	343	do.
	10*	90	22	236	72	420	do
	11	94	20	204	90	408	do.
	12	65	18	190	58	331	do.
	13	53	21	170	64	308	do.
	Total.	(19.72%) 357	(6.02%) 109	(55.46%) 1,004	(18.78%) 340	1,810	do.

Parentage.	Eggs.	Number of worms.					Cocoons.
		Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
No. 11.	14	88	24	233	81	426	white and yellow.
	15	67	26	230	67	390	do.
	16	69	18	154	62	303	do.
	Total.	224	68	617	210	1,119	do.
	Grand total.	(19.72%) 1,078	(6.34%) 347	(55.57%) 3,038	(18.35%) 1,003	5,466	do.

The total number of worms derived from sixteen parent-moths was 5,466, of which

1. 'Striped whites'.....1,078 (19.72%).
2. 'Pale whites'..... 347 (6.34%).
3. 'Striped yellows'.....3,038 (55.57%).
4. 'Pale yellows'.....1,003 (18.35%).

The same relation obtains for the offspring of each parent.

The relation between the colour of cocoons.

Total number of worms.....5,466,

of which

1. White cocoon spinners 1,425 (26.07%) in which { striped ones 1,078 (75.64%). pale ,, 347 (24.35%).
2. Yellow cocoon spinners 4,041 (73.92%) in which { striped ones 3,038 (75.17%). pale ,, 1,003 (24.82%).

The relation between the striped and the pale worms.

Total number of worms.....5,466,

of which

1. Striped worms.....4,116 (75.3%) { 1. yellow 3,038 (73.8%). 2. white 1,078 (26.1%).
2. Pale worms.....1,350 (24.6%) { 1. yellow 1,003 (74.29%). 2. white 347 (25.7%).

b. Offspring of the parent which produced two kinds of worms in the last generation.

	Parentage.	Eggs.	Number of worms.					Cocoons.
			Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
No. 18.	Striped yellow.	1	33	12	120	40	205	white and yellow.
		2	26	11	105	27	169	do.
		3	18	6	56	18	98	do.
		4	45	19	166	58	288	do.
		5	40	14	133	39	226	do.
		Total.	(16.42%) 162	(6.28%) 62	(58.82%) 580	(18.45%) 182	986	do.
	Pale yellow.	6	0	64	0	215	279	do.
		7	0	72	0	202	274	do.
		8	0	38	0	99	139	do.
		9	0	38	0	116	154	do.
		10	0	40	0	115	155	do.
		Total.	0	(25.22%) 252	0	(16.42%) 162	999	do.

The offspring from the striped parents, as in the last case, consist of four kinds of worms :—

The total number of worms.....986,

of which

1. 'Striped whites' .....162 (16.42%).
2. 'Pale whites' ..... 62 (6.28%).
3. 'Striped yellows'.....580 (58.82%).
4. 'Pale yellows' .....182 (18.45%).

The same relation holds good for the offspring of each parent.

The relation between the colours of cocoons.

Total number of worms.....986,

of which

1. White cocoon spinners 224 (22.71%) in which { 1. Striped worms 162 (72.32%). 2. Pale worms 62 (27.67%).
2. Yellow cocoon spinners 762 (77.28%) in which { 1. Striped worms 580 (76.11%). 2. Pale worms 182 (23.88%).

The relation between the striped and the pale worms.

Total number of worms.....986,

of which

1. Striped worms...742 (75.25%) in which { 1. yellow 580 (78.16%). 2. white 162 (21.83%).
2. Pale worms.....244 (24.74%) in which { 1. yellow 182 (74.59%). 2. white 62 (25.40%).

On the other hand, those from pale worms spinning yellow cocoons split up into two kinds, the 'pale whites' (about 25%) and the 'pale yellows' (about 75%). Thus of the nine hundred and ninety worms (to speak in round numbers), we find two hundred and fifty two (25.22%) pale worms spinning white cocoons and seven hundred and forty four pale worms spinning yellow cocoons (74.77%).

The same relation holds good for the offspring of each parent.

Series 2.

Offspring of the parent which produced only striped worms spinning yellow cocoons in the last generation.

Parentage.	Eggs.	Number of worms.					Cocoons.
		Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
No. 7.	1	45	17	164	42	268	white and yellow.
	2*	74	32	248	71	425	do.
	3	85	18	225	72	400	do.
	4	75	12	228	71	386	do.
	5	61	18	164	62	305	do.
	Total.	(19.05%) 340	(5.43%) 97	(57.82%) 1,029	(17.82%) 318	1,784	do.

Parentage.	Eggs.	Number of worms.					Cocoons.
		Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
No. 9.	6*	60	23	205	59	347	white and yellow.
	7	66	20	225	69	380	do.
	8	57	14	161	68	300	do.
	9	67	19	198	65	349	do.
	Total.	(18.16%) 250	(5.52%) 76	(57.33%) 789	(18.96%) 261	1 376	do.
No. 10.	10	54	18	171	60	303	do.
	11	46	20	160	59	285	do.
	12	78	27	212	75	392	do.
	13	68	26	210	67	371	do.
	14	71	18	196	74	359	do.
	Total.	(18.53%) 317	(6.37%) 109	(55.49%) 949	(19.59%) 335	1,710	do.
	Grand total.	(18.62%) 907	(5.79%) 282	(56.81%) 2,767	(18.76%) 914	4,870	do.

The phenomenon of the segregation of the parental characters is the same as in the case of the last series.

Total number of worms derived from fourteen parents.....4,870,

of which

1. 'Striped whites' ..... 907 (18.62%).
2. 'Pale whites' ..... 282 (5.79%).
3. 'Striped yellows' .....2,767 (56.81%).
4. 'Pale yellows'..... 914 (17.76%).

The same holds good for each litter.

The relation between the colours of cocoons.

1. Yellow cocoon spinners 3,681 (75.59%) in which { 1. striped worms 2,767 (75.16%). 2. pale worms 914 (24.83%).

2. White cocoon spinners 1,189 (24.4%) in which { 1. striped worms 907 (76.28%). 2. pale worms 282 (23.71%).

Conversely, the relation between striped and pale worms was as follows :—

1. Striped worms 3,674 (76.44%) of which { 1. yellow cocoon spinners 2,767(75.31%). 2. white cocoon spinners 907(24.68%).

2. Pale worms 1,196 (24.55%) of which { 1. yellow cocoon spinners 914(76.42%). 2. white cocoon spinners 282(23.57%).

It is very interesting to see that in this generation, the striped yellow form which appeared in the first cross generation splits up into four different kinds of worms, (1) the striped worms spinning white cocoons, (2) pale worms spinning yellow cocoons, (3) pale worms spinning white cocoons and (3) striped ones spinning yellow cocoons, the latter two being quite new combinations of the characters, already present. The respective number of these worms present in a brood was quite constant, always keeping the following proportion :—

1. 'Striped whites'.....about $\frac{3}{16}$ or 18.75%.
2. 'Pale whites'.....about $\frac{1}{16}$ or 6.25%.
3. 'Pale yellows'.....about $\frac{3}{16}$ or 18.75%.
4. 'Striped yellows'.....about $\frac{9}{16}$ or 56.25%.

With respect to the colours of cocoons and larval markings, they kept a constant relation independently of the other characters, that is,

The recessive one about $\frac{1}{4}$ or 25%.
The dominant one about $\frac{3}{4}$ or 75%.

Section III.

The third generation.

The four kinds of worms which appeared in the second generation were reared separately and they exhibited the following phenomena of the segregation of the parental characters :—

Series 1.

No. 1.

Parentage.	Eggs.	Number of worms.					Cocoons.
		Striped white.	Pale white.	Striped yellow.	Pale Yellow.	Total.	
Striped white.	1	301	(25.0%) 99	0	0	400	white only.
	2	252	(22.9%) 75	0	0	327	do.
	3	383	0	0	0	383	do.
	4	203	(22.5%) 59	0	0	262	do.
	5	291	0	0	0	291	do.
Pale white.	6	0	1.380	0	0	1.380	do.
	7						
	8						
	9						
Striped yellow.	10	(26.1%) 93	0	(73.9%) 262	0	355	white and yellow.
	11	0	0	288	0	288	yellow only.
	12	0	0	(74.48%) 219	(25.51%) 75	294	do.
	13	(26.8%) 78	0	(73.2%) 216	0	294	white and yellow.
	14	0	0	(67.21%) 162	(32.78%) 79	241	yellow only.
	15	(15.44%) 59	(7.59%) 29	(56.2%) 215	(20.6%) 79	382	white and yellow.
Pale white.	16	0	0	0	356	356	yellow only.
	17	0	0	0	369	369	do.
	18	0	(21.1%) 85	0	312	397	white and yellow.
	19	0	(21.0%) 69	0	259	328	do.
	20	0	(24.3%) 101	0	314	415	do.

	Parentage.	Eggs.	Number of worms.					Cocoons.
			Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
No. 6.	Striped white.	1	182	(23.84%) 57	0	0	239	white only.
		2	157	(21.5%) 43	0	0	200	do.
		3	225	0	0	0	225	do.
	Pale white.	4	0	214	0	0	214	do.
		5	0	226	0	0	226	do.
	Striped yellow.	6	0	0	205	(21.59%) 59	264	yellow only.
		7	0	0	218	0	218	do.
		8	0	0	187	(22.5%) 53	240	do.
		9	0	0	186	(27.9%) 72	258	do.
		10	(15.27%) 31	(6.89%) 14	(54.67%) 111.	(23.15%) 49	203	white and yellow.
	Pale yellow.	11	0	0	0	258	258	yellow only.
		12	0	0	0	255	255	do.
		13	0	60	0	171	231	white and yellow.
No. 10.	Striped white.	1	93	0	0	0	93	white only.
		2	130	(25.28%) 44	0	0	174	do.
		3	74	0	0	0	74	do.
	Pale white.	4	0	212	0	0	212	do.
	Striped yellow.	5	0	0	181.	(23.62%) 56	237	yellow only.
		6	0	0	45	0	45	do.
		7	0	0	240	0	240	do.
	Pale yellow.	8	0	0	0	11	11	do.
		9	0	0	0	272	272	do.

SERIES 2.

	Parentage.		Eggs.	Number of worms.					Cocoons.
				Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
No. 2.	Striped white.	1	96	(20.66%) 25	0	0	121	white only.
		2	125	0	0	0	125	do.
	Pale white.	3	0	185	0	0	185	do.
		4	0	83	0	0	83	do.
	Striped yellow.	5	(20.45%) 45	0	175	0	220	white and yellow.
		6	0	0	150	(25.37%) 51	201	yellow only.
		7*	0	0	143	0	143	do.
		8	0	0	135	0	135	do.
	Pale yellow.	9	0	(27.65%) 39	0	102	141	white and yellow.
		10	0	(22.44%) 55	0	190	245	do.
No. 6.	Striped white.	11	225	0	0	0	225	white only.
		12	221	(20.78%) 58	0	0	279	do.
		13	156	(22.00%) 44	0	0	200	do.
	Pale white.	14	0	122	0	0	122	do.
		15	0	228	0	0	228	do.
	Striped yellow.	16	0	0	185	68	253	yellow only.
		17	(25.74%) 69	0	199	0	268	white and yellow.
		18	0	0	93	0	93	yellow only.
	Pale yellow.	19	0	(23.18%) 48	0	159	207	white and yellow.
		20	0	0	0	214	214	yellow only.
		21	0	0	0	192	192	do.

From the results of these reciprocal crossings we learn firstly, that the 'striped whites' give rise to two kinds of offspring, one producing two kinds of worms, pale (about 25%) and striped (about 75%), the other producing only one kind of worms, striped; secondly, that the 'pale whites' remain constant, as in the case of the white against the yellow; thirdly, the 'striped yellows' produce four kinds of offspring, the first producing 'striped yellows' (about 75%) and 'striped whites' (about 25%), the second, 'striped yellows' only, the third, 'striped yellows' (about 75%) and 'pale yellows' (about 25%) and the fourth, four kinds of worms in a brood, 'striped whites' (about $\frac{3}{16}$), 'pale whites' (about $\frac{1}{16}$), 'striped yellows' (about $\frac{9}{16}$) and 'pale yellows' (about $\frac{3}{16}$). This is a repetition of what was found in the second generation. Lastly, the 'pale yellows' produce two kinds of offspring, one 'pale whites' (about 25%) and 'pale yellows' (about 75%), the other 'pale yellows' only.

The phenomena of heredity observed in this generation remind us of those of the worms of Group A, Kind 4 of the Experiment Case II, and we believe that both cases are due to the same law, which will be discussed in the chapter on general consideration.

Section IV.

The fourth and further generations.

In the fourth generation, we kept only those forms which produced one kind of worms in the last generation.

Parentage.		Eggs.	Number of worms.					Cocoons.
			Striped white.	Pale white.	Striped yellow.	Pale yellow.	Total.	
No. 2.	Striped white.	1	172	0	0	0	172	white only.
		2	121	(21.93%) 34	0	0	155	do.
		3	208	(19.37%) 50	0	0	258	do.
		4	183	0	0	0	183	do.
No. 7.	Striped yellow.	5	0	0	185	0	185	yellow only.
		6	0	0	130	0	130	do.
		7	0	0	115	0	115	do.
		8	0	0	153	0	205	do.
		9	0	0	138	0	138	do.

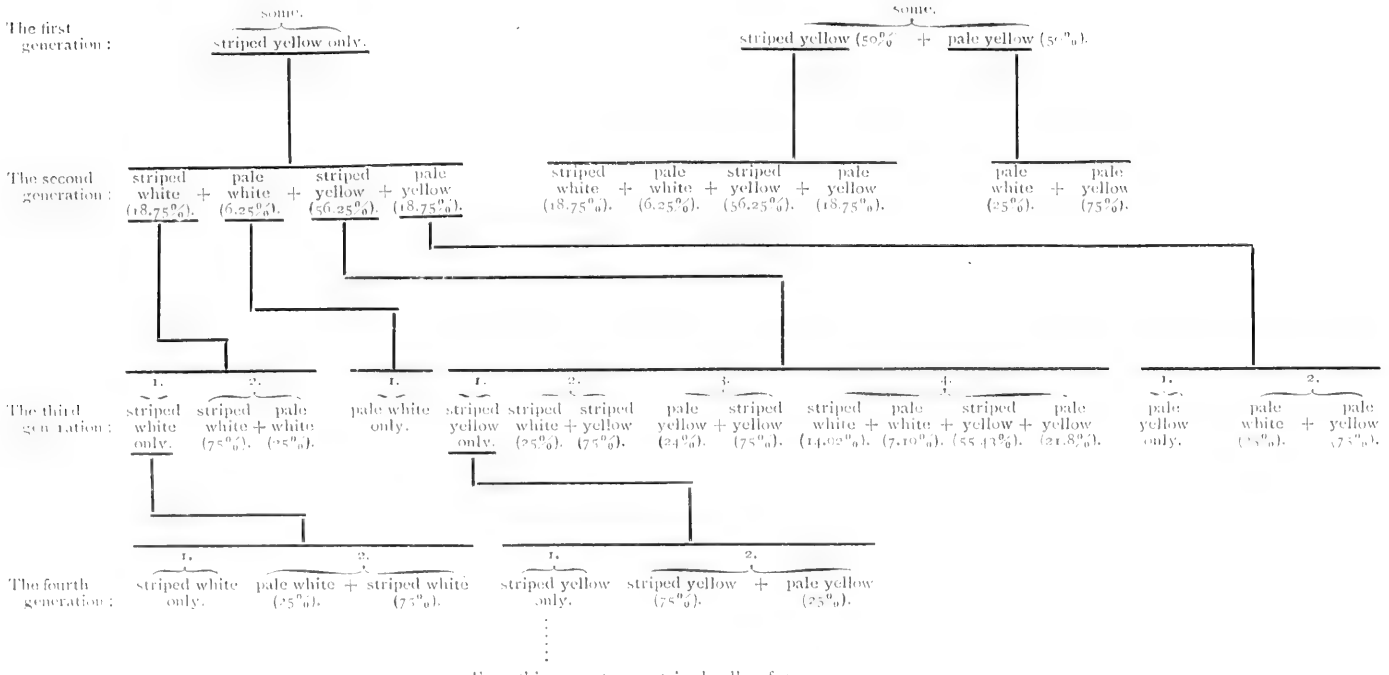
A result already familiar to us.

After weeding out all other forms during three successive generations we got an entire brood of the striped worms which spin yellow cocoons.

We are now able to say that from the crossing between the pale breed spinning yellow cocoons and striped breed spinning white cocoons we can obtain two new breeds, the striped worms spinnings yellow cocoons and the pale worms spinning white cocoons.

Now we will give, as usual, a diagram showing the inheritance of the various colours of cocoons and the larval markings.

CROSSING BETWEEN 'PALE YELLOW' AND 'STRIPED WHITE' BREEDS.



From this, we got pure striped yellow form.

As the diagram shows, the striped yellow form furnishes four combinations of characters; the striped white or the pale yellow ones, two combinations; and lastly the pale white ones only one kind of combination.

CASE VII.

As the last of our series of experiments, we will cite another instance of crossing between two impure breeds; one parent being the common Japanese (Pl. X, III, *c*) breed, and the other striped Siamese breed (Pl. X, III *a*). Both of these breeds, however, contain the "pale" character in a dormant state.

SECTION I.

The first generation.

SERIES 1.

♀ Common Japanese white race. (Pl. X, III, Fig. *c*.)

♂ Striped Siamese white race. (Pl. X, III, Fig. *a*.)

Eggs.	Number of worms.				Cocoons.
	Striped.	Common.	Pale.	Total.	
1	375	0	0	375	all white.
2	316	0	0	316	do.
3	138	(56.19%) 177	0	315	do.
4	153	(51.42%) 162	0	315	do.
5	(47.34%) 116	(30.20%) 74	(22.44%) 55	245	do.
6	(50.46%) 109	(25.92%) 56	(23.61%) 51	216	do.
7	(47.21%) 127	(24.53%) 66	(28.25%) 76	269	do.
Total.	(48.21%) 352	(26.84%) 196	(24.93%) 182	730	do.

SERIES 2.

♀ Striped Siamese white.

♂ Common Japanese white.

Eggs.	Number of worms.				Cocoons.
	Striped.	Common.	Pale.	Total.	
1*	280	0	0	280	all white.
2	191	(51.3%) 202	0	393	do.
3	364	0	0	364	do.

In this generation, as the tables show, we obtained three kinds of offspring: the first producing only striped worms; the second, striped (about 50%) and common marked worms (about 50%; and the last, three kinds of worms, striped (about 50%), common marked (about 25%) and "pale" ones (about 25%). The last one is a new combination of characters appearing for the first time in our series of experiments.

SECTION II.

The second generation

In this generation, we kept the offspring from the parent which produced only striped worms in the last generation.

Series 1.

Parentage.	Eggs.	Number of worms.				Cocoons.
		Striped.	Common.	Pale.	Total.	
No. 1.	1	(75.72%) 156	(18.93%) 39	(5.33%) 11	206	all white.
	2	(72.9%) 113	(21.93%) 34	(5.16%) 8	155	do.
	3*	(76.35%) 226	(17.56%) 52	(6.08%) 18	296	do.
	4	(74.77%) 169	(18.14%) 41	(7.07%) 16	226	do.
	5	(76.07%) 159	(18.18%) 38	(5.74%) 12	209	do.
	6	(70.08%) 157	(23.16%) 53	(6.25%) 14	224	do.
	7	(76.53%) 150	(17.85%) 35	(5.61%) 11	196	do.
	8	(73.93%) 156	(19.43%) 41	(6.63%) 14	211	do.
	9	(77.29%) 177	(13.10%) 30	(9.60%) 22	229	do.
	Total.	(74.94%) 1,463	(18.59%) 363	(6.39%) 126	1,952	do.

Series 2.

Parentage.	Eggs.	Number of worms.				Cocoons.
		Striped.	Common.	Pale.	Total.	
No. 1.	1	(76.77%) 205	(17.22%) 46	(5.99%) 16	267	all white.
	2	(76.20%) 253	(16.26%) 54	(7.53%) 25	332	do.
	3	(73.12%) 166	(22.34%) 53	(3.52%) 8	227	do.
	4	(72.28%) 180	(20.85%) 52	(6.82%) 17	249	do.
	5	(71.73%) 99	(21.01%) 29	(7.24%) 10	138	do.
	Total.	(74.44%) 903	(19.29%) 234	(6.27%) 76	1,213	do.

Here we again meet with a new combination of characters in the offspring from a litter, that is to say, each striped parent produced three kinds of worms, the striped (about $\frac{12}{16}$), the common ($\frac{3}{16}$) and the pale (about $\frac{1}{16}$).

Section III.

The third generation.

We kept only striped worms for breeding in this generation.

Series 1.

Parentage.	Eggs.	Number of worms.				Cocoons.
		Strtped.	Common.	Pale.	Total.	
3.	1*	(73.81%) 155	(19.04%) 40	(6.14%) 15	210	all white.
	2	(74.9%) 102	(25.%) 34	0	136	do.
	3*	(62.6%) 154	(18.29%) 45	(19.10%) 47	246	do.
	4*	(62.92%) 129	(20%) 41	(17.07%) 35	205	do.
	5	(76.86%) 103	0	(23.13%) 31	134	do.

They exhibited different proportion of various worms in a litter, as below :—

(1) the striped (about $\frac{12}{16}$), the common (about $\frac{3}{16}$) and the pale (about $\frac{1}{16}$); (2) the striped (about $\frac{3}{4}$), and the common (about $\frac{1}{4}$); (3) the striped (about $\frac{3}{4}$) and the pale (about $\frac{1}{4}$); (4) the striped (about 60%), the common (about 18%) and the pale (about 18%).

Section IV.

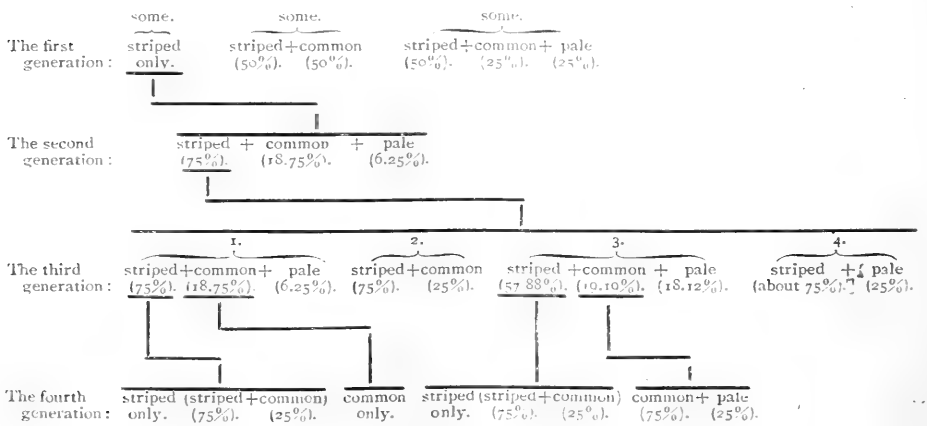
The fourth generation.

We kept, in this generation, only the offspring of the striped and the common forms.

Parentage.		Eggs.	Number of worms.				Cocoons.
			Striped.	Common.	Pale.	Total.	
1.	Stripe.	1	175 (73.43%)	0 (26.56%)	0	175	white only.
		2	97	34	0	128	do.
	Common.	3	0	261	0	261	do.
3.	Stripe.	4	269	0	0	269	do.
	Common.	5	0	(74.75%) 154	(25.24%) 52	206	do.
		6	0	(78.57%) 143	(21.42%) 39	182	do.
4.	Stripe.	7	(78.23%) 115	0	(21.76%) 32	147	do.
		8	156	0	0	156	do.

Some of the striped parents produced only striped worms; others, striped (about 75%) and common (about 25%) worms; while the rest striped (about 75%) and pale (about 25%) worms. Similarly, some of the common marked parents produced uniform and others mixed (common worm, about 75%; pale worms about 25%) offspring.

This is a phenomenon very familiar to us in the previous seisei of experiments. We will give, as before, a short résumé as follows :—



Now we see with certainty that of the three larval markings under consideration, the striped character is first in the order of dominancy, the common ranks next and the "pale" comes last. The respective hereditary relation is quite the same as observed in the case of the various colours of cocoons.

It will be of some interest to note here that the striped form obtained from the crossing between the common Japanese form and the Siamese striped breed exhibits a pair of the common curved markings on the dorsal part of the eighth segment (Pl. X, II.), where no such markings can be found in the striped Siamese breed. Some of them will remain true in the successive generations, and, therefore, it is possible to combine the two different markings into one form by crossing the two breeds.

CASE VIII.

On a mosaic form obtained by crossing the common Japanese white and the striped French yellow breeds.

Before leaving the subject of the hereditary phenomena of the larval markings of the silk-worm, let us consider an interesting instance met with in one of the experiments before mentioned.

In the Spring of 1901, we reared some worms derived from the cross between the striped European breed (♀) (Pl. VI, Fig. I, *b*.) and the common Japanese one (♂) (Pl. VI, Fig. I, *a*). Among them, we found two larvae (Pl. VI, Fig. II, *a*.) of great interest, that is to say, the left half of the body exhibited maternal striped markings and the right half the common paternal markings.

The 'mounting' took place on the 29th, May, but one of them was destroyed by *Ujimia*. The other moth emerged on the 18th, July.

In the following, we will try to give an account of the larvae and the moth above referred to.

1. The larva. (Pl. VI, Fig. II, *a*.)

The body in the fifth age is creamy white in colour; the dorsal shield of the first segment is light pinkish-brown. On the dorsal portion of the

first segment, we find a dark median line beginning at the middle of the second segment and gradually tapering towards the anterior end, where it nearly touches the base of the dorsal shield, and where there is a light dark spot on the left side but none on the right.

The second segment possesses dorsally a pair of the eye-like markings and a centrally placed trapezoidal marking commonly found both in the common and the striped worms.

The joints of the remaining segments are embroidered with a dark band in its left half, while the right half has the common markings. The dark dots or patches normally met with on the basal line of the body of the striped worm are present only on the left side, the one on the first segment being very faint, but nothing of this maternal character is to be found on the right side.

Other markings common to the two parents can be observed on both sides of the body.

2. The Imago. (Fig. II, *b*.)

When the moth emerged, it exhibited its male nature, fluttering its wings and trying to find a chance for pairing.

On a closer examination, it was found that the two halves of the body showed different characters, as in the larval stage.

The following description will serve to illustrate it :—

a. The antennae.

The colour of the right antenna was brownish on its dorsal side where it is covered with scales, while that of the left was grey or much lighter than the former.

With respect to size, the former was larger than the latter ; the former consisted of thirty-five and the latter of thirty-six segments, excluding the basal joint. The pectinated branches arising from each of the antennal segments were longer and larger on the right than on the left. The figures representing the length of the pectinated branches on the tenth segment of both antennae will confirm it :

$1\frac{5}{100}$ mm. in the left ; $\frac{7}{100}$ mm. in the right.

b. The wings. (Figs. II and III.)

The wings had also a peculiar structure on either side. The size of the left fore wing (Fig. III, *c*, *d*) was larger than that of the right one (Fig. III, *a*, *b*); the length being 22 mm. for the former and 20 mm. for the latter. The left hind wing (Fig. III, *d*.) did not attain its normal development, exhibited an abnormal form, and was much smaller than its fellow.

As regards the markings, the right wings (*a*, *b*), both fore and hind, had deeper and clearer markings than the left ones (*c*, *d*), which had lighter and faint markings, showing only a trace of the eye-spot on the fore-wing and a dark spot on the inner margin of the hind wing.

c. The abdomen. (Figs. IV, V.)

In the left half seven somites could be counted and in the right, eight segments—these numbers are typical of the abdominal segments of the normal male and female.

d. External genital armature. (Figs. IV, V and VI.)

Before describing the mosaic form, it will be convenient for comparision to give a brief account of the normal form.

1. Female. (Fig. VII). The outer genital aperture is provided with several chitine-plates: dorsally it is guarded by a narrow and curved plate —the dorsal plate (*a*). This is arched down at both ends to surround an area, where a spherical process (*b*) covered with some short hairs—the ovipositor—projects. On the ventral side, there are two plates (*c*, *d*) placed one over the other; in the space situated between these two plates we find a pore, the copulating pore.

In the centre of the space surrounded by those dorsal and ventral plates, the ovipositor is situated, as above mentioned. On its apex, there is a narrow median slit in which two openings, one dorsal (the anus) and the other ventral, (the ovipositing aperture) may be seen, and from both sides of its basal portion a pale-yellow bladder-like sac is everted in the same manner as the osmetrium in *papilio* larvae. This is the alluring glands

which secrete the odoriferous fluid to entice the male. And moreover, there is another thin plate situated at its ventral.

2. Male. (Fig. VIII.) It is surrounded dorsally by the thick margin (like a lip) (Fig. VIII, *a*) of the integument of the last segment. This may be called "dorsal lip" and serves as a tactile organ. Ventrally, it is guarded by the chitinous expansion (*b*) of the integument as in the case of the female. It has a triangular projection (*h*) which is well chitinised, and is situated on both sides.

In the space guarded by these two portions of the integument, complicated armatures are present.

First, we will mention the anal armature. It consists of a dark square-shaped frame (*d*), over which a well-developed chitine plate—the cover plate (*c*)—is situated. The latter is joined to the former at the dorsal base, where it is supported by a dark plate (the basal plate) and may be lifted up at will. In the central soft space surrounded by the frame the anus is situated.

Secondly, the sexual armature. At both sides of the anal area, we find a strong ventrally curved chitinous hook—the clasper (*e*). Near the ventral base of the clasper, there is again a projecting triangular plate (*f*) slightly curved inward. These two are the accessory apparatus for copulation.

Lastly, the sexual apparatus. It is situated in the median portion, under the anal area and projects as a needle-shaped organ—the penis, around the base of which a chitinous area is well developed.

Having thus given a brief outline of the structure of the genital armature of the normal moth, we may now proceed to the observation of the structure of the mosaic form.

Fig. IV represents the dorsal portion of the abdomen. In the left half, we can enumerate seven and in the right, eight segments, the latter projecting a little beyond the former. In the terminal segment of the left half (Figs. IV and V) we can observe the dorsal chitine plate (*d c*) and one-half of the ovipositor (*ovi*), from the left base of which an alluring gland (*g*) is present. On the other hand, we can see those male apparatus, such as the clasper (Figs. IV, *e*, and V, *e*) the cover plate (Fig. IV, *c*) the triangular projection (*h*) of the ventral plate etc. on the right side.

The details concerning these various apparatus in the two halves are shown in Fig. VI. It represents the frontal view of the terminal portion of the abdomen.

On the left side of the figure, the median line being the boundary, we see the dorsal portion (*a*) of the seventh segment, under which the dorsal plate (*c*.) is situated. It ends abruptly in the median line, while on the left side it arches down around the basal portion of the ovipositor (*g'*). Near the middle of the median line, we find the half of the ovipositor (*g'*), at the left base of which the alluring gland (*d*) projects. Ventrally situated to the ovipositor and the alluring gland there are two plates (*e*. *f*) which also end near the median line. These are the two ventral plates commonly met with in the normal female moth (compare Fig. VII, *e*, *d*).

Now we will turn to other half. First, we observe the well developed dorsal lip (*b'*) on the dorsal margin. The anal apparatus which can be observed only in the male come next. As the figure represents, the square frame (*s*), the cover plate (*p*) and the basal plate (*c'*) are developed only on the right side and exhibit one-half of the normal form.

In the right side of the anal area, there is a strong and well developed clasper (*d'*) below which we find the triangular accessory plate (*k*). Different from the other apparatus it is represented on either side by a small triangular projection (*k*. *k*).

In the median portion below the ovipositor and the anal area, there is a trace of chitinous portion (*i*) guarded by a chitinous area (*h*) on its right side. There is the rudiment of the penis. On its left side, we meet with a thin plate (*n*) which commonly occurs in the normal female.

Now we see clearly that in this case one half of the worm shows the maternal, and the other half the paternal, characters.

Similar cases have been enumerated by Darwin, who says :—

"According to Rengger, the hairless condition of the Paraguay dog is either perfectly or not at all transmitted to its mongrel offspring; but I have seen one partial exception in a dog of this parentage which had part of its skin hairy, and part naked, the parts being distinctly separated as in a piebald animal." He proceeds still further saying that "when Dorking fowls with five toes are crossed with other breeds, the chickens often have

five toes on one foot and four on the other. Some crossed pigs raised by Sir. R. Heron between the solid-hoofed and common pig had not all four feet in an intermediate condition, but two feet were furnished with properly divided, and two with united hoofs."

With bees, similar cases have been observed by Kraepelin ('73) and others.[1]

From the above facts, we may safely conclude that when a commingling of two characters takes place as a result of crossing, it may occur that all the parental characters separately occupy one half of the body of the offspring, even sexual characters being no exception.

CASE IX.

On the colour of the eggs.

When we got four different kinds of cocoons, white, light greenish white, pale-pinkish yellow and pure yellow, from a crossing of the Japanese white and the Siamese yellow, we kept them separately, as before stated. The following observations were then made.

The eggs of each of these kinds have a characteristic colouration, that is to say, those of the pure white form are always light pale-yellow, while those of the yellow form are clear yellow. Curiously enough, however, those of the pale-pinkish-yellow are of a deep yellow colour, with some brownish shade. Those of the greenish white have a deeper colour than that of the pure white, but its difference from the latter is so slight that we can not always distinguish them clearly.

During the whole series of our experiments, we paid particular attention to this point and have convinced ourselves that this relation holds good in every case. Thus it becomes very easy for us to foretell from the colour of the eggs what kind of cocoons the worms hatched would spin.

[1] Standfuss describe analogous cases in some Lepidopetra in his "Synopsis of Experiments in Hybridization and temperature made with Lepidoptra."

Caspari II. made a description regarding a similar case of *Sat. Pavonia.*

Coutague ('02) also found three silk-worms which were black on one side and white on the other.

Of these four colours of eggs, the yellow predominates over the other three. Next comes the brownish-yellow, and these colors when brought together by crossing are governed by the same law that regulates the hereditary phenomena of the various colours of cocoons. For instance, when we crossed the yellow breed with the pale-pinkish-yellow, the eggs laid by the cross-bred offspring were yellow, and these in the next generation split up into two, one yellow (75%) and the other brownish-yellow (25%).

CASE X.

On the cocoon.

Leaving, then, the question of heredity as regards the colours of cocoons and eggs, and the larval markings, we shall next enquire how the construction of the cocoons is affected by crossing.

The following statements which are an epitome of the results of experiments, Case II and III will give some idea about it.

Before entering into the subject, it seems better to give an account of the cocoons of the parent-breeds.

The cocoon of the Japanese breed (Pl. VII, I, *a*.) is white in colour; cylindrical or oblong in shape, with rounded ends, and a constriction in the middle. The floss is very small, amounting only one or two milligrams in each cocoon; the texture is compact and strong, with nice wrinkles or "grains' on the surface. Its size, quantity of silken matter and fineness of the filament are as follows:—

Size of cocoons { average length.. .....29.3 mm.
average breadth .....15.1 mm.

An average silken matter .....0.19 gr.

Diameter of the filament { floss .....0.021 mm.
middle of cocoon...0.033 mm.
'telette' .....0.020 mm.

That of the Siamese breed (Pl. VII, 1, *b*.), on the contrary, is spindle shaped, with the two ends, or only one of them, pointed; without any constriction in the middle. The texture is very loose, showing no wrinkles

on the surface. Around the cocoon there is abundant floss silk (0.02–0.03 gr.) which forms a loosely wound fibrous matrix in which the cocoon is embedded. The following figures give the size, quantity of silken matter etc.

Size of cocoon { average length .....28–30 mm.
average breadth .....12–13 mm.

Average silken matter.....0.07–0.10 gr.

Diameter of the filament { floss.....0.0195 mm.
middle of cocoon...0.0262 mm.
'telette'...0.0164 mm.

We will now examine the cocoon of the cross-bred form.

a. The form of the cocoon.

In the first cross generation (Pl. VII, II), most of the cocoons spun were spindle or conical in shape, very few of them being oval or ellipsoidal; none of an oblong form with rounded ends, like the Japanese breed.[1] In the next generation (Pl. VIII, I), however, we obtained various forms of which we may first mention those of conical or spindle shape, which are most abundant, next comes those of an oval or ellipsoidal shape. It was sometimes found that both forms had a trace of constriction in the middle. Besides these there were some intermediate forms which could not exactly be distinguished from one another.

The various forms of cocoons appeared in the second generation, as far as we have experimented, have the inclination to separate from one another and segregate into several constant forms, when reared separately from one another.

Thus after selection for three consecutive generations, the oval or ellipsoidal form became a constant form (Pl. VIII, and IX, II); on the other hand, it is very difficult to get a pure spindle form, since some other forms always appeared among them (Pl. IX, I).

With respect to the Japanese oblong form which appear very rarely as an active character, we have failed to trace its ultimate fate by inheritance.

[1] In the reciprocal crossing between 'Changhai Blanc' and 'Jaune Var,' Coutagne ('02) recorded some similar facts.

Now we may say that of the forms enumerated above, the spindle or conical form predominates over the other forms. Next ranks the oval or ellipsoidal form, and lastly comes the oblong or cylindrical form with rounded ends.

b. Floss.

The floss gave the following figures in the first cross generation :—

Pure Japanese parent.....0.001–0.002 gr.
Pure Siamese parent.....0.020–0.030 gr.
Mongrel parent.....0.007–0.017 gr.

In the second and further generations, a great deal of individual differences among the worms reared from the same parent or among the worms derived from different parents, appeared.

The following figures obtained in the second, third and fourth generations will bring out this point :—

The second generation (From the same parent.) .....
1. 0.0065 gr.
2. 0.0045 gr.
3. 0.0085 gr.
4. 0.0035 gr.
5. 0.0155 gr.
6. 0.0220 gr.
7. 0.0380 gr.
8. 0.0250 gr.
9. 0.0115 gr.
10. 0.0110 gr.

The third generation.....
Parent, No. 1... 0.012–0.026 gr., average 0.0176 gr.
Parent, No. 2... 0.006–0.012 gr., average 0.009 gr.
Parent, No. 3... 0.007–0.0195 gr., average 0.0127 gr.
Parent, No. 4... 0.009–0.019 gr., average 0.0162 gr.

Parent, No. 1... { 0.010 –0.0185 gr.
average 0.0152 gr.

Parent, No. 2... { 0.027 –0.009 gr.
average 0.0152 gr.

Parent, No. 3... { 0.0151–0.029 gr.
average 0.0183 gr.

Parent, No. 4... { 0.014 –0.033 gr.
average 0.024 gr.

Although we have failed to establish a constant form from these crossings, yet we have good reason to believe that they will follow the same law regulating the hereditary phenomena of the various forms of cocoons, and that the character to spin much floss is a dominant one.

c. The constriction.

This is one of the most unstable characters of the cocoon. In the first cross, very few of the cocoons have a trace of it. In the second and further generations we have observed its rare occurrence as an active character, but it is always not well developed. In consequence of the scarcity of materials we could not arrive at a definite conclusion with respect to its hereditary phenomenon.

d. The size of the cocoons.

It is pretty constant as the following figures shows :—

The first generation..... { average length ...29.9–33 mm.
average breadth...12.5–15.5 mm.

The second generation... { average length ...29 –36 mm.
average breadth...13 –16 mm.

From the third generation on, however, there were found some individual differences, which may be mentioned as follows :—

Cocoons spun by the worms reared from a parent.	Length. mm.	Average length. mm.	Breadth. mm.	Average breadth. mm.
No. 10.	29–31	30	12.5–14.5	13.3
No. 11.	30–34	32.3	14–15	14.5
No. 12.	28–31	29	12–13	12.6
No. 13.	27–31	29.1	12.5–13.5	13.16

e. Silken matter.

It is intermediate in quantity and remains in a pretty constant state. In the first generation, we got the average figure 0.1329 gr. which in the second and further generations varied between 0.1264 and 0.1028 gr.

f. The diameter of the filament.

This also showed an intermediate character between those of the parents.

In the first and second generations we obtained the following average figures :—

Generation.	Diameter of the floss.	Diameter of the filament in middle.	Diameter of telette.
First.	0.0145–0.0266	0.0218–0.0303	0.0096–0.0218
Second.	0.0121–0.0230	0.0218–0.0314	0.0096–0.0218

Worms were kept until the fifth generation, when we got the following figures :—

Diameter of outer floss.....0.012–0.022 mm.
Diameter of the filament of
the middle of cocoon.....0.0218–0.030 mm.
Diameter of 'telette'.....0.010 – 0.020 mm.

g. The texture of the cocoons.

The texture of the cocoons becomes correspondingly compact and strong with the increase of the silken matter.

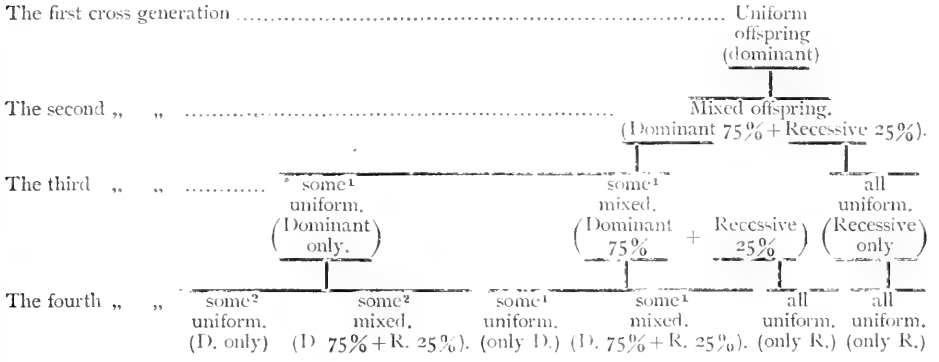
h. Wrinkles or 'grains'.

This is also one of the most unstable characters. During the first three generations, a trace of it could be observed in some cocoons. In the fourth and fifth generations it nearly disappeared.

Of these characters, those mentioned in the paragraphs *e*—*h* are much influenced by the quality of the leaves given, management, climatological conditions etc., and we could not know exactly how much of the change produced was due to the crossing and how much to the influence of management, nutrition etc., and therefore, it is very difficult to draw any definite conclusion as regards the effect of the crossing.

B. SUMMARY AND GENERAL CONSIDERATION.

I. *Monohybrid.* Looking back through the whole course of the hereditary phenomena of the various colours of the cocoons and larval markings of the silk-worm, we may summarise them, in the case of *monohybrid*, as follows :—



This shows such a close parallelism with the law of heredity first enunciated by Mendel (65) in *Pisum* and *Phaseolus*, that without quoting his statement here we would not be able to get a better comprehension of the matter.

In the first cross generation,[3] Mendel says : „ das eine der beiden Stammmerkmale ein so grosses Übergewicht, dass es schwierig oder ganz unmöglich ist, das andere an der Hybride aufzufinden." In the second generation, „ treten nebst den dominirenden Merkmalen auch die recessiven in ihrer vollen Eigenthümlichkeit wieder auf, und zwar in dem entschieden ausgesprochenen Durchschnittsverhältnisse 3 : 1, so das unter je vier Pflanzen aus dieser Generation drei den dominirenden und eine den recessiven Charakter erhalten." He further says that „ Jene Formen, welche in der ersten

[1] These numbers are not constant, sometimes 55% : 45%, sometimes 75% : 25%, etc.

[2] These are also not constant. We once got figures ; 80% of uniform and 20% of mixed offspring.

[3] Our first cross generation corresponds with Mendel's hybrid, his first generation with our second cross generation.

Generation den recessiven Charakter haben, variiren in der zweiten Generation in Bezug auf diesen Charakter nicht mehr, sie bleiben in ihren Nachkommen constant."

With respect to the dominant form appearing in the second generation, he finds „dass von jenen Formen, welche in der ersten Generation das dominirende Merkmal besitzen, zwei theile den hybriden Charakter an sich tragen, ein theil aber mit dem dominirenden Merkmale constant bleibt." And therefore, „Das Verhältniss 3 : 1, nach welchem die Vertheilung des dominirenden und recessiven Charakters in der ersten Generation erfolgt, löst sich demnach für alle Versuche in die Verhältnisse 2 : 1 : 1 auf."

Thanks to the interest taken in the subject by such eminent botanists as De Vries ('00, '03 *Agrostemma*, *Chelidonium*, *Hyoscyamus*, *Lychnis*, *Oenothera*, *Papaver*, *Zea*, *Datura*, *Trifolium* etc.), Correns ('00, '01, *Pisum*, *Zea*), Tschermak ('00, '01, *Pisum*, *Phaseolus*) and others a great deal of light has been recently thrown on this aspect of the problems of heredity.

In animals, studies of poultry (Bateson '02), mice, rats (Cuénot '02, '03, '04; Castle '03; Allen '04; Davenport '04), rabbits (Castle '03), *Helix* (Lang '04) etc. have yielded further evidences of this principle.

Our results with silk-worm crosses, as will be seen, correspond very well with the Mendelian principle above cited, the discrepancy being seen only in some points of detail and is to be attributed to the sexual union of different individuals.

Firstly, Mendel and others proved that one-third of the dominant forms appearing in the second generation remain uniform and constant, and the remaining two-thirds split up again into the parent forms in subsequent generations. This is not the case with the silk-worm. We have not been able to get the constant proportion 1 : 2 between them.

Secondly, in plants, the dominant form appearing as uniform offspring in the third generation remains true, generation after generation. With the silk-worm, on the contrary, some of the uniform offspring remain constant while others break up into the parent forms, like the dominant form derived from a mixed offspring.

Such differences concerning the separation of the pure dominant and hybrid dominant forms may be illustrated as follows :

In the second generation, as Mendel taught us, there are two kinds of dominant forms, one pure or D and other hybrid or DR. As there is no means to distinguish D from DR, mating between these two forms take place at random. From these random matings we may reasonably expect to get the three combinations.

1. D × D, 2. D × DR, 3. DR × DR.

Both D × D and D × DR produce only dominant offspring, while one of them (D × DR), when mated *inter se*, will produce mixed offspring.

Similarly from such random matings it will not be expected to produce pure yellow or D and hybrid yellow or DR in a constant proportion.

Now we may justifiably assert that the yellow character is dominant and the white recessive and the phenomena of the segregation of the two characters are quite the same as in the case of plants observed by Mendel and others.

Cuénot ('02) and Allen ('04) tested the pigmented mice of the second cross generation and found the Mendelian expectation 1 D : 2 DR : 1 R realised.

It is highly interesting to note that Coutagne ('02) obtained a result diametrically opposed to mine by crossing white and yellow breeds, such as 'Blancs de Alpes', 'Bagdad', 'Jaune Var', 'Jaune Defends', 'Petit blanc Pays' etc. In most cases, the white colour dominates the yellow and the offspring is white, yet the resulting hybrid-white, when bred *inter se*, produced both white and yellow offspring, in some cases in the ratio of 1 : 3, in others 1 : 1.

There are, however, many irregular cases recorded by him. For instance, in reciprocal crosses between 'Changhai blanc' and 'Jaune Var', the yellow character behaved as dominant. While 'Jaune Defend × Petit blanc Pays' produced uniform white offspring, the same yellow race mated with 'Bagdad' (a white race) yielded only yellow cocoons.

In dihybrids, he observed more interesting phenomena of segregation and combination of the parental characters.

In crosses between 'Blanc des Alpes (worms white, cocoons white) and 'Jaune Var' (worms striped, cocoons yellow), the first cross produced four kinds of worms :

O′ =striped worms spinning yellow cocoons. 116.
O″ =white worms spinning yellow cocoons. 124.
O‴ =white worms spinning white cocoons. 111.
O⁗ =striped worms spinning white cocoons. 108.

This is an instance of (D+R)R.

In the next generation mated among similars, O′ yielded four kinds of worms in the following proportion :

Striped worms spinning yellow cocoons 236=53.75%,
Striped worms spinning white cocoons 80=18.22%,
White worms spinning yellow cocoons 89=20.27%,
White worms spinning white cocoons 34= 7.74%,

O″ yielded

White worms spinning yellow cocoons 441,
White worms spinning white cocoons 120=24.24%,

O‴ yielded

White worms spinning yellow cocoons 140=25.97%,
White worms spinning white cocoons 399,

And lastly

O⁗ produced

Striped worms spinning yellow cocoons 109=26.45%,
Striped worms spinning white cocoons 180=43.63%,
White worms spinning yellow cocoons 36= 8.73%,
White worms spinning white cocoons 87=21.11%.

In the former two forms, O′ and O″ the yellow character is dominant while in the latter two, O‴ and O⁗ the white dominant. Thus he says "les mnemons du caractère 'Cocon blanc' n'ont pas, dans toutes les races à cocons blancs, la même force de transmission hereditaire. Les mnémons des races 'Blanc des Alpes' et 'Petit blanc pays des Cévennes' sont plus forts que les mnémons de la race, 'Jaune Var'; mais inversement les mnémons de la race, 'Jaune Var' sont plus forts que les mnémons de la race 'Bagdad'.

Similar cases may be quoted from the albino character (which is ordinarily recessive) of Mice and *Matthiola* etc. (Bateson and Saunders, '02,

Castle, '03, Cuénot, '02, Allen, '03, '04 etc.), since it sometimes behaved as dominant.

Hence we may say that such instances occur not rarely in animals and plants.

The principal cause of the discrepancy between the results of Coutagne and of mine, however, seems to rest on the neglect of ancestry of the breeds, because in the former case the breeds used for experiments were derived from various breeds having different ancestry, even hybrid ones are used, while in the latter the lineage of the breeds is quite pure and simple.

It is also very instructive to quote Castle and Allen's hypothesis of "impure recessives" in which case the pigment-forming character (which is dominant) is either partially or completely latent. The white character studied by Coutagne might belong one of such instances.

II. *Back-crossing*. Let us next consider a case of back-crossing a cross-bred form with one of the pure parent-breeds, for which Experiment Case IV furnish a good illustration.

a. First crosses of the cross-bred yellow with the pure white gave two kinds of offspring, one producing uniform yellow worms, another a mixture of yellow and white worms in the proportion of 1 : 1.

In the next generation paired *inter se*, all the yellow forms were disintegrated into their parent-forms in the proportion of three yellows to one white, while the white form remained true to the parents.

A precisely similar result was obtained with the various larval markings of the silk-worm (Case V).

Of the larval characters, Coutagne's ('02) results also confirmed that both black colour and transverse striping are evidently dominants to the normal whitish colour.

b. On the other hand, from crosses between the cross-bred yellow and the pure yellow forms, uniform yellow offspring resulted through two consecutive generations.

According to the Mendelian principle, they should be that:

a. When cross-bred dominant forms are mated with pure recessives, The first generation,

(D+R)R=one half hybrid dominant or yellow and the other half pure recessive or white.

and

The second generation mated among similars,

1. (D+R)×(D+R)=one-third pure recessive or white, two-thirds dominant or yellow.
2. R×R =all recessive or white.

b. When cross-bred dominant forms are mated with pure dominants,

The first generation.....(D+R)D=all dominant or yellow.

The second generation paired *inter se*,

(1) D×D; (2) (DR×DR); (3) D×DR,

or some (1) pure yellow offspring, some (2) mixed offspring of white, and yellow, and the rest (3) a mixture of pure and hybrid yellow worms.

Such aberrations may be explained as follows :—

In our experiments, the cross-bred yellow forms were derived from a mixed offspring of the fourth cross generation between white and yellow breeds and consequently some of them were pure yellow or D while the others were hybrid yellow or (D+R).

By crossing with white or R, therefore, there will be produced the combinations,

1. D×R=hybrid yellow

and

2. (D+R)×R=DR+R=a mixture of hybrid yellow and pure white in the proportion of 1 : 1.

Thus the yellow forms raised from both formulae, when bred together will certainly break up into their components according to the normal monohybrid formula, (D+R)(D+R)=D+2DR+R=a mixture of three dominants and one recessive.

The results actually obtained agree very satisfactorily with this assumption.

In the cross with the pure yellow, on the other hand, we expect to have the following combinations :—

1. D×D or pure yellow.
2. (D+R)×D=D+DR or uniform yellow consisting of one pure yellow and one hybrid yellow.

The former combination D×D will remain constant through further generations, while the latter may produce the three combinations of the parental characters in the next generation :

1. D×DR=D×(D+R)=D+DR=a mixture of pure yellow (50%) and hybrid yellow (50%).

2. DR×DR=D+2DR+D=a mixture of two hybrid yellows, one pure yellow and one pure white.

3. D×D or pure yellow.

There might be chance only for the first and the third combinations to be reared in the second generation, if we keep for the experiment a portion of the offspring descended from the first generation.

This assumption may serve as an illustration for the second case.

From these considerations, we are led to the Mendelian principle cited before.

III. *Dihybrid.* From the reciprocal crosses between the 'pale yellow' and the 'striped white' breeds (the latter is not pure, sometimes pale worms appear), we have obtained interesting combinations and segregation of the parental characters, in the offspring (Case VI).

In the first cross there appeared two kinds of offspring,

the first being all 'striped yellow' worms,

the second being a mixture of 'striped yellow' (50%) and 'pale yellow' (50%) worms,

which is a common phenomenon in a back-crossing already described.

With respect to these two kinds of worms thus raised we learn that the 'pale yellow' form was disintegrated, in the next generation, into two forms, 'pale yellow' (75%) and 'pale white' (25%), as in the second generation of monohybrid, while the 'striped yellow' form produced four kinds of worms, 'striped yellow', 'pale yellow', 'striped white' and 'pale white' in the following proportion :—

In the first group :

'Striped yellow' worms (new form).	55.57%.
'Pale yellow' worms (parent form).	18.35%.
'Striped white' worms (parent form).	19.72%.
'Pale white' worms (latent form).	6.34%.

In the second group
- 'Striped yellow' worms (new form). 58.82%.
- 'Pale yellow' worms (parent form). 18.45%.
- 'Striped white' worms (parent form). 16.42%.
- 'Pale white' worms (latent form). 6.28%.

In the third generation, each of these four forms produced the following kinds of offspring, when bred together with similars in the same brood.

1. From 'striped yellow' forms
   - *a*. A mixture of 'striped white' (25%) and 'striped yellow' (75%) worms.
   - *b*. Only 'striped yellow' worms.
   - *c*. A mixture of 'striped yellow' 74.48%. and 'pale yellow' (25.51%) worms.
   - *d*. A mixture of 'striped yellow' (36.2%), 'striped white' (15.44%), 'pale yellow' (20.6%) and 'pale white' (7.59%) worms.

2. From 'pale yellow' forms
   - *a*. All 'pale yellow' worms.
   - *b*. A mixture of 'pale white' (25%). and 'pale yellow' (75%) worms.

3. From 'striped white' forms
   - *a*. All 'striped white' worms.
   - *b*. A mixture of 'pale white' (25%) and 'striped white' (75%) worms.

4. The 'pale white' form produced only offspring like the parents.

Results closely similar to these have been disclosed by Mendel in *Pisum*, and by De Vries, Correns, Tschermak, Saunders etc. in various kinds of plants.

As regards some discrepancies existing between our results and those of some botanists above referred to, the following formulae derived from the Mendelian conception would give a clear explanation and serve as a verification of our result.

Let the characters be represented by

A = yellow character,
B = striped character,
a = white character,
b = pale character.

Then the 'pale yellow' breed would be represented by Ab, the 'striped white' by aB. As the latter form is not a pure strain it may be either $a\,B$ or $(a\,B+a\,b)$.

In the first reciprocal cross between them we shall get the combinations,

a. $Ab \times aB$ or uniform 'striped yellow' offspring.

b. $Ab(aB+ab)$[1] = a mixture of 'striped yellow' and 'pale yellow' in the proportion of 1 : 1.

In the second generation, $(Ab+aB)$ or the 'striped yellow' form, when bred *inter se*, will give the combinations,

$$(Ab+aB)(Ab+aB)=(A+2Aa+a)(b+2bB+B)=Ab+$$
$$ab+AB+Ba+2Aab+2AaB+2aBb+2ABb+4AaBb,$$

From which we estimate the following figures :—

'Striped yellow' form (AB, 2A*a*B, 2AB*b* + 4A*a*B*b*). 56.25%.

'Pale yellow' forms (A*b* + 2A*ab*)..... 18.75%.

'Striped white' forms (B*a*, 2*a*B*b*)..... 18.75%.

'Pale white' forms (*ab*)..... 6.25%.

If mating takes place among similars at random, various kinds of offspring may be expected in the next generation, since worms of similar external appearance may have different combinations of parent-characters, as the formulae show.

Thus, with the 'striped yellow' forms, we expect to have some or all of the following combinations in the next generation.

a. $AB \times AB$ or uniform 'striped yellow' offspring.

b. $Aa\,B \times Aa\,B$ or a mixture of three 'striped yellows' and one 'striped white'.

c. $AB\,b \times AB\,b$ or a mixture of three 'striped yellows' and one 'pale yellow'.

d. $Aa\,Bb \times Aa\,Bb$ or a repetition of the last generation.

e. $AB \times Aa\,B$ or seemingly 'striped yellow'.

f. $AB \times Aa\,Bb$ or seemingly 'striped yellow'.

g. $AB \times AB\,b$ or seemingly 'striped yellow'.

h. $Aa\,B \times AB\,b$ or a mixture of 'striped yellow' and 'pale yellow'.

[1] $Ab(aB+ab)=(Ab+aB)+(A+a)b$.

i. $Aa\mathrm{B} \times Aa\mathrm{B}b$ } From these we may produce a mixture of 'striped
j. $A\mathrm{B}b \times Aa\mathrm{B}b$ } yellows' and 'striped whites' or 'striped yellows', 'pale yellows' and 'striped whites' etc.

With the 'pale yellow' forms,

a. $Ab \times Ab$ or constant pale yellow forms.

b. $Aab \times Aab$ or a mixture of three 'pale yellows' and one 'pale white'.

c. $Ab \times Aab$ or a mixture of pure and hybrid 'pale yellows'.

The 'striped whites' ($B\alpha$ and $\alpha\mathrm{B}b$) also produced two kinds of offspring, one mixed, consisting of 'striped white' and 'pale white' worms in the proportion of 3 : 1 and others, pure ones. Lastly the 'pale white' form which has only one combination '*ab*' will remain as a constant uniform breed.

Now we see clearly that the estimated numbers exactly agree with those actually obtained.

IV. *Modified dihybrid.* We have now to consider the crosses between the Japanese white and the Siamese yellow breeds (Experiment Case II) which showed some very interesting phenomena of heredity.

As already stated, the first cross gave rise to uniform yellow offspring which when paired *inter se* broke up into four different forms, yellow (parent form), white (parent form), pale-pinkish-yellow (new form) and greenish white (new form), their respective numerical proportion being 70 : 12 : 21 : 24.

On the Mendelian theory, we should expect all the four forms to result from them in the proportion of yellow 72 : white 8 : pale-pinkish-yellow 24 : greenish white 24 with which the observed numbers agree fairy well.

Each of these four forms strictly obeys the Mendelian law of monohybrid when bred *inter se*.

With the yellow form which has the highest dominancy of the four characters we have obtained the following combinations and separation of the parental characters.

The first generation. Uniform yellow form.

The second generation 1. Uniform yellow form.

2. A mixture of yellow (75%) and white (25%) forms.

3. A mixture of yellow (75%) and pale-pinkish-yellow (25%) forms.

4. A mixture of yellow (56.43%), pale-pinkish-yellow (19.57%) and white and greenish white (23.98%) forms.

The third generation.

Mating took place between the yellow forms derived from the parent which produced four kinds of cocoons in the last generation (No. 4). They again produced four kinds of offspring :

1. Uniform yellow form.
2. A mixture of yellow (75%) and white (25%) forms.
3. A mixture of yellow (75%) and pale-pinkish-yellow (25%) forms.
4. A mixture of yellow (60.99%), pale-pinkish-yellow (17.02%) and white and greenish white (21.98%) forms.

When we crossed *Theophila Mandarina* with *Bombyx mori*, a similar phenomenon of the disintegration of the parental characters took place.[1]

As early as 1865, Mendel observed similar cases in the crosses between *Phaseolus nanus* and *Ph. multiflorus* and advanced the opinion „dass die Blumen—und Samenfarbe des *Ph. multiflorus* aus zwei oder mehreren ganz selbständigen Farben zusammengesetzt sei, die sich einzeln ebenso verhalten wie jedes andere constante Merkmal an der Pflanze."

De Vries ('03) confirmed Mendel's opinion in various crosses of *Antirrhinum*, *Papaver*, *Mephisto*, *Silene* etc.

Like results are recorded by Allen ('03, '04), Castle ('03), Bateson Cuénot etc. in various coat-colours of mice, guinea-pigs and rabbits.

Our results above referred to constitute an excellent evidence of the truth of Mendel's opinion.

In plants, however, De Vries obtained only one kind of offspring exhibiting dihybrid phenomena, while in the silk-worm we met with four kinds of offspring, each of which exhibited different combinations of the parent-characters. Thus the first, second and third offsprings belonged to

[1] A full account of this investigation will, I trust, be shortly published as a separate paper.

the monohybrid, while the fourth was dihybrid which may be explained as follows :—

White ..... 6.25%) 25%.
Greenish white..... 18.75%)
Pale-pinkish-yellow..... 18.75%.
Yellow ..... 56.25%.

The discrepancy between the results obtained in Plants and the silk-worm is due to the fact of the sexual union between different individuals and may be easily explained by the considerations discussed in paragraph III, dihybrid.

In his noble work, "the variation of animals and plants under domestication" Darwin referred to some similar phenomena and says: "the crossing of distinct forms, which have already become variable, increases in the offspring the tendency to further variability, by the unequal commingling of the characters of the two parents, by the reappearance of long-lost characters and by the appearance of absolutely new characters."

The disintegration of the parent-character as a result of crossing has also been observed by Tschermak ('01) in *Phaseolus*. Focke ('81) gives many similar instances in various species of plants. Correns ('00) has noticed in *Matthiola* crosses that „ in der zweiten Generation treten neben den Blüthenfarben der Eltern zwei neue Farben auf." [1]

Thus we are led to the conclusion that there are many compound characters which behave exactly like a single independent character, but when crossed with new characters they are decomposed into their components.

Before leaving this subject, it will be of some interest to consider the parentage of the silk-worms used for our experiments.

As already described, both the Japanese white and Siamese yellow races bred true for several generations. In the aboriginal races reared by the Laos, however, we have often observed a greenish white form mixed among the yellow race. It is not the case with the pale-pinkish-yellow form. We have never seen any pale-pinkish-yellow form among the Siamese yellow

[1] Examples may be multiplied if we enumerate those observations made by Bateson and others ('05) with animals and plants.

race or among the crosses between the Siamese yellow and the Siamese greenish white breeds. Yet, when we crossed the yellow with the Japanese white, a pale-pinkish-yellow form normally appears. This form is not, however, an intermediate form derived from the union of the two parent-characters.

Similarly the crosses between the French yellow race (Var), and any one of the Japanese white forms, such as 'Usuaka', 'Kuni-ichi', or some of the divoltine races, gave a pale-pinkish-yellow form.

If we adopt the opinion of Castle ('03) and Allen ('04) who say that "whatever may be the nature of the pigment-producing elements, it is plain that the albino possesses these, and that they undergo segregation as the pigmented animals", we may say that the pale-pinkish-yellow character was transmitted through the Japanese white parents, in which it was lying dormant.

At any rate, it is certain that there are many compound characters which behaved exactly like a single character, when paired *inter se*.

V. *Another instance of modified dihybrids.* We have another interesting case of dihybrids produced by crossings between striped and common breeds (neither is pure and "pale" character lies latent in them), (Case VII).

Contrary to the other cases, they gave three kinds of offspring in the first cross.

1. A mixture of { Striped .....(48.21%) or 2. / Common.....(26.84%) or 1. / 'Pale'.....(24.93%) or 1.
2. A mixture of { Striped .....(about 50%) or 1. / Common .....(about 50%) or 1.
3. Striped only.

This may be also explained by the Mendelian principle.

As both striped and common parents have a recessive character in the latent state, the former may be represented by (S+N) or S and the latter by (C+N) or C. S=stripe, C=common, N='Pale'.

If mating takes place among them at random, we should expect to have some or all of the following combinations of characters :—

1. (C+N)×S=a mixture of two kinds of seemingly striped worms.
2. (S+N)×C. = a mixture of seemingly striped and seemingly common worms.
3. S×C = seemingly striped worms.
4. (S+N)×(C+N). In this combination, we take for granted that both the characters in the parenthesis sometimes act as a whole character, sometimes as separate characters. In the former sense, the result would be uniform striped worms in which the other two recessive characters lie dormant, that is to say, (C+N)+(S+N). In the latter, it would be (C+N) ×(S+N)=CS+SN+CN+N or two striped, one common and one pale worms.

Thus, three kinds of offspring may be produced from this cross :—

1. Uniform striped offspring.
2. A mixture of striped and common worms in the proportion of 1 : 1.
3. A mixture of three kinds of worms in the proportion of two striped, one common and one pale worms.

These precisely agree with the actual figures given on the preceeding page.

In the next generation, the uniform striped offspring broke up into three kinds of worms in the proportion :

Striped worms in one case 74.9%, in another 74.44%.
Common worms in one case 18.59%, in another 19.29%.
Pale worms in one case 6.39%, in another 6.29%.

This may be due to the fact that in the first generation, as we have seen, there is a combination (C+N)+(S+N) for the striped form. By pairing them we can produce the following combinations and disintegration of the parental characters :

(C+2CN+N)×(S+2SN+N)=CS+2CNS+NS+2CNS+4CNS+2SN +CN+2CN+N=12 striped+3 common+one pale,

from which we estimate

Striped $\frac{12}{16}$ or 75%.
Common $\frac{3}{16}$ or 18.75%.
Pale $\frac{1}{16}$ or 6.25%.

This may serve to illustrate the phenomenon of the segregation of the parental characters in the second generation.

The striped form raised from this brood when paired *inter se* produced many kinds of offspring, each of which has a different proportion of the various kinds of worms, as in the case of the colour-characters of the cocoon before mentioned. In the latter case, however, the combination "yellow + pale-pinkish-yellow + greenish white and pure white" is natural while in the former the combination "striped + common + pale" is artificial, but the resulting phenomena are quite the same in both cases.

Furthermore, we see that as a result of crossing between the Japanese common form and the Siamese striped form a new constant form having both parental characters commingled has been produced[1] (Pl. X, II).

Coutagne ('02) observed a parallel case in some crosses between "Bagdad vers noirs" and striped "Jaune Var" in which both black and striped characters appears as an active character. We observed the same phenomenon in crossing various Chinese races of the silk-worms.

The facts above enumerated together with De Vries' results with *Antirrhinum* may afford excellent illustrations for the hybridological analysis and synthesis of plants and animals.

With regard to the spotted mice, Castle and Allen ('03, '04) developed the 'mosaic' theory of gametes, but whether it may be adopted to our worms we must wait the result of further experiments.

These very facts and considerations referred to in the preceding paragraphs furnish a further welcome proof for the correctness of the Mendelian theory and confirm that his theory may be applied, with equal exactness, both for animals and plants.

[1] Such an example is seen in the case of the 'walnut' comb of poultry, produced by crossing rose-comb with pea-comb poultry. (Bateson '05).

VI. *Non-Mendelian phenomena of heredity.* Leaving the Mendelian cases, we come now to consider the non-Mendelian group of hereditary phenomena.

Mendel ('69) has already confirmed in the *Hieracium* crosses that two or more different forms appear in the first cross; each of which when bred *inter se* come true to its parent in subsequent generations.

Millardet's false hybrids ('94) which ordinarily show only the characteristics of one of their parents and come true to this type in later generations may be mentioned as another example.

Recently a good number of non-Mendelian crosses together with Mendelian cases[1] have been discovered by many eminent naturalists.

De Vries ('01, '03,) who has done some epoch-making works on the physiology of heredity has observed many cases where the Mendelian law does not hold good and says that „Den Mendel'schen Spaltungsregeln folgen im Allgemeinen nur phylogenetisch jüngere Eigenschaften, sogennante Rassenmerkmale; von diesen aber wiederum nur ein Theil. Welcher Theil, weiss man aber auch jetzt noch nicht."

Correns (01,[a] 01[b]) has crossed various kinds of *Zea mays* and enumerates four different types of hereditary phenomena of various characters: the first is the *pisum* type in which „das Merkmalspaar ist heterodynam und schizogon; the second, „das Merkmalspaar ist heterodynam und homöogon; the third *Zea* type in which „das Merkmalspaar ist homödynam und schizogon" and the last *Hieracium* type in which „das Merkmalspaar ist homodynam und homöogon".

Tschermak ('00, '01) has observed some similar phenomena in crossing various kinds of peas and beans.

In crossing *Lychnis*, Saunder ('02) also finds that the leaf-characters obey Mendel's law while the colour of the seeds and corolla, the position of the capsule teeth do not follow it.

Davenport ('04) also enumerated some non-Mendelian cases and says: "while Mendelian principles seem applicable to some cases of crosses

[1] A good illustration of blending inheritance is found among rabbits and guinea-pigs by Castle ('05).

between sports and the normal species, there seem to be others where neither Mendel's nor Galton's law of inheritance holds."

Our results with the silk-worm crosses may add another example to the above category,[1] since, as has been said before, some characters, such as the colour of the cocoon, the larval markings, are governed by Mendel's law, whilst others, such as the form of the cocoon, exhibit quite different phenomena of heredity.

The brood-character of the silk-worm, such as univoltine, divoltine, multivoltine etc. is another example of non-Mendelian characters.

Thus when we crossed a multivoltine with univoltine breed, the eggs laid by the moth were either pure maternal or pure paternal, very rarely a mixture of both parents as the following table shows :[2]

1. *a*. Japanese univoltine ♂ × Divoltine ♀.
   Eggs laid were all divoltine or maternal.
   b. Japanese univoltine ♀ × Divoltine ♂.
   Eggs laid were all univoltine or maternal.

2. *a*. European univoltine yellow ♂ × Japanese divoltine ♀.
   All divoltine, or maternal.
   b. European univoltine yellow ♀ × Japanese divoltine ♂.
   All univoltine or maternal.

3. Japanese univoltine ♀ × Divoltine ♂. All divoltine or paternal.

4. Japanese divoltine ♀ × Siamese multivoltine ♂.
   All multivoltine or paternal.
   Japanese divoltine ♂ × Siamese multivoltine ♀.
   All univoltine or paternal.

Those forms raised from the first cross do not remain true to the parents in subsequent generations. Even when we selected multivoltine forms for five generations we failed to get any constant multivoltine breed.

Summing up the results of this very hasty survey, we may assert that of various characters of a species or variety of animals and plants some are

[1] According to the experiment of Coutagne ('03) the character "richesse de soie" shows, to all appearances, a non-Mendelian instance, not undergoing any sharp gametic segregation.

[2] The results of further experiments show that the first cross is always maternal in crossing pure breeds. (Note, added May, 1906).

governed by Mendel's law, while others follow other laws which can not be so clearly formulated as Mendel's.

There are, however, many irregular cases observed by many naturalists. Even those characters which are governed by Mendel's law exhibit apparent exceptions from the general rule, as Correns ('02) has observed it in Maize. From his extended experiments with poultry, Bateson ('02) also says; "even those characters which follow the Mendelian law, sometimes produce aberration or oscillation and that not only different individuals similarly bred may give different proportion, but that these proportions may change also at different times".

Some coat colours of mice possibly come under the similar category. While Cuénot ('02) states that the gray and white characters follow Mendel's law, Darbishire ('03) finds that in crosses between a peculiar race of partial albino mice and true albinos, the albinism does not entirely disappear in the offspring. Moreover, his results indicate that not all albinos breed alike when crossed with the same pigmented stock. Similar results are recorded by Castle ('03), Allen ('03, '04) who distinguished two kinds of recessives, pure and impure. Spotted mice, guinea-pigs, rabbits[1] recorded by Castle and Allen ('03, '04) and others may add another example, since spotted rats or mice are crossed either with gray individuals (dominants) or with albinos (recessives) the offspring are commonly all gray or black in colour, none spotted. Such an individual is called a "mosaic" by Castle and Allen. A like result also is recorded by Davenport ('04).

The result obtained by Wood ('03) with rabbits[2] is far from the Mendelian expectation.

Much more complicated phenomena have been discovered by Saunders ('02), who says that in the *matthiola* crosses the result obtained are so complex that it is difficult to draft statements which shall give a precise and comprehensive view of the phenomena. In some extreme cases, two

[1] See Castle's works "Heredity of Coat characters in guinea-pigs and Rabbits, and Recent discoveries in heredity and their bearing on animal breeding." 1905. (Added May, 1906).

[2] Castle ('05) finds that there are some coat-characters which conform in their inheritance to Mendel's law of heredity, while the lop-eared condition is probably a non-Mendelian character in its relation to normal ears (Note, added May, 1906).

sister plants belonging to the half-hoary type (dominant form) when crossed with the glabrous form (recessive) produced 72 plants all glabrous, although when crossed with another glabrous strain the same two individuals gave the usual results.[1]

Furthermore, she says that with respect to the leaf-character and seed-colour the dominancy is not absolute in *Matthiola* and thus in crosses of pure dominant forms intermediate or recessive sometimes appear.

Examples may be increased when we quote the statement of Tschermak ('01) who says that in peas and beans „in der ersten Generation die Langform der Hülse in dem einen Falle Dominanz, in anderen Gleichwerthigkeit, ähnlich die Schmalform. Die langspitze Form war gar in einer Combination dominant, in der Anderen (fast) recessiv. Die Walzenform des Samens (Zweiter Generation) einerseits dominant, anderseits recessiv, in einer dritten Verbindung gleichwerthig: die Langform das einemal recessiv, das anderemal dominant: das Merkmal „gedrückt" recessiv, beziehungsweise gleichwerthig."

Correns ('00) also enumerates many non-Mendelian cases in *Matthiola*.

Quite recently McCracken ('05) has published an interesting paper concerning some crosses of *Lina Lapponica*. Although he says that it shows no exact parallelism to the Mendelian result, yet when his result is compared with ours, we shall at once be struck with the similarity between them. The principal discrepancy lies in two points: firstly that in the first cross (Table I) the recessive parents produced some dominant offspring and secondly that in the expected case of (D+R)R (Table VIII) an unexpected proportion of recessive forms appeared. Such may happen from the impurity of the character chosen for the experiments.

Lastly we shall consider the results of Standfuss ('96, '00), who has crossed various species of *Saturnia*, such as *S. pavonia*, *S. pyri*, *S. spini*. etc. He did not refer to the Mendelian principle, yet his illustrations (Plate III)

[1] Bateson, Saunders and others ('05) find that even 'albino' plants gave coloured offspring and they consider it to be reversion. We are inclined to believe that concerning the character 'albinism' there exists a close parallelism between the results obtained by Bateson and Saunder (with plants) and those obtained by Castle and Allen (with Mice and Rabbits). (Note, added May, 1906.)

give us some notions concerning the hereditary phenomena of various characters of these insects.

With respect to the larval markings, we see that the *spini-form* predominates over those of *pavonia* and *pyri*, and that of *pavonia* over that of *pyri*. The produced forms, however, do not exhibit uniform dominant character but certain variations are to be observed. A similar phenomenon has been observed by myself in the crosses between *Bombyx mori* and *Theophila Mandarina*.

With regard to the structure of the cocoons we may again observe that the characteristic architecture for the exit-hole of the cocoon of *Pavonia* or *pyri* predominates over that of *spini* (see his Figs. 1, 2, 3, 4, 5), while the general form is intermediate.

Owing to the scantiness of the worms reared by him from a brood, we can not draw any exact conclusion with regard to this interesting problem.

Phenomena of heredity being so complicated and irregular in some crosses, Weldon ('02, '03) has advanced the opinion concerning the ambiguity of the Mendelian categories and says "that segregation of seed-characters is not of universal occurrence among cross-bred peas, and that when it does occur, it may or may not follow Mendel's law. The law of segregation, like the law of dominance, appears therefore to hold only for races of particular ancestry. In special cases, other formulae expressing segregation have been offered, especially by De Vries and by Tschermak for other plants, but these seem as little likely to prove generally valid as Mendel's formula itself".

From our experience in silk-worm rearing we are struck, however, with the belief that the irregularity may be due to the impurity of the characters and strains chosen for the experiment, and to arrive at any satisfactory conclusion concerning the irregular cases, further extended investigations are needful.

The facts and considerations above enumerated afford some help for the explanation of the well-known fact that the offspring of the first cross generation are generally uniform, while the subsequent generations produced by these hybrids display a diversity of characters.

Darwin ('88) attempts an explanation of it and says: "hybrids in the first generation are descended from species (excluding those long cultivated) which have not had their reproductive systems in any way affected, and they are not variable; but hybrids themselves have their reproductive systems seriously affected, and their descendants are highly variable".

Weismann's view ('92, '04) based on the phenomena of 'reducing division' deserves much attention. In "Germ plasm", he says "as this halving of the germ-plasm occurs, in a different manner in different instances, we may presuppose that it will also exhibit differences with regard to the proportion of paternal and maternal idants which come together in each germ-cell in consequence of the reducing division; and this supposition is most satisfactorily borne out by the facts, for it is well known that the offspring of hybrid plants produced by fertilization with their own pollen, become very variable in the following generation. It is evident, indeed, that they must vary greatly, according to whether each one has received a greater number of maternal or paternal ids, or an equal number of both, from the two germcells which combine in the process of fertilization to produce this particular individual".

According to the Mendelian principle, we may ascribe them to the dominancy and recessiveness of the characters and their segregation.

The dominance of some characters over other antagonistic ones certainly produce slight variability in the first cross, since in this cross only dominant characters appear as active, while when the cross-bred form is paired *inter se*, most of the parent-characters appear as active components. Even in the case of dihybrid we may get four different kinds of offspring. If plenty of different characters are mingled together we may safely expect to produce abundant combinations of characters according to the law of combination.

The State of things would become much more complex and diversified when other characters which do not follow Mendel's law are commingled.

In addition to these cases, the frequent occurrence of compound characters may be mentioned as a factor to produce a diversity of forms in the second or further generations.

From the industrial point of view the results obtained with the silk-worm may have some economic importance, since they would give some help for

the selection of cocoons and larvae which is one of the difficult and most important things for silk-worm breeders.

To get pure white race from mixed yellow race, sixty-five years' selection have been practised in France (after Darwin) and the white race called "Sina", by careful selection during the last 75 years, "est arrivé á un tel'etat de pureté, qu'on net voit pas un seul cocon jaunes dans des millions de cocons blancs."

Hutton ('64) met with a similar difficulty when he tried to select the dark brown or blackish brindled worms from the common race.

Our silk-worm breeders have similar experiences concerning the selection of the cocoons and the larval markings.

This difficulty will be overcome if we follow Mendel's law, which is highly to be recommended to worm-breeders for the selection of their breeds.

The writer wishes to express here his indebtedness to Prof. Ishikawa. He is also indebted to Messrs. Y. Takano and Y. Nagashima, assistant in the Royal Sericulture Department in Bangkok who helped him in rearing the worms.

Summary.

1. Of the various characters of the silk-worm, some strictly follow Mendel's law of heredity while others are governed by other laws.

The colour of the cocoon and the egg and the various larval markings belong to the former category, and the shape of the cocoon and the brood-characters such as uni, di and multivoltine etc. to the latter.

2.[1] Among the various larval markings which we have tested, the striped marking comes first in its dominancy, next the normal marking and last the "pale" one. With regard to the colour of the cocoon, yellow comes first, next ranks pale-pinkish-yellow or flesh, then greenish white, and pure white[2] in succession. Hence in the highest dominant form, all the others may lie dormant for a generation, sometimes more.

[1] A parallel case regarding the Coat-characters of guinea-pigs is recorded by Castle ('05).

[2] That albinism is a recessive character in mice, rats, and guinea-pigs has been proved by many authors. Farabee's observations (Castle, '03) indicate that the same is true even in Man.

In plants the depigmented condition is generally recessive. It is probable, therefore, that the recessiveness of albinism is a general law of heredity in animals and plants.

3. Thus, the yellow form in which other recessive characters are lying latent, when paired *inter se*, exhibit complicated phenomena of the segregation of the parent-characters which possibly give a verification of the Mendelian principle :

The first generation, Uniform yellow form.

The second generation,
1. Uniform yellow form.
2. A mixture of white (25%) and yellow (75%) forms.
3. A mixture of pale-pinkish-yellow (25%) and yellow (75%) forms.
4. A mixture of greenish white + white (23.98%), pale-pinkish-yellow (19.57%) and yellow (56.43%) forms.

The third generation, The yellow form derived from the mixed offspring which produced four kinds of worms again repeated the same phenomenon of the disintegration of the parental characters, producing the four kinds of offspring as the last generation.

The same phenomenon has been repeated in subsequent generations. (See Case II).

4. Phenomena similar to those cited above have been observed in the crosses of various larval markings. (See Case VII).

5. In crossing striped race with common one (both having the character 'pale' in the latent state), an interesting instance of modified dihybrids were obtained. It is quoted as follows :

The first cross generation, Uniform striped offspring.

The second cross generation, A mixture of striped (about $\frac{12}{16}$ or 75%), common (about $\frac{3}{16}$ or 18.75%) and 'pale' (about $\frac{1}{16}$ or 6.25%) forms.

This may serve as an another verification of the Mendelian principle.

6. The crosses between the Japanese oblong and the Siamese spindle forms give in the first cross two kinds of forms, the spindle and the ellipsoid. Most of them, however, belong to the former. In the second generation

paired among similars, they split up into various forms, of which some become constant while others again produce various forms when they were mated with similars. But after selection, we can gradually sift out the inconstant ones.

Of those various forms, the spindle or conical shape stands highest in dominancy, next comes the oval or ellipsoidal form and lastly the oblong or cylindrical form rounded at both ends.

7. With regard to the brood characters, the same character sometimes behaves as dominant, sometimes as recessive, according to the sex of the parent, and the order of segregation is not so regular as in other cases.

8. There are many compound characters[1] which behave exactly like a single independent one, while when crossed with new characters they are disintegrated into the component ones. Each component character thus produced behaves as an independent one, breeding true to the parent.

9. Conversely two independent characters belonging to the parents or different breeds may be combined into one form when we cross them and the resulting form breeds true, as if it is single independent character.

10. When commingling of two characters takes place, as a result of crossing, sometimes it occurs that the characters of both parents or breeds even sexual characters occupy each half of the body of an individual. (See Case VIII).

11. Those facts and considerations above referred to may serve to explain the well-known phenomenon of crossing: the slight variability of hybrid in the first cross and the greater variability in subsequent generations. (P. 384—385).

June 20th, 1905.

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[1] Morgan's interpretation ('05) concerning the yellow mice obtained by Cuënot deserves much attention. (Added May, 1906).

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EXPLANATION OF PLATES.

PLATE VI.

Fig. I. Represents larvae of Japanese common (*a*) and French striped (*b*) races. Nat. size.

Fig. II. Represents mosaic larva (*a*) and moth (*b*) produced from crossing of above races. Nat. size.

Fig. III. Represents wings of the same mosaic moth. *a*. *b*, right wings; *c*. d, left wings. Little magnified.

Fig. IV. Dorsal view of the same moth. 8th, eighth abdominal segment; *dc*, dorsal chitine plate; *ov*, ovipositor; *g*, alluring glands; *c*, cover-plate; *e*, clasper; *h*, projection of ventral plate.

Fig. V. End of the abdomen of the same moth, more magnified. Lettering as in preceding figure.

Fig. VI. Frontal view of the sexual armature of above moth. Little magnified. *a*, dorsal portion of the seventh segment; *a'*, dorsal portion of the eighth segment; *c*, dorsal plate; *g'*, ovipositor; *d*, alluring gland; *e.f.* two ventral plates; *b'*, dorsal lip; *p*, cover plate; *c'*, basal plate; *d'*, clasper; *k*, triangular accessory plates; *h*, chitinous area; r, rudiment of penis; *u*, thin plate.

Fig. VII. Female genital armature. Little magnified. *a*, dorsal plate; *b*, ovipositor; *c*. *d*, ventral chitine plates.

Fig. VIII. Male genital armature. Slightly magnified. *a*, dorsal lip; *b*, ventral plate; *h*, triangular projection of ventral chitine plate; *c*, cover plate; *d*, anal frame; *e*, clasper; *dc*. basal plate; *f*, triangular plate; *p*, penis.

PLATE VII.

No. I. Represents cocoons of Japanese (*a*) and Siamese (*b*) races; *c*, yellow cocoon; *d*, pale-pinkish-yellow cocoon.

No. II. Represents cocoons (all perforated) produced by worms raised from a cross between above races.

PLATE VIII.

No. I. Represents cocoons (yellow and white) spun by worms of the second cross generation of the above cross. Some cocoons are perforated.

No. II. Represents cocoons of pale-pinkish-yellow form (fourth generation) which become constant.

PLATE IX.

No. I. Represents cocoons of conical form which produce various kinds of cocoons. (Third generation).

No. II. Represents cocoons of pale-pinkish-yellow form (third generation) which become constant after the selection.

PLATE X.

No. I. Represents yellow and white cocoons produced from a mother-moth. Each "mounting basket" represents a whole brood from a parent.

No. II. Japanese common marked worms and new worms derived from the crossing between striped and common worms.

No. III. Represents three kinds of worms. *a*, Siamese striped worm; *b*, pale worm having no markings; *c*, Japanese common worm.

PLATE XI.

A. Represents Siamese pale worms having no markings.

B. Represents Siamese striped worms.

Fig. I.

Fig. II.

Fig. III.

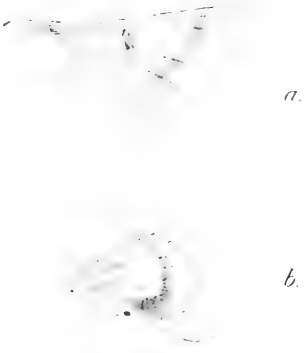


Fig. IV.

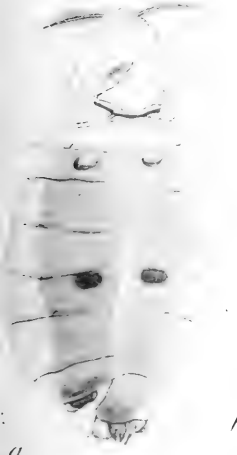


Fig. V.

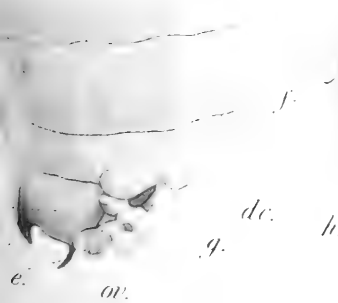


Fig. VII.

Fig. VIII.

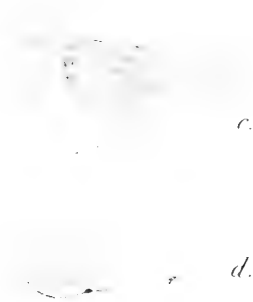
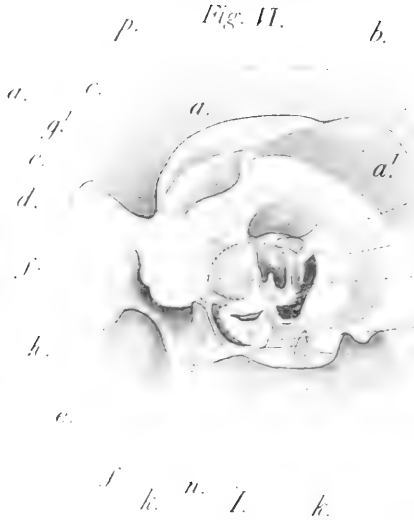
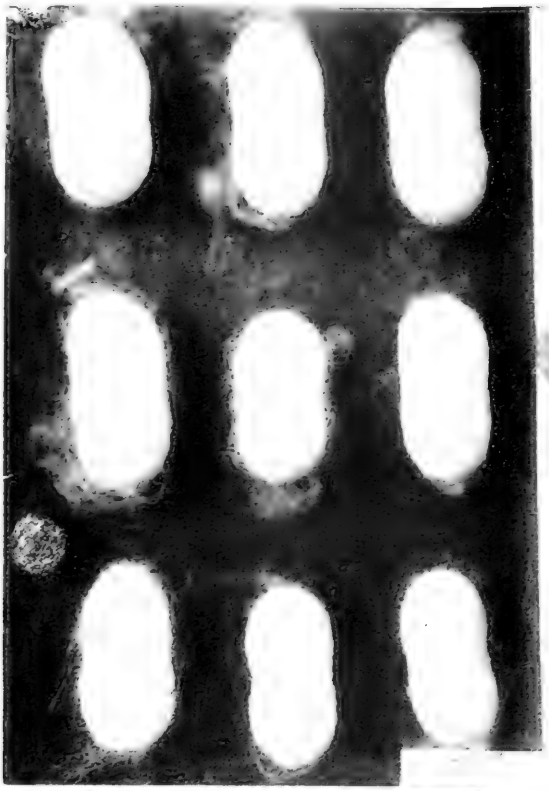


Fig. VI.



I.

a.



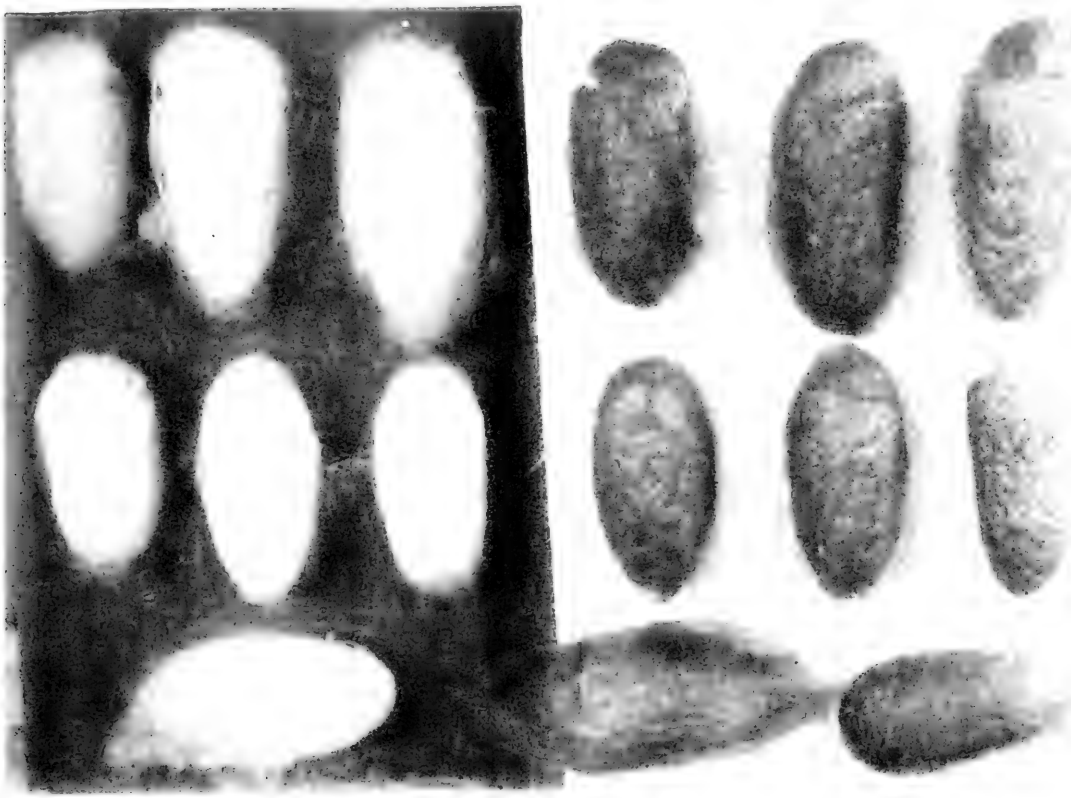
b.

c.

d.

II.

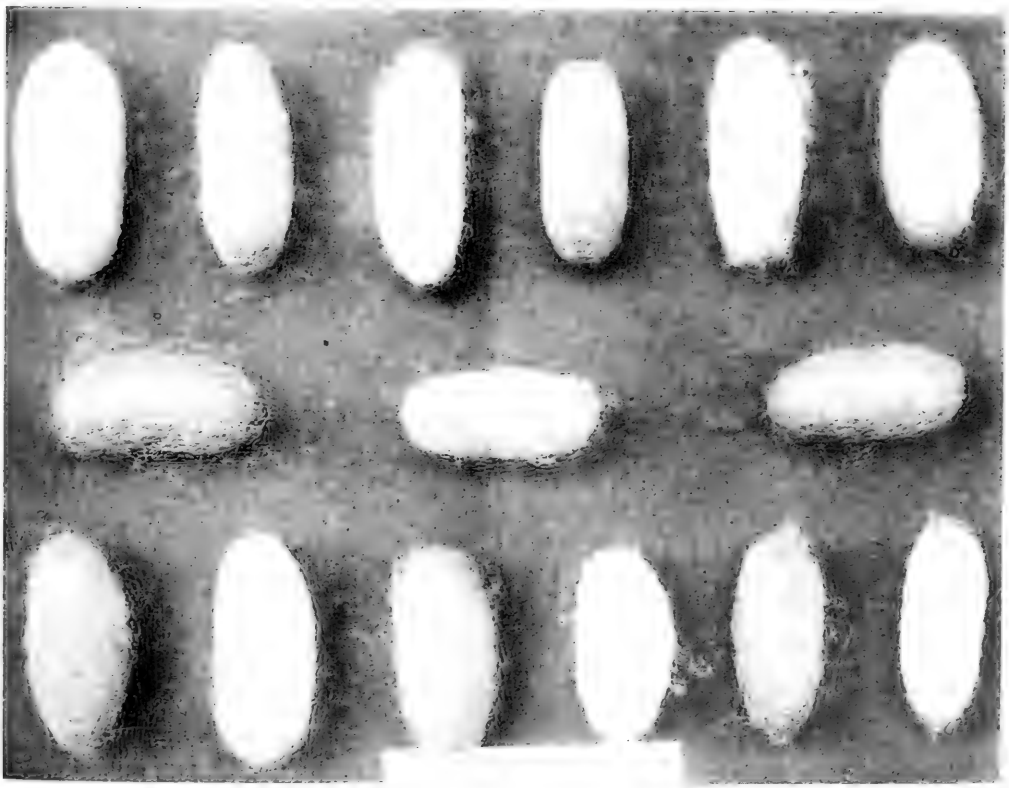
I.



II

I.

II.



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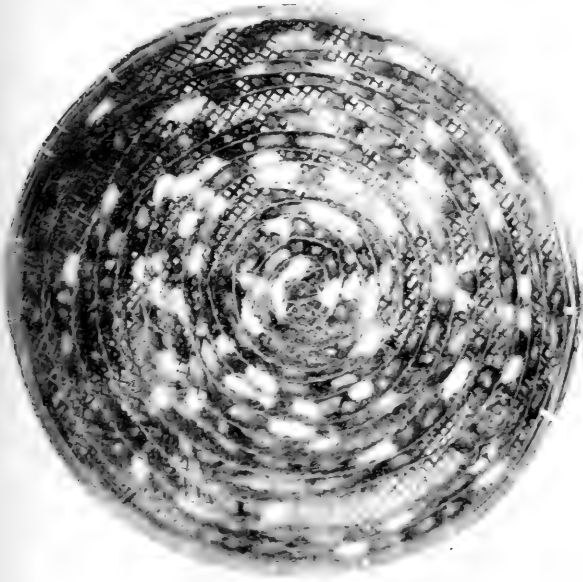
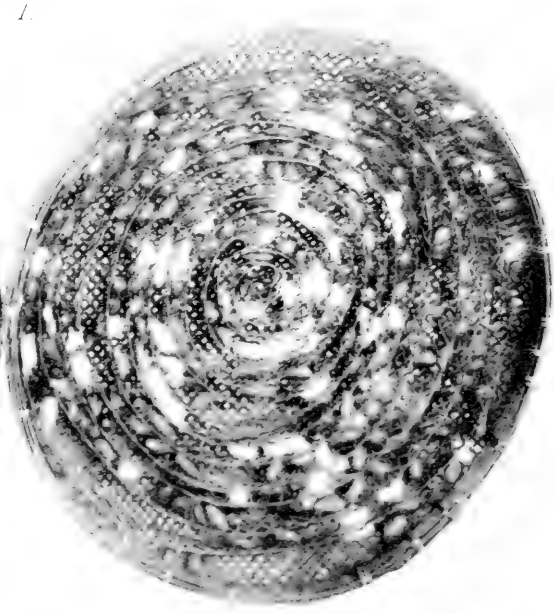




II.

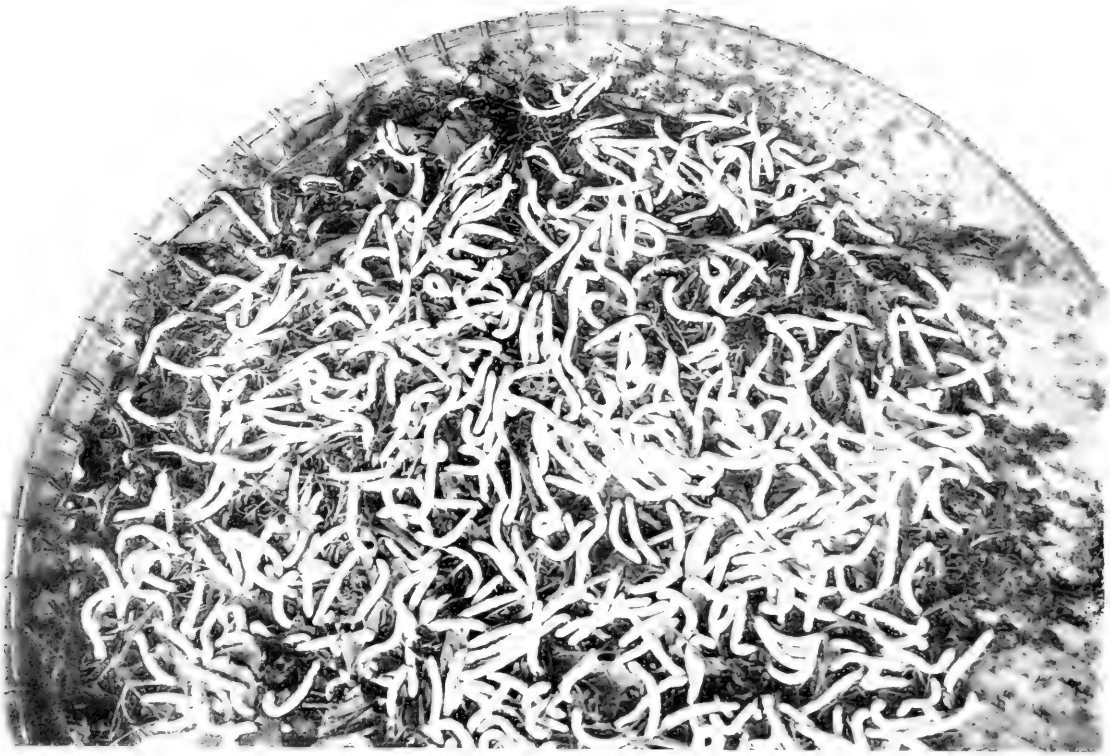
III.

a.

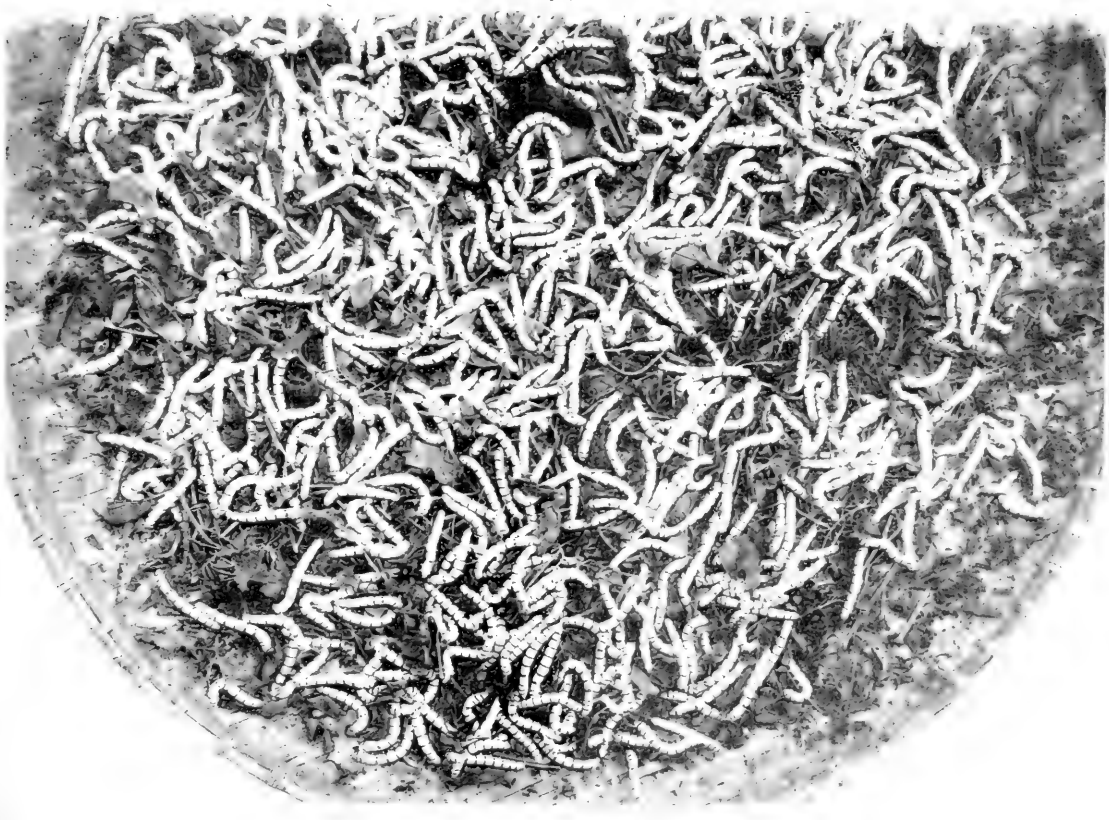
b.

c.

A.



B.



On Physiologically Balanced Solutions.

BY

O. Loew and K. Aso.

About half a century ago various authors have carried out experiments in order to find a solution in which plants could be grown to perfection which are cultivated in soil. After many failures *Knop* succeeded to compose a culture solution of the desired qualities, it was superior to all others, that of Sachs not excepted. In other words, it was a *physiologically balanced solution;* the injury by a one-sided nutrition was prevented by the proper quantity of other nutrients.

It must have been doubtless recognised by Knop, altho it was not pronounced with emphasis, that *the ratio of the different nutrients to each other is of fundamental importance for the best development of the plants* and that this principle of the water culture must hold good also in regard to soil and manure for the field crops.[1] In studying the cause of the toxic action of magnesium salts we were led to infer that special consideration is necessary for the regulation of the relative amounts of lime and magnesia available to the roots.

Numerous experiments have shown beyond any doubt that the injurious action which magnesium salts exert on plants from the higher algæ upwards can only be prevented by lime salts and that the important function of magnesium salts can therefore only be realised in the presence of lime salts.

[1] It is true, some few adhere to the opinion, only holding good for aquatic plants, that the osmotic laws determine the amount and kind of the necessary nutrients to be absorbed. But the current of transpiration plays a more important rôle than that for the land plants and it brings into the plant body much more mineral matter than needed.

Our investigations have further demonstrated, that the most favorable development of plants depends among other things upon a certain quantitative ratio of lime to magnesia available to the root.[1]

We have proved by water—, sand—, and soil culture that an excess of lime as well as an excess of magnesia beyond that best ratio,—the lime factor —depresses the yield of various crops more or less and have pointed out that the determination of magnesia in partial soil analyses is as important as that of lime—but thus far not much attention was paid to this important principle. The law of *physiologically balanced solutions* was clear before our mind, and no doubt also was this law regarded by Godlewski, Schrötter and others when they tried to find by field experiment the best ratio of nitrogen to phosphoric acid and potassa for certain crops.[2]

"Heavy doses of strongly nitrogenous manures also necessitate heavy doses of phosphoric acid to annihilate the injurious effect of an excess of nitrogen," is a statement copied from a book just before us; similar utterances are numerous in agricultural reports. We must call attention to this, because that *law of physiologically balanced solutions was recently claimed as a new discovery*.[3]

There may be a slight distinction made between a physiologically balanced solution for the maintenance of life only and one which would insure the best development of plants; only the latter is of course of importance.

As that author further did not distinguish different phenomena relating to this subject, we must enter upon a further discussion.

That there exist very intimate, special relations between lime and magnesia in their rôle as plant nutrients becomes evident from the fact that

[1] Cf. Flora, 1892 p. 381; ibid. 1903 p. 498 and 1905 p. 336; Landw. Vers.-Stationen 1892, vol. 41 p. 467; Landw. Jahrbücher 1902 p. 561; ibid. 1905 p. 131 and 1906 p. 527; Zeitschrift f.d. Landw. Versuchswesen in Oesterreich, 1905. Cf. further Loew and May, Bul. No. 1 Bureau of Plant Industry, Washington 1901; and the Bulletins of this College, vol. IV p. 361-381; ibid. V p. 495-502; ibid. VI, p. 97-124 and p. 347; ibid. VII p. 8-12 and p. 57-65.

[2] Also here at this College some years ago an experiment was made by *Bahadur* to find the most suitable ratio of N to P_2O_5 for barley in soil culture (cf these Bulletins VI, No. 4).

[3] As physiologically balanced solutions were mentioned by that author blood and sea water.

magnesium salts are not poisonous at all for those lower forms of algæ and fungi which do not require lime for life and propagation.[1]

In perfect accordance with this behavior is that to oxalates which only are poisonous for plant life from the higher algæ upwards, but not for the lowest forms of algæ, flagellatæ and fungi. The most characteristic property of oxalates being the withdrawal of lime from lime compounds[2] it becomes clear that lime must assume a very important position in the organised structure, as soon as a certain stage of differentiation to higher forms is reached.

In regard to marine algæ which doubtless belong to the higher algæ Duggar[3] in a series of interesting investigations has observed that magnesium salts exert but a very weak toxic effect. But it must be taken into account that in his experiments magnesium sulphate was *dissolved in sea water* which contains already *lime*, further that a relatively small amount of lime can depress the toxic action of a considerably larger amount of magnesia and finally that the marine algæ contain more lime than magnesia.[4] This surplus of lime in the plants can also depress the toxic effects of entering magnesia. It must be born in mind that sea water is richer in magnesia than in lime (ratio = 3.8 : 1) and that marine algæ, in order to adapt themselves to this unfavorable condition, must accumulate lime in their cells, which may be done in the form of organic salts.[5]

The theory of one of us as to the functions of lime and magnesia in plants assumes the existence of calcium-protein compounds in the tectonic of the

[1] Lower forms of algæ do not require "physiologically balanced solutions" since they can develop in a 4% solution of magnesium sulphate in presence of mere traces of N, K_2O and P_2O_5 (*Palmella*, *Ulothrix*). These forms even can develop in a 5% solution of manganese sulphate and can adapt themselves gradually to a 4% solution of Na Cl.

[2] On the similar behaviar of sodium fluorid, cf. Flora 1905, p. 336.

[3] Trans. Acad. Sc. of St. Louis, vol. XVI, No. 8.

[4] Gödechens, Ann. Chem. Pharm. 1854. Also Bul. No. 1., Bureau of. Plant Industry, Washington 1901.

[5] We avoid here the term "ion," since this may confer a wrong idea. In regard to the electrolytic dissociation theory compare the important investigations of Louis Kahlenberg.—

nucleus[1] and chloroplasts of the higher plant forms and ascribes to magnesia the rôle to mediate in the assimilation of phosphoric acid when nucleoproteids and lecithin are to be formed from anorganic phosphates. The theory further has pointed out that a certain excess of magnesium salts will act on the lime compound in the nucleus, replacing calcium by magnesium and changing thereby the capacity of the nucleus for imbibition, leads to disorganisation and death, while on the other hand an undue excess of lime will retain the phosphoric acid and prevent the formation of magnesium phosphate, of this important compound for the assimilation of phosphoric acid.[2]

We never had observed such intimate relations as evidently exist between the physiological functions of lime and magnesia, also to exist between potassa and magnesia. However recently not only a toxic action of potassium salts for plants was assumed to exist but also an antitoxic action of potassa to magnesia. These observations were, however, not made with phenogams but only with *Spirogyra* and gemmæ of *Lunularia*, further only with one potassium salt, the chlorid.[3]

When one of us made his first studies in this line (1892) the behavior of magnesium salts to potassium salts and sodium salts was of course compared

[1] The view of some authors that lime salts are only required for certain processes of metabolism in the plants cannot be upheld. It might be objected, e.g., that in this case strontium salts should be capable to replace calcium salts, which is however impossible; these act injuriously, in absence af lime salts. Cf. O. Loew, The Physiological Rôle of Mineral Nutrients, II Edition, pp. 46 and 54 U. S. Dept. of Agriculture, 1903; and U. Suzuki, these Bulletins IV, No. 1. Manganese salts act evidently in the same way poisonously as magnesium salts do. In accordance therewith a poisonous effect for all plants from the higher algæ upwards is noticed and no poisonous effect for lower algæ and fungi. Thus Palmella-forms and Ulothrix-like filaments can grow in a 5% solution of manganese sulphate, while Spirogyra is killed by solutions weaker than 0.1%.

[2] Since it was recently shown by Willstätter (Ann. Chem. 350, p. 46) that the molecule of chlorophyll contains magnesium, it follows that magnesium has still another function to perform. Willstätter ascribes to it a rôle in the assimilation of carbon. Since, however, potassa is also indispensable for the assimilation process, as has been shown long ago by *Nobbe*, it may be possible that both these metals must be present in the transformation of CO_2 into organic compounds. It deserves mentioning, that Berthelot (1906) has observed, especially in the leaves, potassium compounds insoluble in water.

[3] Cf V. Osterhout, vol. II, No. 11 of the Publications of the University of California, 1906.—

with that to calcium salts. No toxic action of potassium salts had been observed however, while a retarding action of potassium salts was observed in one case and an accelerating action in an other, on the toxic action of magnesium salts. The experiment was the following. In a 0.2 per mille solution of magnesium sulphate *Spirogyra communis* died in 5–7 days, while upon addition of 0.1 per mille dipotassium phosphate in 15–18 days and on the other hand upon addition of 0.1 p.m. monopotassium phosphate in 3 days. In a solution of 0.2% monopotassium phosphate and even on further addition of 0.2% KNO_3 the alga can remain alive for a series of weeks.[1] But already at a concentration of 1% and a temperature of 12–20° various salts are injurious which are harmless at 0.2–0.5 per cent. At 4–6° C the resistance power is greater, especially with the larger kinds. Further, a gradual adaptation may be reached. Spirogyra cells that had been kept in 0.5% Na Cl solution can resist a 1% solution longer than otherwise.

Effects of physiologically *not balanced* culture solutions on algæ (Spirogyra) were observed years ago by one of us. Thus it was noticed that a considerable preponderance of lime over magnesia retarded the cell division; an undue preponderance of phosphoric acid and nitrogen over potassa rendered starch accumulation in the chloroplast impossible, all carbohydrate produced by assimilation of carbon being at once transformed into protein required for the rapid growth; on the other hand a surplus of potassa led to a considerable accumulation of starch, *when nitrogen was present in a minimum amount*, while an undue simultaneous maximum of nitrogen and potassa led to the accumulation of much protein[2] in vacuole and cytoplasm, and but little starch becomes visible.

[1] The salts applied should be chemically pure. Often a very faint trace of copper is present, when the salts had been recrystallised from common distilled water. Only water distilled from glass vessels should serve for recrystallisation. In such distilled water Spirogyra can remain alive for a very long time. The flasks for the tests with Spirogyra should be first washed with hydrochloric acid, then with this distilled water. The amount of solution applied should not be too small. Generally 100 cc. served for a small number of filaments, because otherwise some dying filaments losing nutrient compounds by exosmosis can thus influence the resistance power of the neighboring filaments.

[2] This protein is of very labil nature. Cf. O. Loew and Th. Bokorny in „Die chemische Energie der lebenden Zellen" by O. Loew.

Under certain conditions the chloroplast grows more rapidly than the cytoplasm, finally filling this out entirely and rendering the nucleus invisible; under other conditions again the cytoplasm grows more than the chloroplast, the latter changing its spiral form finally to a straight line.[1]

Again in certain culture solutions the cytoplasm is rendered turbid from fine precipitates of phosphates, in others again the filaments break up into single cells, which remain perfectly healthy. This phenomenon is in many cases due to increased turgor.[2]

Some of the many trials[3] may here be mentioned. As favorable culture solutions served the following:

	a	*b*
KH_2PO_4	0.1 p. mille	0.1 p. mille.
KNO_3	0.5 „ „	0.2 „ „
$Ca(NO_3)_2$	0.2 „ „	0.5 „ „
$MgSO_4$	0.2 „ „	0.1 „ „
$FeSO_4$	trace	trace
$Mg H_2(CO_3)_2$	—	0.2 „ „

When in solution (a) the potassium nitrate was replaced by 0.3 p.m. mono-ammonium phosphate the development was somewhat abnormal, some

[1] During such observations, attention must be paid to the presence of *Chytridia*, parasites which easily perforate the cell-walls of *Spirogyra*. Brown has observed over 20 species attacking various algæ. Sometimes Spirogyra is attacked also by *Pseudospora*, which is a mixomycet according to Zopf. These parasites may often be destroyed by placing the algæ for 1–2 days in a 1 per mille solution of phenol in well water. Parasites will doubtless become most abundant after a portion of the Spirogyra cells present had died, furnishing by exosmose from the vacuole organic nutrients for the parasites outside and attracting them to the filaments. The presence of infusoria is favorable as they (especially *Vorticella*) devour *Chytridia*.

[2] This phenomenon was also observed when Spirogyra was kept in very moist air, i.e. under a bell-jar spread on moss, thoroly moistened. Once it was observed by us also by touching the filaments with very dilute OsO_4. W. Benecke made especial studies on this subject. J. w. Bot. 1898.

[3] Spirogyra is very sensitive to ammonium salts, especially in weak alkaline culture solutions, while nitrates may serve well as source of nitrogen even at concentrations of 0.2%. Mono-ammonium phosphate which is of acid reaction, may at a concentration not higher than 0.05% serve, however, as a source of nitrogen in weak acid culture solutions.

cells reaching a great length before cell division took place. Also unusually much tannin accumulated.

In the following solution some Spirogyra cells showed a change of the cylindrical shape to a barrel shape, globular formations appeared in the cells, and numerous rhizoids were produced. Death resulted after a few weeks. That solution was :

$Na_2 H PO_4$	1 per mille.
$Na H CO_3$	0.5 „ „
$Mg SO_4$	0.5 „ „
$Ca (NO_3)_2$	0.1 „ „
$K N O_3$	0.5 „ „
$Fe SO_4$	trace.

It was of weak alkaline nature, and with lime in the minimum. Potassa did *not counteract* the toxic effects of magnesia, as an increase of lime would have done, in accordance with our former observations.

In the following solution the effect of an excess of lime on the growth of the chloroplast became especially noticeable :

$K_1 H_2 PO_4$	0.1 per mille.
$Ca SO_4$	1.0 „ „
$Ca (NO_3)_2$	1.0 „ „
$Mg SO_4$	0.1 „ „
$Fe SO_4$	trace.

The growth of the cytoplasm and the cell division were here much retarded, the increase of the number of cells was slow but the chloroplast continued to grow so that it filled out all available space in the cytoplasm and in some cells it grew beyond that, causing an irregular form of the spiral by the pressure of growth.

In the following solution with a relative preponderance of potassa and nitrogen a great deal of protein was formed and stored in the vacuole and cytoplasm, the starch produced by assimilation of carbon being rapidly utilised for that purpose, therefore only little was seen of it in the chloroplast. Growth of the filaments was not very energetic, as phosphoric acid and magnesia were in the minimum. That solution was :

KNO_3 .....0.5 per mille.
$Ca(NO_3)_2$ .....0.3 ,, ,,
$MgSO_4$ .....0.05 ,, ,,
$K_1H_2PO_4$.....0.05 ,, ,,
$FeSO_4$ .....trace.

We have recently also made further observations on the effect of *imperfect solutions* on *Spirogyra nitida*, one of the larger species. The concentrations of these solutions were mostly below 0.5% and did in no case reach 1%. A small number of filaments of 6–10 cm. length was placed in 100 cc. of the solutions prepared with water distilled from glass vessels. The temperature varied from 8–22° C. The flasks were exposed to direct sunlight, later on only to diffused but bright daylight.—

The figures in parenthesis in the following table signify the percentage of anhydrous salt; they stand mostly in simple relation to the molecular weights.

$MgSO_4$ (0.2)	
$Mg(NO_3)_2$ (0.2)	All cells killed in 2–4 days.
$MgCl_2$ (0.2)	
KCl (0.1)	All cells healthy after 10 weeks.
KCl (0.3)	All cells healthy for 3 weeks, then a gradual change of the chloroplast-spiral took place, it contracted, moved to the cell ends and formed no longer starch. Gradually also the nucleus suffered.[1]

[1] Such injured cells had still the normal turgor, but the nucleus and very probably also the chlorophyll body were killed. The nucleus had contracted to an irregular shaped mass and was lying on the side. Such cases were observed years ago by one of us when highly diluted solutions of oxalic acid acted on the cells. These cells with the cytoplasm alive and the nucleus killed recalled Gerassimows Spirogyra cells without any nucleus, obtained by the influence of low temperature or

KNO_3 (0.15)	All cells normal and rich in starch after 15 days; later on gradual death; some cells alive after 42 days.
K_2SO_4 (0.3)	A number of cells still perfectly healthy after 50 days. Injury commenced after 28 days. Parasites numerous, like in the former case.
$CaCl_2$ (0.2)	All cells alive after 80 days. In a few cells the nucleus has moved to the wall. Much starch, no parasites. Gradual death afterwards.
$Ca(NO_3)_2$ (0.2)	All cells healthy after 50 days, no parasites.
$CaSO_4$ (0.2)	All cells healthy after 80 days, no parasites. Later on the filaments became yellowish. Much starch.
KCl (0.15) + $MgCl_2$ (0.1)	Most cells killed after 10 days; the still living show chlorophyll body attacked, its lobes being retracted and sometimes the spiral torn into fragments. But such injured cells were still alive 4 weeks later.
K_2SO_4 (0.3) + $MgSO_4$ (0.2)	All cells healthly for 30 days; half the cells dead after 50 days. The living cells have now received nutrients and perhaps also lime from the decaying dead cells, as was clearly evinced by the cell-division taking place here and there. Much starch noticed in these cells after 60 days. Some rhizoids.[1]

anæsthetics after the cell division had made a start. The substance contained in the chlorophyll body may serve to sustain the life of the cytoplasm in case the assimilation of carbon in the former had ceased. Such cells without nucleus are capable to live for six weeks (Gerassimow).

[1] Thus far *rhizoid formations* were observed by us in solutions containing:

$Ca(NO_3)_2 + (NH_4)_2SO_4$ (0.002%)

or $CaSO_4 + K_2SO_4$

or $MgSO_4 + K_2SO_4$

but in no case in any of these compounds alone. Sulphates seem to be essential for that phenomenon. It deserves to be mentioned that in the numerous cases of imperfect culture solutions we observed only in lime salts and in 0.1% KCl solution that the filaments of Spirogyra showed the phenomenon of *geotropism*.

Spirogyra sometimes shows the phenomenon of *heliotaxis*. One of us (L.) has seen once that *Spirogyra* filaments lying on the bottom of a flask moved with great rapidity into a nearly vertical position, when the first rays of the morning sun reached them.

KNO_3 (0.15) + $Mg(NO_3)_2$ (0.2) (1 Mol. : 1 Mol.)	Most cells killed in 17 days; the injured cells have living cytoplasm but dead nucleus; all cells killed after 30 days.
KNO_3 (0.5) + $MgSO_4$ (0.2) (3 Mol. : 1 Mol.)	After 25 days healthy. After 50 days about half the cells killed, while the living cells show swollen nucleus. Chlorophyll body attacked, forming no starch in sunlight, hence probably dead.
Na_2SO_4 (0.23) + $MgSO_4$ (0.2)	About 10% of the cells alive after 3 days, while without Na_2SO_4 all killed in 3 days.
KNO_3 (0.15) + $Ca(NO_3)_2$ (0.2)	Most cells alive after 50 days; nucleus normal in all cells.
K_2SO_4 (0.3) + $CaSO_4$ (0.2)	*Many rhizoids* had formed. Cells almost all alive after 50 days, they have grown in length more than in any one of the cases mentioned here; nucleus in most cells normal but Chlorophyll body often somewhat emaciated, with change of the spiral shape.
$Mg(NO_3)_2$ (0.2) + $Ca(NO_3)_2$ (0.2)	Almost all the cells after 50 days perfectly normal and healthy, starch present. Very few Chytridia.
$MgSO_4$ (0.2) + $Ca(NO_3)_2$ (0.4)	All cells healthy for 30 days, a few filaments injured after 50 days, showing emaciated and disrupted chlorophyll body and displaced contracted nucleus. The healthy cells show starch.
$MgCl_2$ (0.1) + $CaCl_2$ (0.2)	About 95 per cent of all the cells after 80 days perfectly normal.
$MgSO_4$ (0.2) + $CaSO_4$ (0.2)	All cells normal after 50 days. Later on a yellowing set in. No rhizoids. No parasites.
$Mg(NO_3)_2$ (0.2) + K_2SO_4 (0.01) + $Ca(NO_3)_2$ (0.04)	Remained healthy for 32 days, but later on many cells died, and those cells that lived after 50 days showed injury to chloroplast and displaced nucleus. No further starch formation was possible. The effect of a relative excess of magnesia was evident. No rhizoids were observed.[1]

[1] It must be not lost sight of in these experiments that a living cell can extract thru the separating wall, from a neighboring cell in a dying condition, various compounds of organic and anorganic nature and thus become able to a prolonged resistance under unfavorable conditions.

It will be seen from this table that the cells remain alive and healthy in solutions of calcium salts at a concentration of 0.2% and further that the poisonous action of magnesium salts can only be prevented by certain doses of calcium salts. It will be further noticed that potassium salts can retard but not prevent the toxic action of magnesium salts, which influence is more noticeable when both bases (or one of them) are present as sulphates than in other cases. *It would be, however, not be justified to give the same explanation for both cases of counteraction without close examination.* One might, e.g., suppose that potassium-protein compounds[1] in the living matter can exchange their potassium against magnesium and that this might lead to a similar disturbance as by the substitution of the calcium of the nucleus or magnesium. Such an explanation would demand the proof that the assumed potassium protein-compound forms really on essential *part of the tectonic* of living matter; it might merely be loosely connected with the structural elements and in that case the substitution of its potassium by magnesium would not lead to a collapse of the tectonic, as is the case of the calcium-protein compound of the nucleus when its calcium is replaced by magnesium. Further, that hypothesis would necessarily imply that calcium salts must also act poisonously, which is not the case. The algæ cells showed even much starch after 2 months in a 0.2% solution of $Ca\,Cl_2$.

It is much more probable that the retardation of the toxic action of Mg-salts by K-salts is due to the property of forming double salts with potassium. These double salts may exert less energy in a similar way as also Mg-bicarbonate exerts less toxical energy on *Spirogyra* than many other Mg-salts do.[2] It is stated (cf. Muspratt's Chemistry) *that a very stable double salt* is formed by both the sulphates of Mg and K, but not by those

[1] The existence of such compounds in the living cells was assumed by one of us long ago, cf: The Physiological Rôle of the Mineral Nutrients, p. 27, Washington 1899, and Die chemische Energie der lebenden Zellen I Edition p. 32, foot note and 2d Edition p. 34. The assumption that such a protein compound would be necessary for the chemical condensation processes *in all cells* does not exclude Willsättters view on the rôle of Mg in the chlorophyllbody.

[2] We have observed that magnesium-potassium sulphate acts on calcium carbonate at 90° much more slowly than magnesium sulphate alone does.

of Mg and Na.[1] This would explain, why the algæ live longer in the mixture of Mg and K sulphates than in that of the nitrates or chlorids; in the latter cases so well defined double salts as with the sulphates have not been obtained but the existence in the solutions of the mixture is more probable than for the mixture of magnesium and sodium salts.

Still another hypothesis may be considered which however does not exclude the former. It is possible that potassium salts can attach themselves to the calcium protein compounds of nucleus and chloroplast and thus rendering the calcium more negative diminish its faculty to be substituted by magnesium. Further investigations are necessary.[2] So much follows from our various experiments with water and soil cultures that the *action of potassium salts, can not be identified with that of calcium salts in counteracting the injurious action of magnesium salts*, altho that retarding action of potassium salts can also be observed with phenogams. Young barley plants of 8 cm. hight were carefully deprived of the endosperm in order to exclude the influence of stored up mineral matter, and placed into the following solutions (3 in each flask):

I 0.4% $Mg(NO_3)_2$,
II 0.4% $Mg(NO_3)_2$ + 0.2% $CaSO_4$,
III 0.4% $Mg(NO_3)_2$ + 0.2% K_2SO_4,
IV 0.4% K_2SO_4.

After 7 days the plants in I were dead, after 15 days two of the plants were dead in III, after 30 days the third was perfectly yellow and 11 days later it died. In IV two of the plants died after 28 days, the last after 36 days, while in II (Ca + Mg) each plant had three green healthy leaves

[1] A double salt of Mg and Na-sulphate can only be obtained in presence of much $MgCl_2$, but as soon as the double salt is treated with water, it undergoes a splitting into the two simple sulphates.—In coincidance therewith is the fact that sodium sulphate cannot essentially (a few days only) retard the toxic action of magnesium sulphate for Spirogyra.

[2] In comparing the peculiarity observed in the mixture of $KCl + MgCl_2$ (see table), that the cytoplasma can remain alive long after the death of nucleus (and chloroplast) it seems probable that potassium salts can also increase the resistance power of the cytoplasm to disturbing influences in the cell.

after 80 days, while the oldest leaves only had died off. The most remarkable difference was however the growth of the root in this case from 6 cm. to 14 cm. while in the other three solutions growth had stopped altogether. These plants were still alive five weeks later, the old leaves died, but young ones started anew.

A similar experiment was made with young pea plants. Here only those plants developed branches and reached the flowering stage, which were placed in the solution II. These plants increased in height 20 cm., those in III only 6–8 cm., while those in I and IV stopped growth and died gradually.

When the endosperm of barley shoots is not removed it will take much longer until the toxic effect of magnesium salts causes death. Thus such barley seedlings of 6–8 cm. height, placed in 0.20% $Mg(NO_3)_2$ were still alive after 18 days, altho the leaves had almost entirely turned yellow. By the simultaneous presence of 0.25% KNO_3 this yellowing had not yet developed so far as in the former case, but it had spread over nearly one half of the leaf area; the former plants died after 31 days, the latter after 40. As to the alleged toxic action of potassium salts it may be mentioned that when barley seedlings are deprived of the rest of the endosperm after they had reached 18 cm., they can remain 2–3 months alive, if they are placed in 0.5% solutions of KNO_3, KCl or K_2SO_4, a sure proof that the assertion of the toxic action of potassium salts is unfounded; the older leaves die, but new ones develop, utilising mineral food from the dying leaves. Similar experiments were made with seedlings of maize, which were still alive 7 weeks after being placed in a 0.5% solution of K_2SO_4.

The antitoxic action of K to Mg is, furthermore, too weak to play any decisive rôle in manuring. We recognised, e.g., the law that the common cereals thrive best when the available amounts of lime and magnesia are about equal. If now potassium salts would exert any notable action in the sense mentioned, the maximum harvest would have been obtained with very much less lime in those cases where the potassium salts of the manure were increased. But as a matter of fact the same lime factor was observed at very different amounts of potassium salts in the manure. Some influence of potassium-sulphate can however be recognised so long as the plants are

young. Six pots each holding 2 kilo of an exhausted loam soil received the following general manure: 0.8g. K_2SO_4; 0.5g. Na_2HPO_4; 0.8g. NH_4NO_3, while the special manure consisted in:

I No further addition.
II 5g. KCl.
III 10g. artificial magnesium carbonate.
IV " " " " + 10g. KCl.
V " " " " + 5g. K_2SO_4.
VI " " " " + 100g. $CaCO_3$.[1]

Two pots, each with 5 barley plants served for each case. The seed was sown Oct. 30. The fresh weight of the young plants on March 16 yielded in average the following figures, g:

I = 9.5	IV = 3.5
II = 7.3	V = 5.0
III = 1.5	VI = 13.7

It will be seen that the increase of the potassa in the form of sulphate exerted some counteraction on the depression by an excess of magnesia but only lime was able to counteract fully that injurious effect. Comp. Plate XII.

A similar experiment was made with spinach. After one month the young plants had reached only 2 cm. in height at the excess of magnesia and at this excess + an extradose of 5g. KCl per pot, the average height was quite the same, while at the addition of calcium carbonate, the average height was 4.6 cm.

Summary.

1. The view recently expressed that "physiologically balanced solutions have not been made use of by botanists," can hardly be sustained, since Knop's culture solution must be regarded as such a solution. Lower forms of algæ and fungi do not require physiologically balanced solutions.

[1] This large quantity was required on account of the great availability of the magnesia in the artificial magnesium carbonate. The original soil contained 0.4% MgO and 0.5% CaO.

2. Potassium sulphate and nitrate are only injurious for plants when the concentration is abnormally high. Potassium chlorid at 0.3% exerts after several weeks a slow injurious effect on Spirogyra, but on phenogams not for many weeks, even at 0.5%.

The final death of Spirogyra cells in dilute solutions of potassium sulphate or nitrate is merely due to the one sided nutrition and exhaustion.—

3. Potassium salts can retard but not prevent the toxic effects of magnesium salts. The cause of this retardation is entirely different from the prevention of this toxic action by calcium salts.

4. Some interesting observations may be made on *Spirogyra* kept in *imperfect culture solutions*. Thus, e.g., in a solution containing only KCl and $MgCl_2$ the cytoplasm can remain long alive after the nucleus is killed, recalling Gerassimow's cells without a nucleus; in a solution containing only K_2SO_4 and $CaSO_4$ an abundance of rhizoids is formed. This rhizoid formation depended in our cases only upon the salts in solution, while in other cases it depends upon the contact with an object, as Borge and Kny have observed. In saturated gypsum solution the tendency to show geotropism is strongly preserved and the cells continue to produce an abundance of starch even after the chloroplasts have gradually turned yellow. This starch formation can be considered as a proof that neither potassium nor magnesium of the chloroplast had been replaced by calcium. This yellowing is not observed in the solution of 0.2% $CaCl_2$ even after three months.

5. Interesting effects can be observed with Spirogyra kept in full, but not balanced culture solutions.

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This plate shows that potassium salts cannot counteract the injurious action of magnesium salts as lime salts can do.

Benzoesäure in Pinguicula vulgaris.

VON

O. Loew und K. Aso.

Es ist eine auffallende Tatsache, dass Insecten, welche auf den schleimigen Blättern von *Pinguicula vulgaris* sich oft in grösserer Menge niederlassen und da absterben, keinen Fäulnissgeruch erkennen lassen. Die Wahrscheinlichkeit, dass eine antiseptische Substanz von den Blättern mit dem Schleim secernirt werde, führte schon vor Jahren den einen von uns (L) zu einen Versuch mit den Blättern. In eine 0.5 procentige, neutrale Loesung von Pepton wurden zalreiche frische Blätter von *Pinguicula* gebracht und nach 15 Stunden die Flüssigkeit in einen Kolben abgegossen. Weder Pepton noch Kolben war sterilisirt worden, der Kolben wurde nicht verschlossen. Selbst nach drei Wochen zeigte diese Flüssigkeit keine Spur von Fäulnissgeruch. Eine geringfügige Bacterienvegetation war zwar vorhanden, dieselbe rief aber nur einen schwachen Geruch nach rohem Leim hervor. Durch Erhitzen auf 75° wurde die antiseptische Wirkung nicht zerstört.

Da die Möglichkeit vorlag, dass Benzoesäure das antiseptische Agens sei, haben wir an der Sonne getrocknete *Pinguicula* Pflanzen mit Wasser extrahirt und die sauer reagirende Flüssigkeit mit Aether ausgeschüttelt. Dieser hinterliess nach dem Verdunsten eine krystallinische Masse, gemengt mit gelber amorpher Substanz und etwas Gerbstoff. Durch zweimaliges Umkrystallisiren aus wenig heissem Wasser konnten jene Krystalle rein erhalten werden. Ihr Schmelzpunkt wurde zu 122° gefunden, während für Benzoesäure 120–121°.4 angegeben wird. Der Habitus der Tafeln und Nadeln glich genau dem der reinen Benzoesäure, ebenso der Geruch. Die Formen des Kalksalzes glichen genau denen des benzoesauren Kalks, so dass

über das Benzoesäure-Vorkommen in *Pinguicula* kein Zweifel mehr obwalten kann.[1]—Die *Pinguicula* lässt es also nicht zu einer Fäulniss der gefangenen Insecten kommen, wie die *Utricularia* es tut.

Dass verschiedene Harze Benzoesäure enthalten, ist seit lange bekannt; der eine von uns fand sie ferner in den Preisselbeeren,[2] und kürzlich wurde sie von *Cotton* auch in *Rhinanthus major* und *Rh. minor* beobachtet. In *Husemann's* und *Hilger's* „Pflanzenstoffe" findet sich angegeben, dass sie auch in den Samen von *Evonymus europæus* und in den Wurzeln von *Acorus Calamus*, *Pimpinella Saxifraga* und *Inula Helenium* vorkomme. Vielleicht findet sie sich noch in anderen Pflanzen; denn *Wiesner*[3] berichtet, dass aus seinen Versuchen das Vorhandensein antiseptisch wirkender Substanzen in *Lysimachia*, *Begonia*, *Tradescantia*, *Ranunculus aquatilis*, *Daucus Carota* und *Chenopodium* gefolgert werden müsse. *Wiesner* vermutet, dass sich Bodenwurzeln und Wasserpflanzen durch antiseptische Mittel gegen Angriffe von Bacterien schützen. In *Daucus Carota* kommt nun ausser einem ætherischen Oel, auch eine sehr geringe Menge einer sich der Benzoesäure ähnlich verhaltende Säure vor. Aus 800g. Wurzeln erhielten wir jedoch nicht genügend, um nach weiterer Reinigung wenigstens eine Schmelzpunktbestimmung ausführen zu können.

[1] Dass diese Säure etwa aus einem amygdalinartigen Glycosid erst durch Spaltung und Oxydation hervorgieng, ist nicht anzunehmen, weil sonst beim Absterben der Pflanzen der Geruch nach Benzaldehyd hätte auftreten müssen. Davon war aber ebensowenig etwas wahrzunehmen, als vom Geruch von Blausäure.

[2] O.L., Journ prakt. Chem. 19, 309.

[3] Wiener Akad. Ber., October 1893.

On the Action of Naphthalene on Plants.

BY

K. Asō.

It has been shown by various authors that after treatment of the soil with certain volatile substances, such as carbon disulphide, ether or chloroform, plants developed more vigorously in such a soil. It seemed to me of some interest to observe also the effect of less volatile substances as e.g. naphthalene. This has the melting point = 79° C and boiling point = 218° and volatilizes slowly at the ordinary temperature.[1] Since naphthalene has long been applied as a means to keep off moths from clothing, and is also recently reported to drive off intestinal worms, an effect on nematodes in the soil might be expected. Hollrung observed that insects may be kept off from plants dusted with naphthalene, but he could not observe any fungicide properties. A mixture of naphthalene and lime is recently recommended to keep off earth fleas, larvæ of *Lema asparagi* and snails from young plants.

An injurious effect on higher plants has thus far been not reported. In contrary, W. Busse observed with barley grains, that had been mixed for a certain time with 1% naphthalene, a preservation of the germinating power for a longer time than with the barley not thus treated.

Before my experiments with phænogams will be described, some tests with bacteria and algæ may be mentioned.

[1] I have observed in this regard the following: 1g. of naphthalene was left covered with 100 c.c. water in an Erlenmeyer flask plugged with cotton at 20° C. After nearly one month the larger portion of naphthalene had sublimed into the upper part of the flask.

To 100 c.c. of culture water 1% and 0.1% naphthalene respectively was added, and some filaments of Spirogyra nitida added. After four days, the algæ were dead in the flask with 1% naphthalene, while they remained alive for several weeks in the flask with 0.1% naphthalene. This led me to the suspicion that the perfectly white naphthalene contained some impurity, and therefore it was treated with sodium carbonate to extract organic acids, but such were not found. Another portion was warmed with hydrochloric acid and this extract evaporated to dryness. A small amount of crystallized substance was thus obtained, which upon addition of caustic potash yielded small droplets of a strong and decisive odor of *quinoline*.

My tests with bacteria showed that 0.1% naphthalene does not prevent entirely bacterial growth but may supress the development to a varying degree when added to bouillon infected with Bacillus prodigiosus, B. fluorescens liquifaciens, B. mycoides, B. pyocyaneus and B. subtilis respectively.[1] B. mycoides is less injured than B. prodigiosus and the B. subtilis.

Experiment with Barley and Pea.

Pots filled with 10 K of unmanured loamy soil received as general manure, g :

	For barley.	For pea.
Ammonium nitrate	5	0.1
Sodium phosphate	5	5
Potassium sulphate	3	3

For each plant, one pot served as check pot, two other pots received each 1 gram of naphthalene well mixed with the soil, while one pot received grams naphthalene. After several months decisive differences were noticed which, however, did not perfectly correspond in both series as will

[1] Since recently the remarkable fact was reported by Rahn (Centr.-Bl. Bakt. II. 76, 382) that a hydrocarbon, like paraffine, can be attacked by a mould fungus and serve as the source of carbon, and further a communication was made by Söhngen (Centr.-Bl. Bakt. II. 15, 513) that also methane can serve as a source of carbon for a kind of bacterium (Bacillus methanicus), I have been led to test whether well purified naphthalene would serve as a source of carbon for certain bacteria, such as B. fluorescens liquifaciens and B. methylicus. The result was entirely negative as I had expected.

be seen from the photographs reproduced on plate XIII ; 1 gram naphthalene caused a stimulation of barley, but not of pea while 5 grams naphthalene per pot caused injury in every case. The plants were harvested and weighed in the airdry state :

Barley, 9 plants per pot.

Naphthalene.	Number of stalks.	Weight of ears g.	Weight of straw. g.	Weight of grains. g.	Total. g.
1	28	44.8	34.0	37.0	78.8
	27	44.9	31.0	37.5	75.9
5	17	39.0	22.9	32.7	61.9
Check pot.	19	41.0	22.0	34.5	63.0

Pea, 10 plants per pot.

Naphthalene.	Number of pods.	Weight of pods. g.	Weight of seeds. g.	Weight of straw. g.	Total. g.
1	49	24.0	21.1	11.5	35.5
	52	24.9	21.8	11.0	35.9
5	36	18.4	17.0	6.2	24.6
Check pot.	59	27.0	23.3	15.0	42.0

Experiment with buckwheat and millet.

In this experiment, two series were observed under essentially the same condition, as with barley.

The quantities of napthalene were 5g., 1g. and 0.5g. per pot. The harvest was weighed in the airdry state with the following result :

Naphthalene.	Weight of fruits. g.	Weight of stalks. g.	Total. g.
0.5	46.0	32.0	148
	52.9	17.5	
1.0	52.9	15.0	133.4
	52.5	13.0	

Naphthalene.	Weight of fruits.	Weight of stalks.	Total.
	g.	g.	g.
5.0	41.5	10.9	109.3
	44.5	12.4	
Check pot.	44.8	13.8	124.6
	52.0	14.0	

Millet, 4 plants per pot.

Naphthalene.	Weight of ears.	Weight of straw.	Total.
	g.	g.	g.
0.5	20.5	19.0	71.0
	16.5	15.0	
1.0	17.5	15.5	68.0
	17.0	18.0	
5.0	12.0	13.0	49.2
	13.0	11.2	
Check pot.	16.0	14.5	62.5
	17.0	15.0	

Experiment with Rice.

Pots holding about 1 K of soil were manured with 1 gram ammonium nitrate, 1 gram sodium phosphate and 0.7 grams potassium sulphate. Two pots received 0.05 grams, other two 0.1 gram and two 0.5 grams while two served as check pots. Each pot received three young rice plants. The air-dry harvest was as follows :

Naphthalene.	Weight of straw.	Weight of grains.	Total.
	g.	g.	g.
0.05	12.0	8.2	42.7
	12.7	9.8	
0.1	12.5	7.0	38.5
	10.0	9.0	

Naphthalene.	Weight of straw.	Weight of grains.	Total.
	g.	g.	g.
0.5	5.8	2.0	16.3
	6.5	2.0	
Check pots.	13.5	9.0	43.5
	12.5	8.5	

The observations show therefore :

1. Naphthalene can prevent the development of various soil bacteria, altho it does not kill them.

2. Naphthalene, in the proportion of 0.005–0.01% added to soil, can cause in some cases a moderate stimulation of growth with phenogams, as with barley, buck wheat and millet, but not with pea and rice. An increase to 0.05% injured the growth in every case. The injurious action must be ascribed to the vapors of naphthalene spreading through the pores of the soil.

3. Since naphthalene injures the plants it cannot be recommended as a remedy against nematodes, at least not in doses of more than 0.005% of the soil.

To page 414. Plate showing the influence of naphthalene on barley and pea.

STUDIES ON HUMUS FORMATION, II.

BY

S. Suzuki.

In a former paper[1] the writer has described an experiment which had demonstrated that not only carbonate of lime but also carbonate of magnesia promote the decomposition of moist leaves by fungi, judged by the amounts of carbonic acid produced. This production may, however, more relate to the respiration of the fungi grown on the leaves than to the humification proper, altho the production of dark black substances was observed in connection with it. W. Hilgard in his extensive studies on soils observed that carbonate of lime promotes the transformation of plant remnants into humus, which however in dry countries accumulates only in clayey soils, otherwise also in calcareous soils.

Proteins as well as carbohydrates[2] are assumed to contribute to the formation of the black substance. However, the frequent nitrogen content of humus is not yet a sure proof that the humus is derived from proteins, as shown by Udransky[3] who heated sugar in presence of urea with sulphuric acid and obtained a humus-like compound containing nitrogen. The nitrogen content observed in crude humus may also often chiefly be due to an admixture of bacteria, of mycelia of fungi or of chitin parts of insects as was

[1] These Bull. VII, No. 1, p. 95.

[2] Pure cellulose does not yield any humus according to Hoppe-Seyler, but lignin substances will do so according to Lange, who gave in this regard, however, only a short note without any experiment being mentioned. (Zeitschrift für physiologische Chemie XIII, p. 84).

[3] Zeitschrift für physiologische Chemie XII, p. 42.

pointed out by P. E. Müller,[1] v. Post and others. But this can not hold good for the humic acid precipitated from solution. André (1900) concluded that lime gradually transforms the amido nitrogen of humus into ammonia, and that the composition of the soluble nitrogenous compounds is very variable. H. I. Wheeler, Sargent and Hartwell (1898) observed that lime diminishes the humus content of soils[2] and renders its nitrogen more available.

The writer has recently carried out some experiments to reveal the nature of the nitrogen content of humus. This investigation is not yet finished, but so much can be regarded as certain, that it was in the case examined due to protein matters, perhaps more or less decomposed.[3] Humus was boiled with concentrated hydrochloric acid for eight hours and the amido-compounds thus obtained treated after E. Fischer's method ; and from the fractions obtained by distillation of the esters, the amido compounds were regenerated and thus obtained : leucin, alanin, glutamic acid, aspartic acid and tyrosin. A full account of this investigation will be shown in the next Bulletin.

Hoppe-Seyler denied the participation of bacteria in the humification process. According to Benni this process consists in a slow oxidation of proteins and carbohydrates.[4]

The writer has examined the behavior of several kinds of bacteria occuring in humus-particles. From a section of loamy soil freshly opened to the depth of about 12 feet rootlets were selected at the depth of 3 feet below the surface which rootlets had turned black by the humification process. These were transfered by means of a sterilized pincet into a test tube containing sterilized bouillon. After several manipulations three kinds of bacteria were isolated from this sample, and from another sample which also was derived from the same place, one kind, showing a greenish fluorescence of its culture. Starting from the fact that pentosans are capable to yield by

1 Natürl. Humusformen, p. 173.

2 Déherain and Demoussy observed a slow oxidation of humus at 40–50°, and more so at 100° C.

3 Close investigation is necessary to examine the cause of this resistance to putrefaction.

4 Chem. Centralbl. 1897, I, 31.

decomposition with hydrochloric acid about half their weight of furfurol which easily can be turned into black matters, the writer supposed that certain bacteria might be capable to accomplish a similar process. Hence the following culture media were prepared: Bouillon, containing:

a. 1.0% Yeast nuclein (moist).
b. 0.5 „ Starch.
c. 0.5 „ Araban.
d. 0.5 „ Xylan.

After sterilization these culture media were inoculated with the isolated bacteria above mentioned and the flasks left several weeks in an incubator at 17° C. But formation of a black substance could not be observed under these conditions.

The possibility existed, however, that the degree of the access of air had an influence on the formation of humus. It had been observed that clay soils and loamy soils form much more humus than loose sandy soils. Hence, since a restriction of access of air seems to be favorable for humus formation, a clay soil was mixed with 10% of araban, xylan, starch, dry egg albumin, glucose, cellulose, tannin, fat (sesam oil), coniferin, vanillin,[1] fine saw dust, and finely powdered straw and leaves respectively, and the mixtures left partly in Petri dishes (13 g. soil in each) and partly in test-tubes (each containing 9 g. of soil) in order to observe the influence of the extent of æration. The amount of water added was equal to that of the soil. In the cases where sugar and other carbohydrates had been added to the soil, and in some other cases two series of mixtures were observed, namely with and without addition of 0.1% of meat extract.[2] The clay soil was selected from a locality in which the humus formation took place extensively, it was taken just below the stratum blackened by extensive humus formation. In order to observe whether with a decrease of clay also the humus formation would be decreased, a further experiment was made in which the same clay soil was mixed with fifteen times of its amount of coarse quartz sand. To this mixture 5% of saw dust and starch respectively with 0.1% of meat

[1] Vanillin and coniferin have a close relation to lignin.

[2] This was of course done for the purpose of nourishing the soil bacteria.

extract were added. In this test however the amount of water was only one half of the other cases in order to imitate the looseness of a sandy soil. The mixtures were prepared Oct. 26 and left in a thermostate at a constant temperature of nearly 30° C. After three days all the tannin mixtures commenced to blacken, but this is not surprising. Since, further, tannin on account of its relatively small quantity in most plants can not play any essential rôle in the humus formation, the tannin mixtures were not further considered.

The phenomena observed after 90 days are recorded in the following table :

I Crude araban
- with meat extract.—Slightly black.
- without „ „ —No blackening.

II Xylan
- with meat extract.—Black, but only in the lower portion of the test-tube.
- without „ „ —No blackening.

III Starch
- with meat extract.—Deeply blackened to a large extent in the test-tube, and at the bottom of the Petri-dish in isolated spots.
- without „ „ —No blackening.

IV Glucose
- with meat extract.—Slightly blackened, both in the test-tube and in the Petri-dish.
- without „ „ —No blackening.

V Cellulose.—No blackening in either case.

VI Saw-dust.—No blackening in either case.

VII Straw.—Slightly blackened in both cases in the test-tube, but only at the bottom in the Petri-dish containing meat extract.

VIII Tannin.—Blackened throughout the whole mass.

IX Vanillin and coniferin.—No blackening in either case.

X Egg-albumin.—Blackened to a large extent in the test-tube, and only slightly at the bottom but not at the surface in the Petri-dish.[1]

[1] A very peculiar odor was noticed there, however not that of putrefaction.

XI Sesam oil.—No reaction whatever.

XII Leaves.—Particles became black in all cases.

The blackening may be due in the first place to the tannin content of the leaves.

XIII Saw-dust in Sand.—No blackening.

XIV Control (soil alone).—No blackening.

Summary.

These preliminary tests show that protein, starch and pentosans can contribute to the black matters of humus but neither fat nor cellulose, and that the restriction of the air is very essential to the humus formation.

The nitrogen content of humus in two cases examined was found due to protein.

Können Phosphate Chlorose erzeugen?

VON

T. Takeuchi.

Bisher war bei Versuchen mit Wasserkulturen noch von Niemanden beobachtet worden, dass lösliche Phosphate ungünstig gewirkt hätten. Deshalb dürfte die kürzlich von Crone gemachte Angabe (Bonner Inauguraldissertation, 1904, (Dez.) und Biedermanns Centralbl, 1906, S. 30.), dass lösliche Phosphate Chlorose erzeugen könnten, wohl einiges Bedenken hervorgerufen haben. Vergleichen wir jedoch die Nährlösung, welche Crone anwandte mit der wohl bewährten Knop'schen Nährlösung, so findet sich, dass jene Nährlösung nicht nur weniger Stickstoff, sondern auch bedeutendere Mengen von Sulfat enthielt. Sie enthielt ferner Dikaliumphosphat, was die Resorbirbarkeit des Eisens herabdrücken musste. Angesichts der zalreichen bisherigen Erfahrungen mit Knop's Loesung wird auch der weitere Schluss Crones kaum auf Zustimmung rechnen können:

„Die Voraussetzung, dass diejenige Nährflüssigkeit die besten Erfolge versprechen müsse, die alle ihre Bestandteile nur in gelösten Zustand enthalte und dadurch der Wurzel der Arbeit des Aufschliessens enthebe, muss jetzt als völlig irrig hingestellt werden."

Lösliche Phosphate sind im Gegenteil zu Crones Behauptung unerlässlich, um Chlorophyllbildung hervorzubringen, wie O. Loew vor langer Zeit folgerte (Über den Einfluss der Phosphorsäure auf die Chlorophyllbildung. Bot. Centralbl., 1891). Er experimentierte mit Algen, welche zunächst in eine mit destillirtem Wasser (2 L.) hergestellte Nährlösung gebracht wurden, welche 0.2 p. mille Calciumnitrat und 0.02 p. mille Ammoniumsulfat enthielt. In die sehr geräumige Flasche wurde hie und da etwas

Kohlensäure eingeleitet. Nach sechs Wochen Stehen im zerstreuten Tageslicht bei 14–16° waren die Zellen gelb geworden, aber trotz der Unvollständigkeit der Nährlösung nur wenige Zellen abgestorben. Hierauf wurde 0.02 p. mille Ferrosulfat zugesetzt und die Loesung mit den Algen in zwei möglichst gleiche Portionen geteilt und zur einen Hälfte noch 0.08 p. mille Dinatriumphosphat gesetzt. Schon nach fünf Tagen ergab sich ein höchst auffälliger Unterschied: Die Phosphat-Algen hatten eine intensiv grüne Farbe angenommen, die Control-Algen aber hatten ihre gelbe Nuance behalten, trotz des Zusatzes eines Eisensalzes.

Dieser Versuch beweist klar, dass trotz des Eisenzusatzes bei den Algen Chlorose fortdauerte, wenn Phosphate mangelten, während bei Anwesenheit von Phosphaten sie schön grün erschienen, dass also lösliche Phosphorsäure hier unumgänglich nötig war zur Chlorophyllbildung.

Um nun zu beweisen, dass in dem Versuche Crone's nicht Phosphorsäure es war, welche Chlorose hervorrief, verglich ich die Crone'sche Nährloesung mit einer, in welcher das Calciumsulfat dieser durch die doppelte Menge Calciumnitrat ersetzt, und das Phosphat nur als Monokaliumphosphat gegeben war, nicht als Gemisch mit Dikaliumphosphat.

Die Nährlösungen (die Salzmengen beziehen sich auf den wasserfreien Zustand) enthielten im Liter g:

	Crone'sche Loesung.	Control-Loesung.
Kaliumnitrat	1.0	1.0
Calciumsulfat	0.5	—
Calciumnitrat	—	1.0
Magnesiumsulfat	0.5	0.5
Eisensulfat	0.005	0.005
Dikaliumphosphat	0.25	—
Monokaliumphosphat	0.25	0.5

Als Versuchspflanze diente Weizen. In feuchten Sägespänen gekeimte und in Brunnenwasser gezogene Keimlinge von ca. 10 cm. Höhe wurden am 19 März in diese Loesungen, je 2½ L. in einem Zylinder eingesetzt. Der geringe Niederschlag von Eisenphosphat wurde von Zeit zu Zeit aufgerührt. Es zeigte sich schon nach 25 Tagen, dass die Blätter der Sprosse in der Crone'schen Nährflüssigkeit ein gelbliche Färbung annahmen, während in der

Control-Loesung sie schön grün erschienen. Auch ein bedeutender Höhenunterschied war bemerkbar. Die Beobachtung am 16 April ergab folgende Data :

	Crone'sche Loesung.			Control-Loesung.		
	I	II	III	I	II	III
Längstes Blatt.	21 cm.	21 cm.	22 cm.	28 cm.	29 cm.	28 cm.
Zahl der Blätter.....	5	5	5	7	6	6
Farbe	gelblich	gelblich	gelblich	grün	grün	grün
Wurzel-Länge ...	17 cm.	20 cm.	26 cm.	26 cm.	35 cm.	32 cm.

Da nun die Blätter in der Crone'schen Nährloesung von Tag zu Tag blasser wurden, und infolge dessen das Absterben bald zu erwarten war, so wurde am 26 April zu sämtlichen Nährlösungen je 15 ccm. einer ziemlich concentrierten Aufschwemmung von künstlichem Ferriphosphat gegeben und wieder von Tag zu Tag der Niederschlag aufgerührt. Es zeigte sich schon nach wenigen Tagen, dass die jüngsten Blätter in der Crone'schen Loesung wieder grün wurden und die Pflanzen weiter wuchsen. Aber auch die Pflanzen in der Control-Loesung, obwohl grün, fingen an, noch etwas dunkler zu werden.

Die Messung am 7 Mai ergab, cm. :

	Crone'sche Loesung.			Control-Loesung.		
	I	II	III	I	II	III
Längstes Blatt.... ..	32	30	33	53	52	51
Zahl der Blätter ...	8	8	8	14	9	9
Wurzel-Länge	32	33	33	44	46	41

Am 9 Mai wurde was Frischgewicht bestimmt, g. :

	Crone'sche Loesung.			Control-Loesung.		
	I	II	III	I	II	III
Frischgewicht	2.61	2.52	2.47	6.51	6.48	7.46
Mittel		2.53			6.81	

Der Versuch wurde nun als beendet betrachtet, da nun erwiessen war,

1. Dass Eisen nicht giftig wirkte, wie Crone meinte.

2. Dass die Nährlösung Crone's der Aufnahme von Eisen bei geringem Eisenzusatz Schwierigkeiten bereitete.

3. Dass entgegen Crone lösliche Phosphate keine Chlorose verursachen können, was allerdings längst bekannt war.

4. Dass entgegen Crone die Pflanzen durchaus normal gedeihen, wenn die Nährstoffe in löslicher Form dargeboten worden, was ebenfalls bekannt war, seitdem Wasserkulturen mit Knop'schen Nährloesungen ausgeführt worden sind.

Does Any Organic Silica Compound Exist In Plants?

BY

T. Takeuchi.

It is a well known fact that various organs of the animals contain among the mineral constituents also some silica, but whether this silica is present in the form of an organic compound and whether it performs any special physiological rôle, is not yet decided. It is thus far only with the feathers that Drechsel (Centralbl. f. Physiol. 11, p. 361.) isolated by extraction with a mixture of alcohol and ether an organic silica compound in small quantities which he supposed to correspond to a cholesterin ester of silica. Whether, however, this compound has anything to do with the growth of the feathers, further whether it occurs also in other forms of keratin, as wool, hairs, hoofs, etc. and whether that organic silica compound is produced in the animal body or is derived as such from vegetable food, has not yet been decided. Drechsel died soon after his discovery and nobody has followed up thus far his observation.

It may, in regard to that question, be of some interest to compare the amounts of silica found in various organs of animals and plants. One thousand parts of ash of egg yolk were found to contain 5.5–14.0 parts silica, while one thousand parts ash of egg white 2.8–20.4 parts (Poleck). The ash of feathers contains 10–30% silica. Young animals contain in the same tissues more silica than old ones. In the ash of the pancreatic gland 12% silica was found by Faulhaber.

In regard to plants Wolf's tables contain the following data: Parts of SiO_2 in 1000 parts dry matter:

Roots and tubers	0.6–16
Leaves of root crops	1.8–18.1
Leaves of Gramineæ	13.6–42.0
Grains and seeds	0.2–7.1
Elodea canadensis	28.9

In order to decide whether in plants or in their products silica is present in an organic form, two oils were tested for the presence of silica, namely rape and sesam oil. 100 c.c. were burned in a platinum basin, finally the minute residue was fused with some sodium carbonate and the solution evaporated to dryness with an excess of hydrochloric acid, but no trace of silica could thus be detected.

Since the leaves of Gramineæ are especially rich in silica, one kilo of hay cut into small pieces was extracted for a week with ligroin, and after filtration the ligroin distilled off. The small amount of fatty residue remaining was fused with some carbonate of soda. After adding now hydrochloric acid in excess to the solution and evaporating no trace of silica was observed, on treatment with water the solution obtained was perfectly clear.

The extracted hay was now left for ten days with alcohol of 90% and this extract treated as before, but only a turbidity was obtained at the final test.

The hay was now extracted with water for ten days, the filtered extract after evaporation mixed with carbonate of soda and the mixture fused at red heat until all carbon had burned away. The fused mass after dissolving in warm water was evaporated with hydrochloric acid in excess and the evaporation residue treated again with distilled water. This solution showed hardly a trace of turbidity, so that the presence of silica in this extract became improbable.

The extracted hay was now left with a solution of 2% crystallised sodium carbonate for eight days, and the filtrate treated as before mentioned. In this case silica was present clearly. The well washed silica, very probably present as hydrate, obtained from this extract weighed 0.062 g.

The small amount of silica observed in the above mentioned Alcoholic extract made a second test with another sample of hay desirable. This time the finely cut hay (500 g., air dry) was directly extracted with alcohol

of 90% (1.4 litres) for 15 days at the ordinary temperature and the filtered extract after evaporation fused with a mixture of potassium and sodium carbonate. After evaporation with HCl of the fused product and treatment of the dry residue with water a not inconsiderable amount of SiO_2 was obtained, it corresponded to 0.065% of the hay.

Since anorganic silicates are insoluble in alcohol, it appears therefore that silica occurs in an organic form in Gramineæ[1] and that its quantity varies considerably. Further tests are necessary to clear up the nature of this organic silica compound.

[1] The sample of hay serving for this test consisted chiefly of Gramineæ.

Can Calcium Carbonate Cause Loss of Ammonia by Evaporation from the Soil ?

BY

T. Takeuchi.

It is often assumed in agricultural circles that in the manuring with ammonium sulphate of soils containing calcium carbonate a loss of ammonia can be caused by the production of ammonium carbonate and the partial volatilization of this compound. Thus differences are often explained when on comparative manuring with nitrate and ammonium salt, the nitrate has produced a higher harvest. However, Stutzer and also Pfeiffer have already called attention to another possible explanation of such a difference, namely to the rapid absorption of ammonium carbonate thus formed by bacteria, since it is a very favorable source of nitrogen for their growth and multiplication ; thus a portion of the nitrogen becoming inavailable, the harvest would become less favorable than expected. These authors further called attention to the fact that ammonium carbonate eventually produced would be saved from volatilization by the absorptive power of the soils. However, there are still other circumstances to consider.

The reason why chili-saltpeter is often (not always) superior to ammonium sulphate might be that the alkalinity produced gradually by the decomposition of nitrate in the soil serves to neutralize the acidity of the superphosphate and thus will render the manure neutral, while ammonium sulphate would in contrary gradually increase to an injurious degree the acidity, caused by superphosphate, when calcium carbonate is absent.

Considering the above question from the plain chemical standpoint, however, it seems very improbable that ammonium sulphate would be capable of reacting easily with calcium carbonate and passing thus into

ammonium carbonate, with the production of calcium sulphate. In contrary, the reverse is true: when *calcium sulphate comes in contact with ammonium carbonate, calcium carbonate is rapidly formed with the production of ammonium sulphate* and Liebig has recommended therefore more than 60 years ago to spread some gypsum on putrefying stable manure to prevent the escape of ammonium carbonate. The reaction in the opposite direction may only be possible under special conditions, as e.g., at high temperature. In which degree this process may be realized at *summer temperature* deserved to be tested.

To a solution of 10 g. ammonium sulphate in 5 c.c. distilled water were added 100 g. precipitated calcium carbonate and kept at 24° C in a well closed flask. Soon after the mixture was made a weak alkaline reaction became noticeable on turmeric paper. After four weeks the flask was provided with a double perforated stopper bearing two glass tubes[1] of which one was connected with a flask of dilute sulphuric acid and the other with 10 c.c. of titrated sulphuric acid. Through the whole apparatus was sucked air which, in passing through the first flask, was deprived of any trace of ammonia that might have been accidentally present in the air. Thus purified air passed then through the main flask[2] and carried the ammonium carbonate into the titrated sulphuric acid, which after two hours was titrated again. A special test showed that the air passed through the apparatus after that time did not give any further reaction with Nessler's reagent. Found by titration = 0.0152 g. NH_3 corresponding to 0.152% of the ammonium sulphate. The test was repeated 8 days later and found this time = 0.0139 g. NH_3 = 0.139% of the ammonium sulphate. The third test after a week yielded 0.0123 g. NH_3 = 0.123% of the ammonium sulphate.[3] The mixture was now subjected to boiling whereby the reaction was accelerated.

[1] This operation was so rapidly performed, that no loss of ammonia was possible.

[2] The main flask was repeatedly shaken and kept in a water bath at 24–30° C.

[3] 10 c.c. of our titrated sulphuric acid = 0.102125 g. NH_3.

Neutralized at the first test	= 1.488 c.c.
At the second test	= 1.362 c.c.
At the third test	= 1.204 c.c.

After three hours 50 c.c. of the titrated sulphuric acid were already completely neutralized. This is however not surprising at all and was expected.

In order to test the rapidity of the normal reaction 20 g. of gypsum were mixed with 4 g. ammonium carbonate dissolved in 100 c.c. water. The smell of ammonia disappeared almost at once; and the alkaline reaction became weaker, but after a certain time it did not decrease any more. The examination of the filtrate and washing showed by titration that there were still necessary 16 c.c. of our titrated sulphuric acid for neutralization. The supposition that this remaining alkaline reaction was due to the lime salt of carbamic acid, was confirmed by the not inconsiderable lime content of the solution.

In a further experiment, therefore, the commercial ammonium carbonate which contains carbamate of ammonia was avoided. The normal ammonium carbonate, produced by a mixture of ammonium sulphate and potassium carbonate in equivalent quantities, was here applied. 5 g. ammonium sulphate dissolved in 10 c.c. water, were mixed with 5.23 g. potassium carbonate dissolved in 5 c.c., whereupon a great portion of potassium sulphate formed was separated as a crystalline powder. The whole mixture was exposed to very low temperature and then filtered. Half of the filtrate, containing 2.16 g. ammonium carbonate was diluted with water to 50 c.c. and the solution shaken with 10 g. of gypsum. This excess of gypsum was used in order to increase the surface of contact. After shaking for some time, and standing 20 hours the mixture was filtered. The filtrate with wash water was slightly alkaline and the titration with our titrated sulphuric acid showed that only 1.7 c.c. were neutralised corresponding to 0.01736 g. $NH_3 = 0.69\%$ NH_3 of the ammonium sulphate present.

We can therefore infer that while gypsum and ammonium carbonate at the ordinary temperature act rapidly upon each other with production of ammonium sulphate and calcium carbonate, the process in the opposite direction can be realized only in a very insignificant measure at average summer temperature, even under the most favorable condition present in our flask.

Hence it may be concluded that *there is no danger of losing any significant amount of ammonia by manuring a soil with ammonium sulphate when calcium carbonate is present.*[1]

[1] This conclusion agrees well with the observation of Wagnik, that no loss of ammonia was observed on a sandy soil containing fully 10% $CaCO_3$.

Relation of Plant Growth to Root Space.

BY

S. Kumakiri.

The causes of the smaller yield of plants when grown in small pots compared with such grown in larger pots have been repeatedly discussed by various authors, most recently again by Lemmermann. The final conclusion at which this author has arrived is that the conditions of the soil nutrients, and especially of the water supply are less favorable in small than in large pots. It is a fact that pots kept in a glass house and manured at the same rates as is usual in the fields, will yield generally less harvest than fields for an equal number of plants. The increased supply of nitrogen by the rain can not fully explain the better growth on the fields—under otherwise equal conditions.

The roots of plants grown in small pots will run to a great extent along the walls of the pots, as Sachs had already pointed out, hence they are on one side not in contact with the soil from which they draw the nutrients.

This unfavorable condition will not be so great in a large pot as in a small pot under otherwise equal conditions.

It is clear that the differences will increase with the number of plants and size of the species. In order to obtain here some data, the yield of a small species, spinach, was compared with that of a larger, viz, barley.

The soil serving for the experiment was a loamy humus soil and was manured per 10 kilo with :

5 g. Double superphosphate.
6 „ $NaNO_3$
4 „ $(NH_4)_2SO_4$
6 „ K_2SO_4.

The small pots held 2 kilo soil while larger pots 10 kilo.

The manure was certainly abundant as the number of plants grown per pot were only two. The objection that there was not enough of mineral nutrient in the small pots would therefore have been impossible.

On October 10, 15 seeds of spinach and 15 seeds of barley respectively were sown in each of the large pots, while the small pots received 8 of spinach seeds and 8 of barley grains respectively.

The young plants were thinned October 28 to two plants of equal size in all the pots.

The spinach plants showed at an early date a considerable difference in height.

The measurements were, cm.:

	December 22.	January 17.	February 2.
Small pots	7.5	8.7	9.7
	8.1	10.0	10.6
Average	7.8	9.3	10.1
Large pots	12.1	13.9	16.9
	12.4	14.2	17.8
Average	12.2	14.0	17.3

These plants were harvested on February 2 with the following result, g:

	Small pots.	Large pots.
Total harvest	17.4	49.0
	23.0	49.3
Average	20.2	49.15

An examination of the roots in both cases revealed an immense difference, as in the small pots a very great number of roots were growing along the walls, very much more so than in the large pots.

The barley plants also showed a very marked difference in height, as will be seen from the following data in cm.:

	December 21.	January 17.	May 29.
Large pots	24.3	28.6	86.7
	23.3	28.0	77.0

	December 21.	January 17.	May 29.
Small pots	14.3	16.8	57.0
	16.1	17.7	63.7

The plants in the large pots flowered earlier and ripened earlier than those in the small pots.

The plants were cut May 29 and weighed in the air-dry state:

	Small pot.	Large pot.
Number of Stalks	8	27
	5	20
Straw, g.	20.7	91.0
	14.5	70.8
Grains, g.....	8.2	41.0
	6.0	36.0

Hence the plants in the large pots produced here 5.4 times more seed than in the small pots.

The examination of the barley roots also showed a very great difference in regard to the amount of root growing along the walls.

Conclusion.

With barley the total yield in the large pots was 4.8 times of that in the small pots, while with spinach the former was 2.5 times that of the latter, hence the extent in which the roots can spread along the walls of the pots has a very great influence in diminishing the harvest.

On the Physiological Effects of an Excess of Magnesia upon Barley.

BY

S. Kumakiri.

It is a well known fact that an excess of magnesium compounds depresses the yield of many crops; it has further been shown that it is especially the ratio of magnesia to lime which determines the degree of depression. But it was also of interest to observe whether special phenomena would characterise the growth at an excess of magnesia in the soil. For this purpose barley was grown at a considerable excess of magnesia.

Six Wagner pots holding 8 Kg loamy humus soil containing 0.5% CaO and 0.4% MgO soluble in HCl of 10% received the following manure per pot, g :

4	Double superphosphate.
4.8	$NaNO_3$
3.2	$(NH_4)_2SO_4$
4.8	K_2SO_4.

Twenty five grains of barley were sown in each pot Nov. 28 and in the following month the young plants were reduced to 15 of equal size per pot.

Two pots A served as check pots and did not receive any further addition.

Two pots B received 10 g. each of crystallized magnesium sulphate in high dilution.[1]

[1] The doses of magnesium sulphate employed here in the pots B and especially in C would certainly have produced a much worse result on a light sandy soil than they did on our loamy humus soil.

Two pots C received each 50 g. of the same salt.[1]

It was noticed in the following month that in the pots C the plants remained in height far behind the plants in pots B and these again remained behind the control plants which showed also much earlier development of ears than the plants of B and C.

On June 8, the check plants were deadripe while the plants in B were in the majority still green and the plants in C had not yet commenced yellowing at all. With these latter plants a very remarkable phenomenon was noticed, namely the sheathes of the leaves had separated from the stems in many cases causing the leaves to hang downwards.

In comparing the number of shoots, it was observed that in the pots C not a single original seed had developed more than one stalk while in the pots B were found 2 plants with 2 shoots, one plant with 3 shoots and one with 4 shoots. In the check pot, however, were observed five plants with two shoots and one plant with three shoots.

The plants were now untied and left exposed to the wind, whereby it was noticed that the plants in C had week stalks, offering but little resistance and bending down heavily.

Summing up it may be stated.

1. With an excess of magnesia over lime growth and ripening process are retarded, the more so, the greater that excess.

2. A moderate excess of magnesia does not diminish essentially the number of shoots, but a larger excess will.[2]

3. An excessive amount of magnesia in the soil diminishes the strength of the leaf sheathes and of the stalks.

[1] If we now take into consideration that according to former experiments carried out here, 14 parts of crystallised magnesiumsulphate are as effective on this soil as 100 parts of magnesite in finest powder and assume the availability of magnesite to be the same as that of the natural magnesium compounds in that soil, the ratio of available lime to magnesia would be in that mixture now $= \frac{1}{4}\frac{1}{4}$ or $= 0\,\frac{2}{1}\frac{3}{7}$.

[2] The number of stalks starting from one seed depends therefore not only upon peculiarities of race or variety, and further upon influence of moisture and temperature, but also upon certain proportions of magnesia in a soil.

On Changes of Availability of Nitrogen in Soils, I.

BY

O. Loew and K. Aso.

Altho the useful as well as the injurious activities of the bacterial flora in soils have been repeatedly investigated and discussed, there still remain various points requiring a further clearing up. Soluble nitrogen compounds often serve to a certain extent to rapidly increase bacterial life,[1] instead of benefitting the crops. A sort of contest takes place in the soil for the nitrogen compounds between the roots and most kinds of the soil bacteria. How does the nitrogen of these bacteria again become available for the roots? When the bacteria die, what agency renders them soluble and where are the probable enzyms derived from which split these bacterial proteids into amidocompounds[2] and ammonia?

The question whether bacteriolytic enzyms are produced by certain soil bacteria has thus far never entered into discussion, altho the existence of such enzyms has been proved for Bact. pyocyaneum and the related Bact. fluorescens liquefaciens.[3] The *pyocyanase*, obtained from cultures of the former was found capable to dissolve typhoid-, pest-, cholera-, anthrax-, and diphtheria-bacilli, gonococci and the cocci of meningitis.

[1] Ammoniacal nitrogen is more favorable for soil bacteria than nitric nitrogen, as Stutzer has observed in several cases.

[2] Various amido-compounds can be absorbed by the root as such and serve directly for nutrition. Bæssler, e.g., has shown that for asparagin. Cf. also E. Schulze, Landw. Vers.-Stat. 1905. Loew and Bokorny have proved that principle for algæ.

[3] Rudolf Emmerich and Oscar Loew, Zeitschr. f. Hygiene 1899. These enzyms were called *nucleases*, since they dissolve the nucleoproteids of microbes.

Recently it has been shown by Heinze[1] that a sterilised mass of Azotobacter furnished available nitrogen to mustard plants but how these bacteria became soluble or how the nitrogen of their protoplasm became available to the roots was not yet decided by this author.

Since yeast cells behave in various chemical relations like bacteria, some observations with yeast cells may be mentioned, as they shed some light also on the behavior of bacteria.

Excretion of protein from living yeast cells. Under certain conditions living yeast *excret a not inconsiderable amount of albumin*, as one of us has observed many years ago, namely on treatment with a current of air at 30–32°. An amount of beer yeast, corresponding to 2 g. dry matter, was suspended in 250 c.c. of a solution, containing:

Canesugar	10%
Ammonium acetate	1%
K_2HPO_4	2%
$MgSO_4$	0.02%
$CaCl_2$	0.01%

and a swift current of air, filtered through cotton, passed thru[2]. After 15 hours some fibrin-like flocculi had separated on the wall of the flask and the filtrate yielded after acidulating with nitric acid and heating a mass of coagulated albumin, amounting in the dry state = 7.6% of the dry matter of the yeast.[3]

Recently M. Müller,[4] studying the behavior of the microbes in the intestinal canal of cattle, observed similiar facts and he declared: „the protein compounds produced by those microbes (of the first stomach or pounch) form only to a small portion real protoplasm, since they are excreted in considerable quantities." It remains to be investigated how far this con-

[1] Landw. Jahrb. 1906, Heft VI.

[2] The experiments are described in the work of C. Nägeli: Theorie der Gärung, p. 81 [1879].

[3] The effect was about the same when the ammonium acetate of the above solution was replaced by nitrate or carbonate of ammonia, while sodium nitrate was not utilized.

[4] Pflüg. Arch. f. d. ges. Physiol. 112, p. 247 [1906]; also Chem. Ztg. 1906.

clusion would hold good also for the microbes of the soil.[1] It would appear, however, that in soils the conditions are not so favorable for energetic protein formation as in the intestinal canal and that usually excretion of protein from the living soil bacteria would not take place to any great extent.

Not only yeast and bacteria, but also green plants produce more protein than immediately needed. This excess, however, is not excreted by them but remains dissolved in the cell-sap, either in the original labile state, active albumin or protoprotein,[2] or in the changed, passive state, ordinary albumin, which is separated in flocculi upon addition of some nitric acid to the filtered juices and heating. The organised proteids of cytoplasm, nucleus and chloroplasts are insoluble in water.

Nitrogenous matters excreted by dying cells. It is a well known fact of plant physiology that in the moment cells die, all soluble matter diffuses from the cells to the surrounding water outside. This is due to the fact, that *in the moment of death* not only the chemical but also the physical properties of the living matter change,[3] and the cytoplasm, playing the rôle of an osmotic membrane as long as the cells are alive, becomes a mere filter, thru the pores of which all soluble matters can make their way.[4]

The writers have proved with yeast cells that the amount of nitrogenous matters, partly consisting of *peptones*, passing to the outside upon the death of the cells, is by no means inconsiderable.

Fresh, pure cultured beer yeast was suspended in water and then by filtration under moderate suction the adhering water removed as far as it was practicable. It contained in this state = 22.8% of dry matter. 63.27 g. of this yeast = 14.42 g. dry matter were mixed with 120 c.c. water and 20 c.c. bisulfide of carbon and with repeated shaking left for 17 hours, after which

[1] Since many microbs excret an enzym and enzyms are closely related to proteins, the excretion of nitrogen in this case is not doubtful.

[2] Loew and Bokorny, in: Loew, Die chemische Energie der lebenden Zellen 2d. Ed. Chapt. VIII.

[3] Ibid. Chapt. II and IX.

[4] This principle holds good also for the animal cells but the physiologists have thus far not paid sufficient attention to it. A suitable object for demonstration is Spirogyra, which, on being killed, gives up at once the tannin content, as can be shown by ferrous sulphate.

time the yeast had assumed the grayish color of dead yeast while the yeast in the control flask with water alone had preserved the normal yellowish color of living yeast. The filtrates from both flasks were now evaporated to dryness and the nitrogen determined in the residue. The result was: the killed yeast yielded 2.9618 g. extractive matters containing 0.2383 g. nitrogen, while the control yeast yielded 0.4106 g. soluble matters, containing 0.0133 g. nitrogen. From this it follows that the original yeast cells with 8.70% nitrogen, on *being killed by bisulfide of carbon had lost 20.52% of the dry matter containing one fifth of the total nitrogen*, while the living yeast had in the same time and at the same temperature (10–15°) only excreted 2.84% of its dry matter, containing only 1.06% of the total nitrogen of the original yeast cells.

In a second test with another sample of fresh beer yeast the amount of mineral matters excreted in the moment of death was determined. Also this sample lost, on being killed with bisulfide of carbon, over seven times the amount of soluble matters than was excreted by the living cells in the same time; from 8.46 g. dry matter of yeast was thus obtained 1.5878 g. extractive matters, while in the control case with living yeast only 0.2172 g. These extracts were incinerated and the ash determined. The original yeast contained 6.79% of ash in the dry matter; 5.64 parts = 80 per cent of the ash were water-soluble phosphates. The matter excreted by the living yeast in 15 hours yielded 0.0514 g. ash = 0.61% of the dry matter of yeast, while the matter excreted from the killed yeast yielded 0.3970 g. ash = 4.69% of the dry matter of yeast = *69% of the total ash of the yeast*. This excreted ash constituents were entirely soluble in water and contained

0.167 g. phosphoric acid

and 0.155 g. potassa

hence consisted chiefly of potassium phosphate. Various authors have analysed the yeast ash; their data vary from 44–59% P_2O_5 and from 28–39% K_2O. It is obvious from the above determination that *dying yeast cells lose by filtration to the outside much nitrogen, potassa and phosphoric acid.* This law holds good also for all plant cells, also for other species of fungi and hence also for bacteria.

These facts throw now some light also on the favorable effects of the

soil treatment with bisulfide of carbon and other bactericidal substances, observed by Nobbe, Hiltner, Richter and others. Moritz and Scharpe[1] write: „*An increased nutrition with nitrogen and mineral matters seems to take place after treatment of the soil with bisulfide of carbon.*" Efforts were made further to discover whether bisulfide of carbon would be able to render mineral matters in the soil more available by forming some sulfuric acid on oxidation, but such was not the case. These last named authors observed also that *sterilising the soil by heat* led about to the *same increase* of crops than the treatment with bisulfide did, what becomes quite intelligible under the point of view developed above.

However soils rich in humus often form acid by sterilisation at 100–125° as Schulze observed and in this case an addition of some lime is necessary to render visible the beneficial effect of sterilizing. By the killing of the microbes, however, not only a part of their nitrogen, potassa and phosphoric acid becomes available to the roots, but also the air in the soil will be less over-charged with carbonic acid, produced by the respiration of the microbes, and better conditions for the respiration of the roots are produced, what becomes more important for clayey soils than for loose sandy soils.

According to the views of several authors small doses of bisulfide of carbon and of other volatile matters act also as stimulants for root growth. Such a view is perfectly correct, as it not only was proved recently by one of us (A) for naphthalene[2] but also observations by Mr. Daikuhara from the Exp. Station at Nishigahara have shown it to take place with bisulfide of carbon.[3] This acts beneficially in several ways.

Summary.

1. Protein matters are excreted under favorable conditions of growth by yeast and bacteria.

[1] Arbeiten aus der biolog. Abteilung am kaiserl. Gesundheitsamt, IV, Heft. 2 [1904].

[2] Cf. page 413 of this Bulletin.

[3] These observations will be published later on by the author.

2. On the death of cells all soluble matters can pass thru the cytoplasm to the outside. Peptones and mineral nutrients are excreted largely by dying yeast cells and very probably also by the microbes of the soil. This phenomenon throws some light on the beneficial action on crops of bisulfide of carbon when applied to soils.

On the Continuous Application of Manganous Chlorid in Rice Culture, II.

BY

K. Asō.

In a former communication[1] the writer had stated that an increase of one third in grains and about one half in straw was obtained with rice by the application of 25 kilo Mn_3O_4 per hectar in the form of manganous chlorid. That experiment was continued in the following year on the same field under the same circumstances as before. Two plots, each of 30 sq. metres, used for the former experiment, served again. 27 kilo barnyard manure, 15.5 kilo rotten human excrements, 230g. double superphosphate and 570 g. wood ash were applied to each plot, and while one plot received 200g. crystallized manganous chlorid, the other served as control. On July 5, the young rice plants were transplanted from the seed-bed[2] and on Nov. 5, the plants were harvested and weighed in the air-dry state with the following result :

	Manganese plot	Control plot.
Total harvest, kilo	27.12	26.52
Grains	12.42	12.17
Straw	14.70	14.35
Full grains	12.12	11.85
Empty grains	0.30	0.32

[1] Bul. College of Agric. Tokyō Vol. VI. p. 131–133. [1903].

[2] Each plot received 306 bundles of twelve equally developed individuals. There was caused no noticeable damage by fungi or insects during the season. According to Fraps (Texas Apr. Exp. Stat.,

These differences (2% of total harvest) are so small that a stimulation by manganese can hardly be inferred. The cause is probably due to the fact that *weather conditions during that year* (1904) *were exceedingly favorable for rice culture*, so that the plants of the control plot reached the most favorable growth possible. The action of manganese as a stimulant proved therefore superfluous ; in fact stimulation was hardly possible.

In the following year (1905) the experiment was repeated on the same paddy field. The quantities of barnyard manure, rotten human excrements and double superphosphate were the same as before ; but 500g. kainit were applied in this experiment instead of wood ash. The manganese plot received again 200 g. manganous chlorid.

On July 7, the young plants were transplanted from the seedbed. The growth proceeded without any disturbances by parasites. In the year of 1905 the weather at the flowering time in August was cool and rainy and interfered with the fertilization process. The plants were cut Nov. 27, and weighed in the air-dry state with the following result :

	Manganese plot.	Control pot.
Total harvest, kilo.....	26.103	25.201
Grains.....	9.343	9.001
Straw	16.760	16.200
Full grains	8.511	8.220
Empty grains.....	0.832	0.781

These figures show in the first place that the stimulating effect of manganese was much smaller than in 1903 ; the increase in straw being only 3.5%

Bull. No. 82) an average rice crop consumes 16 pounds P_2O_5, 42 pounds N and 55 pounds potash per acre. Rice straw carries with it, when removed, 14 pounds N and 31 pounds of potash per acre. In burning the rice stubble nearly 5 pounds of nitrogen goes up in smoke and requiring about 70 pounds of rape cake to restore the loss. In burning rice straw 14 pounds of nitrogen per acre pass off (which are contained in about 200 pounds of soy-bean cake). The ashes of one acre of rice straw contains nearly 3 pounds of P_2O_5 and 37 pounds of potash. An average rice crop consumes more nitrogen than an average crop of cotton, oats or corn. If the rice straw ashes are restored the loss of potash is only 5 pounds per acre, the amount contained in the grain.

and that in grains 3.8% over the yield on the control plot. This result might have been due to the fact that by the experiment of 1903, much more of the mineral nutrients of the soil and manure were absorbed by the increased production on the manganese plot than on the control plot leaving the latter richer than the former. However the manganese plot was not only left poorer in nutrients, but also must have increased in acidity. In order to decide the question of the cause of that phenomenon, the experiment was modified in the following year (1906). The relative amount of manganous chlorid applied remained the same as in the previous years, but it was partly applied in conjunction with lime, and partly with an increase of manure. Six plots served for this trial,[1] each measuring 9.5 sq. metre. Three of them received crystallized manganous chlorid in high dilution, 66.6 g. each, July 11, while the other three served as check plots. Two plots were limed and two received 33% increase of manure. The plan of the experiment is seen from the following sketch :

No increase of manure.[2]	
A.	D.
Manganese chlorid.	Check.
No increase of manure.	
B.	E.
Manganese chlorid + lime.	Check. Lime.
33% increase of manure.	
C.	F.
Manganese chlorid.	Check.

200 g. calcium carbonate were applied to each of the two central plots B and E, May 20. The transplantation of the young rice plants took place

[1] The former manganese plot was subdivided into three manganese plots, the former check plot into three check plots.

[2] The general manure (applied July 10) consisted for the plots A, B, D and E of 166 g. Kainit, 77 g. double superphosphate, 9 k. barnyard manure and 5 k. rotten human dung respectively. The plots C and F received one third more.

July 12, 126 bundles for each plot, each bundle of twelve healthy plants. The weather of this year was not favorable for rice growth, still not so inferior as the previous year. The plants were harvested on Nov. 21 and weighed in the air-dry state four weeks later. The result was in kilograms :

	Total harvest.	Grains.	Straw.	Full grains.	Empty grains
A	7.249	1.969	5.280	1.580	0.389
B	7.318	2.373	4.945	2.000	0.373
C	7.300	2.490	4.810	2.140	0.350
D	5.868	1.361	4.507	0.890	0.471
E	7.006	2.178	4.828	1.760	0.418
F	7.322	2.327	4.995	1.960	0.367

It will be learned from this table :

1. Liming was favorable, the total production being increased by 19% (compare the check plots D and E).

2. Increase of manure at 33% increased the total harvest for 24.8% (compare F and D).

3. On the manganese plots the increase was relatively greatest where the manuring conditions were the least favorable ; the increase in total harvest being 23.5% (compare the check plot D with the manganese plot A).

4. On the limed plots the manganese has led to an increase of 4.4% only of the total harvest and of 13.6% in full grains (compare the limed check plot E with the manganese plot B).

5. On the plot with increased manure the manganese exerted no effect as to the total harvest (Difference—0.3%), but as to full grains an increase of 9% took place, there being relatively more straw produced on the check plot F than on the manganese plot C.

6. Since the considerable effects of manganese of 1903 have not been reached again neither after liming nor after increase by manure, the experi-

ments will be continued[1] until a satisfactory answer to the question is obtained, under which conditions the effects of manganese are the most favorable? Finally the increase of total harvest in the different years by the action of manganese may be compared:

1903	41.8%
1904	2.2 ,,
1905	3.5 ,,
1906	23.5 ,,
1906 (limed plots)	4.4 ,,
1906 (increased manure)	0 ,,

[1] Also the similar experiments started by Prof. Nagaoka in frames (cf. these Bulletins VII, No. 1) will be continued.

Observations on Stimulation of Plant Growth.

BY

S. Kakehi and K. Baba.

Effect of manganese carbonate. In former experiments carried out at this College stimulating effects have been observed on plants by manganese, applied in the form of sulphate and chlorid, which salts are of course changed in the soil to humate, silicate, or phosphate. In order to exclude the influence of such a change, whereby also original compounds of potassium or sodium are transformed to sulphate or chlorid respectively, manganese was applied in our test in the form of artificial carbonate in a dose of 1 g. per pot of 10 kilo soil. As general manure per pot served : disodium phosphate 10 g., sodium nitrate 5 g., ammonium sulphate 5 g., potassium sulphate 6 g. Two pots were sown with pea, two with barley (Oct. 10). To each case were two check pots without manganese. The young plants were thinned to 10 per pot of equal size (Nov. 2). The pea plants were ripe May 14, the barley May 26. The harvest was weighed in the air-dry state. The result was :

	Pea.		Barley.	
	Mn-Plants.	Check.	Mn-Plants.	Check.
Total weight, g.....	122	110	89	84
	118	84	84	79
Seeds, g.....	41	34	43	40
	36	31	43	39

Hence there was exerted a moderate stimulation with pea, the plus yield being 24%, while the small difference with barley (6%) is not very

decisive. Also in former cases pea had responded more to stimulation than barley.

Comparison of the stimulating effect of fluorine and manganese. Soil and general manure were the same as in the former experiment. Two pots received 0.002% manganese sulphate, (=40 kilo per ha) one further pot 2 milligrams sodium fluorid and another 20 mg., corresponding to 0.5 and 5 kilo NaF per ha, respectively. The stimulants were applied as *top dressing* in two fractions. (Feb. 20 and March 12). Eight wheat plants were allowed to grow in the pots. Since towards end of April danger from fungi developed, the plants were cut before the ripening of the seeds and weighed in the fresh state with the following result :

	$MnSO_4$	NaF 0.002 g.	NaF 0.02 g.	Check.
Total weight, g.....	345 352	328	332	313 298
Weight of ears, g.....	49 50	47.8	49.0	48.0 46.5

This gives the following ratio :

Average of the 2 Check pots ..... = 100

Manganese sulphate in top dressing at the rate of 40 kilo per ha ..... = 113

Sodium fluorid, at the rate of 0.5 kilo per ha ... = 107

Manganese sulphate had therefore in this case produced a better result than sodium fluorid ; however this may change on other soils.[1]

[1] In certain soils sodium fluorid may be much more quickly transformed into the but little active calcium fluorid, than in others.

On Different Forms of Phosphoric Acid in Press Cakes.

BY

T. Funatsu.

Since refuse press cakes are frequently used as manure, it is of some importance to determine the amounts of phosphoric acid present in different forms, as the availability for plants differs very much in different compounds.

A portion of the phosphoric acid in such cakes is soluble in water another in dilute acids while a further portion is dissolved by alkaline liquids. This last mentioned portion is due to lecithin and nucleoproteids. As to lecithin, it is probably easily decomposed in the soil by microbs, but a certain part of nucleoproteids might resist destruction and contribute a share to humus which contains not only some nitrogen but sometimes also some P_2O_5 in a form not easily available to the plants.

From this point of view the writer has determined in several press cakes and for comparison also in fish guano the amounts of phosphoric acid soluble in different solvents.

Soybean cake: Soybean cake is imported from Manchuria to Japan where it serves extensively as manure.

Soybeans contain about 1.6% lecithin (Schulze) and yield 14–16% of oil[1]. Soybean cake shows according to a report from Experiment Stations in average:

$N = 6.5\%$
$P_2O_5 = 1.0\%$
$K_2O = 2.0\%$

Our soybean cake contained 12% hygroscopic moisture. In determing total phosphoric acid 3 g. air dry cake were incinerated and the phosphoric

[1] This oil serves in China for cooking purposes.

acid determined as usual ; obtained 0.057 g. $Mg_2P_2O_7 = 1.211\%$ P_2O_5 of the air dry cake.

To determine phosphoric acid present in the form of lecithin 10 g. air dry cake served for extraction with ether and alcohol. After evoporating these extracts to dryness and fusing with KNO_3 and Na_2CO_3 was obtained 0.027 g. $Mg_2P_2O_7 = 0.16\%$ P_2O_5 of the air dry cake.

To determine phosphoric acid present in the form of nucleoproteid, the residue of this extraction was treated with dilute hydrochloric acid, (*a*) and the well washed residue incinerated with addition of carbonate and nitrate of soda. Obtained : 0.031 g. $Mg_2P_2O_7 = 0.197\%$ P_2O_5. The acid liquid and wash water (*a*) were filled up to 250 c.c., one fifth of this liquid was evaporated and the residue incinerated as before. Obtained :

0.31 g. $Mg_2P_2O_7 = 0.197\%$ P_2O_5, present in inorganic form and as so-called anhydro-oxy-methylen-phosphoric acid. Calculated for the cake, dried at 100°, the result is :

Total phosphoric acid	$= 1.38\ \%$
P_2O_5 as lecithin	$= 0.174\%$
P_2O_5 as nuclein	$= 0.226\%$
P_2O_5 soluble in 4% HCl	$= 0.980\%$
Sum	$= 1.380\%$

The same determinations were carried out with cotton seed cake, rape cake and herring guano dried at 100° with the following result, to which we add for comparison the soybean cake analysis mentioned above.

	Soybean cake.	Cotton seed cake.	Rape cake.	Herring guano.
Total P_2O_5	1.38%	2.25%	2.82%	4.56%
P_2O_5 as lecithin	0.17%	0.12%	0.20%	0.35%
P_2O_5 as nuclein	0.23%	0.30%	0.26%	0.66%
P_2O_5 sol. in dilute HCl	0.98%	1.80%	2.37%	3.55%

The relative amounts total $P_2O_5 = 100$.

	Soybean cake.	Cotton seed cake.	Rape cake.	Herring guano.
P_2O_5 as lecithin	12.4	5.0	7.0	7.7
P_2O_5 as nuclein	16 5	13.2	9.0	14.4
P_2O_5 sol. in dilute HCl	71.0	81.7	84.0	78.0

These results show that the amounts of phosphoric acid present in the form of nucleoproteids are comparatively small.

As to the better availability of phosphoric acid of fish guano, observed by Nagaoka, this may be due to the forms soluble in water (K_2HPO_4) and in dilute hydrochloric acid.

In seed cakes there exists much more phosphoric acid in organic combinations than in fish guano.

A special test was further carried out in order to observe whether a part of phosphoric acid would be easily split off from nucleoproteids by soil bacteria Nucleoprotein (from beer yeast) freshly prepared and corresponding to 9.9 g. dry matter was mixed with sand, infected with soil bacteria and moistened with a 0.5% solution of potassium acetate containing 0.2 g. $MgSO_4$. After two months in the thermostete at 27° only 39% of the original phosphoric acid had become soluble. The mass had become almost black and had an ammonical and fecal odor, showing a certain degree of putrefaction.

[1] Cotton seed cake and also poppy cake contain often more phosphoric acid than observed in the above samples of cake. Thus in U. S. the avarage amount of P_2O_5 in cotton seed cake is considered $= 2.88\%$, and as to poppy cake, as much as 3.5% P_2O_5 was observe l in one sample by Mach.

On Bat Guano from Marianne Islands.

BY

S. Kanamori.

The caves of Rota and Saipan in the Marianne Islands contain a loose dark brown mass, resembling somewhat dry peat, in strata of several meters in thickness.[1] There occur small white particles and occasionally crusts of about the size of a walnut in that formation but whether these admixtures are spread uniformly through the strata is not yet known. Since the microscop reveals numerous particles of wings and legs of insects and even occasionally wing-scales of butterflies, there can hardly exist any doubt that this deposit represents bat-excrements which probably have undergone partial changes in course of time. Warm dilute caustic potassa extracts a humus like substance with evolution of some ammonia. The solution becomes at the same time very slimy, rendering filtration rather difficult. Hydrochloric acid precipitated from this solution a brown flocculent mass, which contained nitrogen. The extracted residue consisting mainly of chitin represented the chief mass of the manure; it was digested with hydrochloric acid of 5% for one hour, washed and dried and served for some further bacteriological tests.

The white particles and crusts contained in average 8.45% P_2O_5 and contained nitrogen and carbonate and phosphate of lime. The latter might have derived and accumulated from the urine of the bats.

The analysis of the loose brown mass yielded the following results:

[1] A sample of this formation was kindly furnished us from Mr. Weinberger of Yokohama wh had received it from a German officer stationed at those Islands.

Hygroscopic water...	14.2%
Organic matter	74.3 ,,
Ash[1]	11.4 ,,

In the air dry mass of two samples[2], % ...	I	II
Total phosphoric acid	7.33	
Total nitrogen	14.89	8.97
Potassa.....	2.47	2.09
P_2O_5, soluble in 1% citric acid	1.50	1.75
Nitric acid	0.22	
N, soluble in acidulated water, chiefly ammonia	0.13	
(*a*) N in humus, soluble in alkali	2.64	
(*b*) N, liberated as ammonia by hot alkali.....	2.87	

The aqueous extract contained besides some nitrate also traces of sulphates and chlorides.

The question arises whether this guano would be a readily available nitrogenous manure. This question can, however, not be answered directly since a good deal of the nitrogen is present in the form of chitin, a substance that putrefies not so readily as protein matters do.[3] Besides chitin, however, there are present doubtless also other nitrogenous compounds, which can

[1] Analyses have been published of bat-guano, found in different parts of the globe, showing great differences in composition of this product. Since the water content was found between wide limits (20-67%) it can easily be explained that these guanos from different localities have undergone a very different degree of putrefaction and extraction. The nitrogen content varied from 2–14%, phosphoric acid from 2–11%, potassa from 1.6–2%, nitric acid from 0.2–1.2%.

[2] Sample I contained many of the white particles mentioned, Sample II was nearly free from them.

[3] Whether common soil bacteria can gradually attack chitin was not yet investigated. Benecke *W. Benecke*, however, has isolated from putrefying chitin (tortoise-shell) a kind of bacterium which destroys chitin very easily. He named it *Bacillus chitinovorus*. It may be that this microb occurs also in soils occasionally which question I am now investigating. The writer has seen mouldfung attacking chitin.

easily be decomposed by boiling with a solution of 1% sodium hydroxid. Thus the air dry manure yielded after boiling for an hour and a half 2.87% N in the form of ammonia, determined by titration of the distillate.[1] Very probably this nitrogen becomes more easily available to the plant than that of chitin. Chitin = $C_{18}H_{30}N_2O_{12}$ contains 6.0% N and would yield after perfect decomposition 7.28% NH_3. The question of the availability of that organic guano-nitrogen had to be decided by a practical test.

Two pots A filled with 8 Kilo unmanured loamy humus soil were manured, each with :

Potassium sulplate	6 g.
Ammonium sulphate	8 „
Secondary calcium phosphate	8 „

Two pots B received 11, 4 g. of the guano, corresponding to the nitrogen amount of pots A.

The difference in P_2O_5 and K_2O in the pots A and B was supplied to the pots B in the form of secondary calcium phosphate and potassium sulphate. Hence the pots B received :

Bat-Guano	11.4 g.
Potassium sulphate	5.5 „
Secondary calcium phosphate	6.7 „

Two further pots C were prepared like A, except that no nitrogen manure was added.

Barley, 20 seeds per pot were sown Nov. 8. The young plants when 15 cm. high were thinned to 10 per pot, all of equal height.

During the vegetation it was noticed that only the plants with full manure were perfectly green while with the bat-manure and in absence of nitrogen the stalks were reddish, due to the presence of authokyan which, as S. Suzuki[2] has shown, appears when either phosphoric acid or nitrogen is wanting. In our case however it only can be ascribed to the lack of nitrogen. Already this phenomenon shows that the nitrogen of the bat-

[1] Taken 2 g. sample ; distillate required 2.8 c.c. titrated sulphuric acid. 10 c.c. of this acid = 0.6982 g. H_2SO_4.

[2] This Bulletin Vol. VII. No. 1.

guano was not easily available, altho there existed bacteria in the soil which can attack it gradually, as my observation have proved.[1] Also a superficial comparison showed that the plants of the fully manured pot were far ahead of the other plants.

Since there existed danger of attack by fungi, the plants were cut Jan. 29 and weighed in the fresh state. The observations were :

	Average number of stalks.	Fresh weight, g. (average).
A Full manure.	42	53.5
B Bat-guano.	35	30
C No nitrogen added.	36	28

This result shows that this bat-guano, containing undecomposed chitin, forms no readily available source of nitrogen.

[1] Nitrogen from chitin, becoming thus nitrogen of the bodies of microbs, cannot at once become available for roots.

On the Composition of the Shoots of Aralia cordata.

BY

T. Takeuchi.

The shoots of Aralia cordata (Jap. Udo) serve as food in Japan. Its branched stems are either consumed in the fresh state with addition of some salt, or after boiling with soy souce. Some time ago, Dr. Fairchild,[1] agricultural explorer of the Department of Agriculture in Washington who on his exploring tours around the globe had also visited Japan, has warmly recommended these shoots for the American cuisine and proposed the cultivation of the plant in the United States. He declared that a fine salad may be prepared by cutting the shoots into long thin shavings and pouring over them the dressing composed in the usual way of oil, vinegar, salt and pepper. "These slices of Udo are crisper than slices of celery and have none of the objectionable stringy fibres of the latter. They have a fresh taste, like the midrib of a lettuce leaf, with a slight but most agreable suggestion of pine flavor. The tenderest young shoots of celery could not be more brittle than these branched stems of Udo."

"From the first of October until the middle of May this vegetable is for sale in the markets of Japan, and in this winter character, aside from its being an excellent salad, lies its great value. It is comparatively cheap and is eaten by the poor Japanese as well as by the rich."

"From its adaptability to winter culture and its excellent quality, this plant deserves to become as well known as asparagus or celery." Indeed, in the cooked state its taste is at least as fine as that of asparagus.

[1] Bulletin No. 42, Bureau of Plant Industry, Washington.

There are two varieties of *udo*, called respectively "*Kan-udo*" and "*Moyashi-udo*," and these, though of similar appearance as they are placed on the market, are quite differently cultivated.

My observations on the chemical composition relate to *Kan-udo*, the shoots of which are in average 45 cm. in height and 1.5 cm. in thickness. For investigation served only that part which also serves as food; hence the lowest part and the tip, consisting in some small branches with leaflets, were cut away. The surface is reddish, due to the presence of antocyan to judge from the change into blue color by alkali. The juice has a slight acid reaction, and contains some soluble albumin, reducing sugar, tannin, further oxidase, peroxidase and catalase. Pentosans, and galactan were observed, but not mannan. The presence of starch was revealed by the iodine test.

The fresh substance consists of 94.5% water and 5.5% dry matter. 100 c.c. of the juice required 8.5 c.c. of $\frac{n}{10}$ NaOH solution for neutralization. The analytical determinations were carried out after the usual methods.

2 g. served for the determination of nitrogen.
2 ,, ,, ,, ,, ,, ,, protein nitrogen.
10 ,, ,, ,, ,, ,, ,, ethereal extract.

5 g. served for the determination of pentosans. In their determination 0.174 g. and 0.1705 g. of the furfurol precipitates by phloroglucin were obtained in two samples. In the determination of galactan, 5 g. yielded on oxidation with nitric acid in 2 texts: 0.0208 g. and 0.0204 g. of mucic acid. In the determination of tannin, 5 g. yielded after Wolff's method 0.039 g. and 0.0382 g. of CuO. Also citric acid is present.

In the following table the results obtained are shown, and for comparison the average of 4 samples of Asparagus officinalis is added.[1]

[1] Data from König's "Chemie der menschlichen Nahrungs-und Genuss-mittel." Vol. II., p. 660.

Shoots of Aralia cordata.			Shoots of Asparagus officinalis.	
	% of dry matter (100°C.)	% of fresh substance.		% of fresh substance.
Water	0	94.50	Water	93.75
Crude protein	19.97	1.10	Crude protein	1.79
Crude fibre	15.38	0.85	Crude fibre	1.04
Ethereal extract[1]	7.67	0.42	Crude fat	0.25
Ash	9.91	0.55	Ash	0.54
Total nitrogen	3.20	0.18	Nitrogen free extract	2.26
Albuminoid nitrogen	2.01	0.11	Sugars	0.37
Non-albuminoid nitrogen	1.19	0.05		
Protein	12.56	0.69		
Dextrose	4.41	0.24		
Sucrose	1.21	0.07		
Starch	2.26	0.12		
Pentosans	7.47	0.41		
Galactan	0.53	0.03		
Tannin	5.95	0.33		

Since the introduction of Aralia cordata into Europe and America, for the purpose of the cultivation of its shoots, might some day take place, we add the following culture notes, taken from Fairchild's Bulletin above mentioned.

"*The Cultivation of Kan-udo. The seeds of this variety are sown broadcast in seed beds*, prepared of rich garden earth, *in the month of March or April*, and are allowed to grow there for one year. The following spring the individual seedlings are transplanted from this seed bed, after the tops, which have died during the winter, have been removed, and they are then set in rows 2 feet apart and 10 inches from each other in the rows. In these rows they are cultivated all summer, or until September, when the leaves begin to turn brown. The stems are then cut back close to the rootstocks

[1] Besides fat and lecithin there was another substance present, which I intend to examine later on.

and the earth is piled up in a mound 2 feet high above the latter. In forty days the new shoots, which begin to form as soon as the old ones have been cut back, appear above the surface of the mound. They are then ready for cutting, and the mound is opened and the marketable shoots cut. Each rootstock produces about five of these blanched shoots, three of which are probably fit for the market at the first cutting, early in October. The remaining small shoots are covered up again and allowed to grow for a second cutting a week or so later. In removing these shoots for market care is taken to cut close to their bases, so as not to leave stubs, as the presence of the latter is said to prevent the rapid growth of the remaining young shoots.

Generally only two crops of shoots are secured of the *Kan-udo*, but occasionally there are three. After the removal of the last crop the rootstocks are buried and allowed to remain over winter. In the spring the mounds are opened and rich manure is applied in trenches running on both sides of the plants. Thru-out the summer the plants are allowed to grow and are again cut down in autumn and treated in a similar way to that just described. The life of the *Kan-udo* rootstock is more than ten years, but beyond that age its use ceases to be profitable.

Altho generally grown from seed, this variety can be reproduced from root cuttings, though the latter method is considered less practicable, owing to the fact that the large root cuttings take up more space in the field.

The season for *Kan-udo* is October and November, and being the earliest variety and occupying the fields to the exclusion of other crops it is also the dearest, sometimes selling for as much as 25 cents for a bundle of 16 shoots. It is not otherwise preferable in any way to the other variety, which first appears in the market toward the end of November."

Note on the Composition of a Chrysanthemum Flower, serving as food.

BY

T. Funatsu.

In the province of Akita in Japan, the yellow flowers of a kind of Chrysanthemum are largely consumed as an addition to fish, or also soaked in hot water and prepared as a salad. These flowers are called "Hoshi-kiku," are of sweet taste and are considered as a delecacy. They are sold, either in the dry state pressed into sheets, or in the salted state.

It was of some interest to determine the composition which was found to be as follows:

Hygroscopic moisture	10.20%
Glucose	20.60 ,,
Cane sugar	3.80 ,,
Crude protein	10.82 ,,
Crude fibre	13.93 ,,
Fat. (ether extract)	7.40 ,,
Ash	5.95 ,,
Starch	1.92 ,,
Extractive matters, pentosans, etc. from diff.	25.38 ,,

Note on Japanese Tobacco from Satsuma.

BY

K. Baba.

The Tobacco raised in Japan has served thus far chiefly for the manufacture of cigarettes. Moderate quantities were also exported for wrapper purposes but no tobacco variety suitable for fillers in cigar manufacture has thus far been produced. The heavy manuring applied in the United States for the production of "gummy" leaves, has thus far not been applied, at least not in central Japan. Recently, however, some attention has been paid to the production of a suitable cigar leaf and while formerly tobacco was only cured but not fermented, the fermentation in bulk has commenced to be practiced. Hence the question whether Japanese tobacco varieties are capable to develop a good cigar aroma has become of interest and the writer has carried out the following investigation in regard to the qualities of a tobacco (Nicotiana rustica) raised in Satsuma province, thus far the most famous tobacco in Japan.

Since the aroma decreases with the increase of fat and protein, it was necessary to determine these constituents. Kissling holds that the resins of tobacco are important for a proper aroma; but also certain compounds, produced from nicotine by oxidation in a thorough fermentation process, may contribute to the formation of aroma.[1] Hence resins and nicotine also were determined, the resins after Kissling's method. For analysis the middle vein of the leaves was discarded, the determinations were made according to the usual methods, the nitrogen after Kjeldahl, protein-N after Stutzer, nicotine by distillation and titration.

[1] Thoms has obtained an ethereal oil from the smoke of tobacco, which results from a product of the fermentation process, by the dry destillation in smoking.

Four qualities served for the test, namely A, lowest or sand leaves (doha), B, lower middle leaves (chuha), C, upper middle leaves (honpa) and D, upper most leaves (tenpa). Here as in other countries the central leaves are considered superior to the lowest, oldest and to the youngest.

The result of the analysis was:

Hygroscopic water.

A. 8.789%
B. ..... 8.148 „
C. ..... 8.112 „
D. ..... 8.062 „

The dry matter contained, %:

		A	B	C	D
	Crude fat.....	6.302	11.549	14.004	12.616
Resin	Soluble in petroleum ether...	3.001	5.839	6.666	6.003
	„ „ ether	0.365	0.432	0.824	0.799
	„ „ alcohol	0.498	0.858	1.133	1.021
	Total nitrogen.....	0.893	1.381	1.525	1.594
	Protein nitrogen	0.5488	0.7754	0.8080	0.8753
	Protein	3.4300	4.8463	5.0500	5.4706
	Amido-nitrogen	0.163	0.272	0.300	0.372
	Nicotine	0.8897	1.4435	1.8112	1.4099
	(corresponding to nicotine N)	0.1541	0.2500	0.3137	0.2442
	NH_3	0.033	0.102	0.126	0.125
	Nitric acid	0.1180	0.1723	0.1691	0.2012
	Ash	22.271	14.811	14.042	12.654

These numbers show a moderate content of nicotine and protein and a unusual high proportion of the resin fraction soluble in petroleum ether. The figures for crude fat in B, C and D are rather high, too high for a good cigar leaf.

In addition I mention some analytical determinations of three other Japanese Tobacco varieties and add for comparison the numbers obtained with the Ibusuki tobacco above analysed.

In dry matter, 100 parts :

		Total N.	Nicotin.	Crude fat.	Nitric acid.	Ash.	K_2CO_3 in the ash.
Shiroume		1.122	1.371	7.821	0.140	14.582	1.990
Takeda produced in Oita.	B	1.632	0.679	8.135	0.200	16.000	2.204
	C	2.072	0.807	8.821	0.353	14.123	2.052
Daruma produced in Ibaraki.	B	1.299	1.187	9.235	0.180	18.465	2.201
	C	1.589	1.273	11.671	0.183	16.121	2.356
	D	1.712	1.002	11.102	0.188	13.111	2.016
Ibusuki produced in Kagoshima.	A	0.893	0.889	6.302	0.118	22.271	2.043
	B	1.381	1.443	11.549	0.172	14.811	2.406
	C	1.525	1.811	14.004	0.169	14.042	2.132
	D	1.594	1.409	12.616	0.201	12.654	1.693

Note on Bacillus Metiylicus.

BY

T. Takeuchi.

This microb occurs in a reddish and in a colorless variety. Moreover the *red* variety when cultivated in bouillon develops in a *colorless* state. Since it seemed to me that the reaction of the solution might have an influence on the formation of the color, a further experiment was made. The original culture solution of Loew contained sodium formate, which by its gradual conversion into sodium carbonate produced a tolerably strong alkaline reaction. In order to avoid this reaction I substituted magnesium formate[1] for sodium formate. After exposing the culture solution for several months in an open Erlenmeyer flask to the air,[2] a considerable formation of *white* films was noticed and further at the bottom of the flask a crystallisation of magnesium ammonium phosphate; the liquid itself had remained perfectly clear. An alkaline reaction was hardly perceptible. The films appeared to consist of a pure culture of Bacillus methylicus.[3]

The composition was:

Magnesium formate	0.5%
Dipotassium phosphate	0.2 „
Diammonium phosphate	0.1 „
Magnesium sulphate	0.01 „

[2] This microb also occurs, as Katayama (These Bulletins V p. 255 and VI p. 191) observed, in the soil at moderate depth, also in rivers and sea water.

[3] This microb is far different from the *Bacillus formicicus* of Omelianski; the latter *can not develop* with formates as an exclusive food and shows other characteristics, as Katayama (l. c.) has already discussed. It is, further, different from *B. methanicus* which has cilia, is motile and can assimilate methan.

In order to observe whether this *colorless* culture of B. methylicus would assume a *reddish* color, when nourished with sodium formate, the films were shaken with sterilized bouillon-gelatine and a plate culture prepared. From one of the numerous colonies which all resembled each other a trace was inoculated in sodium formate culture solution and in sterilized bouillon; further the growth on potatoes and in stab culture in bouillon gelatine was observed.

The result was that in sodium formate solution the microb showed again a *red* color, while on the control media the development was quite characteristic for B. methylicus.

Hence not only a red and white variety of B. methylicus must be distinguished, but also it must be admitted that the red variety can grow in a colorless state when care for neutral reaction is taken.

Ueber die chemische Zusammensetzung der japanischen Soja-Sauce oder "Schōyu."

VON

U. Suzuki, K. Aso und H. Mitarai.

Da die japanische Soja-Sauce oder "Schōyu" ein unentbehrliches Volks-Speise-Mittel ist, dessen Verbrauch in ganz Japan jährlich über vier Millionen Hektoliter steigt, so bietet es physiologisch, wie technisch ein wichtiges und interessantes Problem, die chemische Zusammensetzung und auch die chemischen Vorgänge, die während des Reifeprozesses der Sauce vor sich gehen, genau kennen zu lernen. Zwar haben sich manche Chemiker bereits mit diesen Fragen beschäftigt, trotzdem bleibt aber noch vieles unklar, besonders das Schicksal der Stickstoff-Verbindungen, die im Ausgangsmaterial (Sojabohnen und Weizen) hauptsächlich als Eiweiss-Stoffe sich vorfinden, und während des Reife-Prozesses entweder durch Enzyme oder durch Mikroorganismen eine energische Spaltung und Veränderung erleiden, was natürlich zu einer ganzen Reihe verschiedener complizirter Verbindungen führen kann. Da die Trennung und das Identifizieren der Eiweisszersetzungs-Produkte eine schwierige Aufgabe ist, so hat man his jetzt in dieser Richtung keine befriedigenden Resultate bekommen. In neuester Zeit hat aber unsere Kenntniss über die Zersetzungs-Produkte der Eiweiss-Körper durch die Untersuchungen von E. Fischer, E. Schulze, A. Kossel u. A. eine neue Gestalt gewonnen, und deshalb schien es uns auch möglich, durch Anwendung dieser neuen Methoden das Problem der "Schōyu" etwas näher aufzuklären. Die nach altbewährten Regeln hergestellte Schōyu-Probe, die wir für diese Untersuchung anwendeten, wurde uns von Herrn K. Mogi, Besitzer einer grossen Schōyu Brauerei in Noda, gütigst geliefert, wofür wir ihm unseren besten Dank aussprechen. Diese Probe wurde aus 3 Maischen hergestellt :—

7 April	1904	3 Hektoliter
7 April	1905	3 Hektoliter
27 October	1905	5 Hektoliter

und die völlig ausgereifte Maische ausgepresst. Als Material wurde teils gewöhnliche Sojabohne teils nackte erwendet und mit dem gleichen Volumen Weizen aus der Higo-Provinz gemischt. Das Kochsalz war aus England bezogen.

Bei der Herstellung der Sauce werden zuerst die Soyabohnen 5 Stunden gekocht, dann nach dem Abkühlen mit gerösteten Weizenkörnern gemischt und diese Mischung nach Infection mit den Sporen von Aspergillus Oryzae auf Gestellen in einer engen, meist halb-unterirdischen Kammer bei 30–40° 3 Tage lang belassen, wodurch ein weit verzweigtes Mycel um alle Körner herum sich entwickelt. Diese nun *Soya-koji* genannte Masse, wird mit der Kochsalzlösung von 15–20% vermischt und in grossen Kufen 1–3 Jahre stehen lassen, wobei die auf der Oberfläche wachsenden Schimmelbildungen von Zeit zu Zeit in die Masse gerührt werden. Jenes Mycel liefert die verschiedenen beim Reifeprocess nötigen Enzyme.

Um eine bessere Qualität Sauce zu erzielen, wird nach längeren Pausen zu der fermentirenden Sauce noch ein oder zweimal *Soya-koji* gesetzt.

Diese Probe hatte folgende quantitative Zusammensetzung :—

Farbe	Dunkel braun.
Reaktion	Ziemlich stark sauer.
Spez. Gewicht	1.197
Wasser	67.15%
Trockensubstanz	32.85%

In 100 Theilen Trocken substanz.

Organische Substanz	49.12
Roh-Asche	50.88
Chlor	27.24
Chlor als NaCl berechnet	44 94

	In 100 gram Schōyu.	In 100 c.c.
Gesammtstickstoff	1.249	1.488
Eiweissstickstoff	0.037	0.044
Ammoniakstickstoff	0.140	0.166

	In 100 gram Schōyu.	In 100 c.c.
Durch Phosphorwolframsäure fällbarer Stickstoff (Ammoniak ausgenommen).	0.330	0.361
Stickstoff in anderer Form	0.742	0.914

	In 100 Theilen Trocken substanz.	Gesammt stickstoff als 100.
Gesammt stickstoff.....	3.802	100.00
Eiweiss stickstoff	0.113	2.96
Ammoniak stickstoff.....	0.462	11.16
Durch Phosphorwolframsäure fällbarer Stickstoff (Ammoniak ausgenommen).	0.965	26.41
Stickstoff in anderer Form	2.262	59.49

Der gesammte Stickstoff wurde nach Kjeldahl und der Eiweiss stickstoff nach Stutzer bestimmt. Für die Bestimmung des Ammoniak stickstoffs wurde 100 c.c. Schōyu mit 200 c.c. Wasser verdünnt, mit Natronlauge beinahe neutralizirt, mit etwa 1–2g. Magnesia usta geschüttelt bis die Flüssigkeit schwach alkalisch reagirte, und bei niederem Druck (15–20 mm.) bei 40–50 c.c. destillirt. Das Destillat wurde in normal Schwefelsäure-Lösung eingeleitet, und in gewöhnlicher Weise titrirt. Für die Bestimmung des Stickstoffs in organischen Basen wurde 10 c.c. Schōyu mit 40 c.c. Wasser verdünnt, durch basisches Bleiacetat gefällt. Zum Filtrat von dem Bleiacetat-Niederschlag wurde nach Entbleien durch Schwefelsäure noch so viel Schwefelsäure zugegeben bis die Flüssigkeit ungefähr 5% der Säure enthielt und so viel Phosphowolframsäure-Lösung zugegeben bis kein Niederschlag mehr entstand. Nach 24 Stunden wurde der Niederschlag abgesaugt, mit 5% iger Schwefelsäure gewaschen bis das Waschwasser keine Reaktion auf Chlor zeigte und gleich darauf der Kjeldahl'schen Methode der Stickstoffbestimmung unterworfen. Während des Kochens des Niederschlags mit conc. Schwefelsäure muss man unbedingt 1–2g. Kupferoxyd oder Kupfersulfat zugeben, damit das heftige Stossen vermieden wird.

Aus dem analytischen Resultate sieht man, dass in Schōyu nur 3% des gesammten Stickstoffs als Eiweissstoffe vorhanden ist d. h. die Spaltung ist so weit vorgeschritten, dass die resultirenden Produkte nicht mehr als Nahrung-

stoff betrachtet werden können. Dies führt zum Schluss, dass Schōyu nicht als ein Nahrungsmittel sondern als ein Genussmittel aufzufassen ist, das keinen direkten Einfluss auf die Ernährung des Menschen hat.

In folgenden Seiten teilen wir die Isolirung der einzelnen stickstoffhaltigen Bestandteile und auch der organischen Säuren mit.

I. Organische Basen.

Zwei Liter Schōyu wurden zuerst durch Eindampfen im Vacuum vom grössten Teile NaCl befreit, dann mit etwa 2 Liter 10% iger basischer Bleiacetat Lösung versetzt, wobei ein dicker Niederschlag entstand. Das klare Filtrat wurde durch Zusatz von Schwefelsäure vom Blei befreit, und dann mit so viel Schwefelsäure angesäuert bis die Flüssigkeit ungefähr 5% Schwefelsäure enthielt, und dann mit einer conc. Lösung von Phosphowolframsäure gefällt. (Man braucht dazu etwa 2 kilo Phosphowolframsäure.) Der Niederschlag wurde abgesaugt und mit 5% iger Schwefelsäure gewaschen. Da die Entfernung von Kochsalz durch Waschen sehr langweilige Arbeit war, wurde der Niederschlag noch mal mit 5% iger Schwefelsäure verrieben, wieder abgesaugt und mit 5% iger Schwefelsäure gewaschen. Als das Waschwasser keine Reaktion auf Chlor mehr zeigte wurde der Niederschlag in Wasser verteilt und mit Überschuss von Bariumhydroxyd verrieben. Das Gemisch wurde öfters umgerührt und bei einer Temperatur von 25–30°C. 24 Stunden stehen gelassen und abgesaugt. Der Rückstand wurde nochmals in Wasser verteilt und mit Baryt verrieben. Diese Operation wurde dreimal wiederholt. Die vereinigten Filtrate wurden durch Kohlensäure vom Baryt befreit und in Vacuum bis auf 1 Liter eingedampft. Das in der Flüssigkeit vorhandene Ammoniak wurde dabei vollständig entfernt. Die alkalisch reagirende Flüssigkeit wurde jetzt mit Kohlensäure gesättigt und mit einer gesättigten wässerigen Lösung von Quecksilberchlorid versetzt, bis die Flüssigkeit schwach sauer reagirte und kein Niederschlag mehr entstand.

A). Der Quecksilberchlorid-Niederschlag wurde in Wasser verteilt, mit Schwefelwasserstoff zerlegt. Die vom Schwefel-Quecksilber befreite Flüssigkeit wurde in Vacuum eingeengt. Es blieb dabei ein hellbrauner Syrup, der nicht krystallisirte. Der Versuch, dicsen Syrup als Methylester

salzsaures Salz zur Krystallisation zu bringen hatte auch keinen Erfolg. Wir haben später gefunden, dass wir hier eine polypeptidartige Verbindung in der Hand hatten. Nach den Untersuchungen von E. Fischer, A. Levene, Siegfried, Pick. u. A. geht die tryptische Spaltung von Eiweisskörpern nie so weit, wie mit conz. Mineralsäuren. Es giebt eine gewisse Gruppe im Eiweissmolekul, die gegenüber der enzymatischen Wirkung sehr wiederstand fähig ist und nur durch die Säure-Wirkung weiter gespalten wird. E. Fischer schlug für solche Verbindungen den Namen "Polypeptide" vor, weil sie mit den von ihm dargestellten künstlichen *Polypeptiden* eine grosse Aehnlichkeit haben und durch starke Säure in die einfacheren Aminosäuren gespalten werden. E. Fischer hat auch darauf aufmerksam gemacht, dass in den Polypeptiden oft Prolin und Phenylalanin vorherrscht d. h. diese beiden Aminosäuren scheinen gegen die enzymatische Wirkung am meisten Wiederstand zu leisten.

Wir haben einen Theil des Syrups mit 10% iger Salzsäure 6 Stunden gekocht und in der That haben wir gefunden, dass etwa 40% des Stickstoffs in Form von Monaminosäuren abgespalten wurde. d. h. sie wurden nicht mehr durch Phosphowolframsäure gefällt und von dem Filtrat vom Phosphowolframsäure Niederschlag nach der Entfernung der Phosphowolframsäure durch Baryt und des Baryts durch Schwefelsäure und Verestern in bekannter Weise haben wir Monaminosäure-Ester bekommen. Leider genügte die Menge nicht, um die Ester zu fraktionieren. Nach dem Verseifen durch Baryt wurde Prolin mit Sicherheit nachgewiesen. (Durch Alkohollöslichkeit der freien Monaminosäure und des Kupfersalzes) und ausser dem eine kleine Menge Krystalle, die wahrscheinlich Phenylalauin waren.

Der Phosphowolframsäure-Niederschlag wurde wieder durch Baryt zersetzt, die alkalische Lösung, die freie Basen enthielt, wurde mit Kohlensäure gesättigt mit Quecksilberchlorid-Lösung gefällt und in folgender Weise behandelt.

a). *Der Quecksilberchlorid-Niederschlag.*

Der Niederschlag wurde in Wasser verteilt, mit Schwefelwasserstoff zersetzt. Die von Quecksilbersulfid abfiltrirte Flüssigkeit gab nach dem Eindampfen in Vacuum farblose Krystalle, die wir anfangs für Histidin-

chlorid gehalten hatten. Die nähere Untersuchung zeigte uns jedoch, dass kein Histidin chlorid vorlag. Wir haben nämlich versucht, diesen Körper zu verestern, indem die Krystalle in Methylalkohol gelöst, und trockenes Salzsäuregas bis zu Sättigung eingeleitet und dann in Vacuum eingedampft wurde. Die dabei ausgeschiedenen Krystalle waren aber kein Ester, sondern waren ganz unverändert geblieben ; es war also sicher, dass der Körper keine Carboxyl gruppe enthielt.

Die aus heissem Methylalkohol durch Zusatz von Methylalkohol und Aether ausgeschiedenen farblosen Krystalle betrugen ca 1.32g.

Für die Analyse wurden sie nochmals aus Methylalkohol umkrystallisirt und im Vacuum bei 100°C getrocknet. Ausserdem wurde die Hälfte der Krystalle aus wenig Wasser umkrystallisirt und analysirt. Die Krystallform Schmelzpunkt so wie auch Chlorgehalt waren genau dieselben wie bei dem aus Methylalkohol umkrystallisirten Präparat.

0.100g. Subst : (Aus Wasser umkrystallisirt) gab 19.3 c.c. N(19° 748 mm.)
0.1490g. „ („ „) 0.1953g. CO_2
0.1490g. „ („ „) 0.0730g. H_2O
0.115g. „ („ „) 0.0422g. Cl
0.0980g. „ (Aus Methylalkohol umkrystallisirt) 0.036504g. Cl

		C	H	N	Cl
$C_6H_9N_3 2HCl$	Berechnet	36.77	5.62	21.45	36.16
	Gefunden	35.75	5.49	21.83	36.70 37.20

Das aus Methylalkohol umkrystallisirte Präparat bestand aus farblosen Prismen, schmolz bei 232-233°C (uncorr.) ziemlich scharf unter Zersetzung. Es zeigte aber keine lebhafte Schäumung wie es bei Estern der Fall ist.

Das salzsaure Salz hat kein Krystallwasser, löst sich leicht in Wasser, reagirt ziemlich stark sauer. In warmem Methylalkohol löst es sich ziemlich leicht, in Aethylalkohol schwer, in Aether Benzol, Chloroform und Petrolether ist es unlöslich.

Das salzsaure Salz wurde in wenig Wasser gelöst, die Salzsäure durch normal Natronlauge genau neutralisirt und die etwa drei fache Menge Pikrinsäure in Substanz zugegeben und so lange erwärmt bis sie klar gelöst wurde. Nach dem Erkalten schieden sich gelblich rothe seidenglänzende

Krystalle in reichlicher Menge aus, die zweimal aus heissem Wasser umkrystallisirt und gereinigt wurden. Für die Analyse wurden sie im Vacuum bei 100°C getrocknet.

0.1624g. Subst. 0.2171g. CO_2
0.1624g. „ 0.0480g. H_2O
0.1035g. „ 19.6 c.c. N(17°. 755 mm.)
0.4073g. „ 0.3227g. Pikrinsäure.

		C	H	N	Pikrinsäure.
$(C_6H_9N_3)(C_6H_3N_3O_7)_2$	Ber.	37.17	2.58	21.69	78.83
	Gef.	36.46	3.31	21.83	79.23 78.98

Das Pikrat bestand aus orangegelben Prismen oder Tafeln und hat kein Krystallwasser. In Capillarrohr rasch erhitzt wurde es bei 200°C allmählich braun und bei 230°C (uncor.) zersetzte es sich unter lebhaftem Schäumen zu einer schwarzen Flüssigkeit. Es löst sich schwer in kaltem Wasser, leichter in heissem Wasser, in Methylalkohol auch leichter als in Aethylalkohol und in Aether, Petrolaether und Benzol ist es unlöslich.

Das salzsaure Salz wurde in wenig Wasser gelöst, eine wässerige Losung von Platinchlorid in genügender Menge zugesetzt und langsam eingedunstet. Es schied sich bald eine orangegelbe Krystallmasse aus, die aus kurzen Prismen oder Tafeln bestand. Für die Analyse wurde sie einmal aus heissem Wasser umkrystallisirt, mit Alkohol und Aether gewaschen und in Vacuum bei 100°C getrocknet.

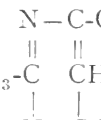
0.1880g. Subst. 0.0903g. CO_2
0.1880g. „ 0.0461g. H_2O
0.1465g. „ 0.0545g. Pt

		C	H	Pt
$(C_6H_9N_3)_2HCl.\ PtCl_4$	Ber.	13.56	2.07	36.50
	Gef.	13.10	2.72	37.20

Das Platindoppelsalz wurde gegen 250° vollständig schwarz, schmolz aber bis 290° nicht. In heissem Wasser ziemlich leicht, in kaltem Wasser schwer, und in Alkohol und Aether ist es fast unlöslich.

Aus den Analysen kann man schliessen, dass die Base der empirischen Formel $C_6H_9N_3$ entspricht. Was die Strukturformel derselben betrifft, so

wissen wir bis jetzt nur drei synthetisch dargestellte Körper, denen die Formel $C_6H_9N_3$ zukommt, nämlich dem Nitril der $\alpha.\alpha'$. Imido-dipropionsäure (C. 1904. (I) 353), dem Di-cyanmethyl-äthylamin, oder Nitril der Aethyl imido-diessigsäure. (B. 37. 4092) und drei isomere Amido-di-methyl-diazine (Amido-di-methyl-Pyrimidin). Nur die letzt genannten Amido-di-methyl-diazine haben nach der Beschreibung grosse Ähnlichkeit mit unserer Base, nämlich : sie bilden krystallinische Salze mit Quecksilberchlorid, Salzsäure ($C_6H_9O_3$. 2HCl) Platinchlorid ($C_6H_9N_3$. H_2PtCl_6) und auch ein Pikrat ($C_6H_9N_3$. $C_6H_3N_3O_7$. od. $C_6H_9N_3$. $(C_6H_3N_3O_7)_2$). Bloss die Schmelzpunkte solcher Verbindungen stimmen mit unserem Präparat nicht. Da noch andere Strucktur-Isomerien möglich sind so ist unsere Base wahrscheinlich eine isomere Form von diesem Diazin-derivat. Besonders interessant wäre es, weil es mit den Nucleinbasen Uracil, Thymin und Cytosin in näherer Beziehung steht.

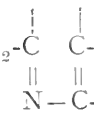
1.  f. 183°

Chloro platinat : Gelbe Nadeln. $C_6H_9N_3H_2PtCl_6$.

Pikrat : Gelbe Nadelbüschel f. 214°. $C_6H_9N_3$. $C_6H_3N_3O_7$.

Quecksilberchloridsalz : feine weisse Nadeln. $C_6H_9N_3HgCl_2$.

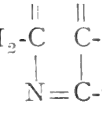
(B. 35 1577. C. 1902. (1). 1236)

2.  f. 214–215°

Salzsaures Salz : lange gefiederte Stäbchen.

Platindoppelsalz : rötlich gelbe Stäbchen und Täfelchen f. 227° unter Aufschäumen schmelzend.

(B. 34. 2819)

3.  f. 150–152°. Wässerige Lösung stark alkalisch.

Chlorplatinat, gelbe Prismen bei 225°C schmelzend.

Salzsaures Salz : $C_6H_9N_3$. 2HCl. Sternförmig gruppierte Nadeln f. 181°C., in Wasser und Alkohol leicht löslich.

Pikrat. $C_6H_3N_3$. $(C_6H_3N_3O_7)_2$ gelbe Blättchen. f. 230°C.

Es sei noch bemerkt, dass die Formel des Histidin $C_6H_9N_3O_2$ mit der unserer Base $C_6H_9N_3$ eine gewisse Ähnlichkeit hat. Da wir keine Spur von Histidin in Schōyu fanden, obgleich diese Base in den sauren Spaltungsprodukten der Eiweisskörper von Soyabohnen und auch von Weizen vorkommt, so dürfte man auch annehmen dass die Base aus Histidin durch Bakterienwirkung entsteht.[1] Wir beabsichtigen später, diese Base etwas näher zu untersuchen und zu entscheiden, ob diese Base durch Säurewirkung aus Soya-oder Weizen-Eiweisskörpen gebildet wird, oder ob ein sekundäres Umwandlungsprodukt vorliegt.

B). Das Filtrat vom Quecksilberchlorid-Niederschlag (a) wurde durch Schwefelwasserstoff vom Quecksilber befreit und im Vacuum eingedampft. Nach dem das Wasser vollständig ausgetrieben war, wurde es mit trockenem Methylalkohol versetzt, trockenes Salzsäuregas bis zur Sättigung eingeleitet, im Vacuum eingeengt, und mit absolutem Alkohol und Aether versetzt. Die dabei ausgeschiedenen farblosen Krystalle betrugen ca 2.2g. Das einmal aus heissem Methylalkohol umkrystallisirte Präparat schmolz gegen 195°C unter lebhaftem Schäumen und enthielt 31.80% Cl, während Lysin-Methylester-salzsaures Salz 30.47% Cl enthält. Die entsprechenden Salze von Arginin und Histidin enthalten noch weniger Chlor (27.2% bezw. 29.2% Cl), desshalb war anzunehmen, dass noch kein einheitlicher Körper vorlag und wurde das Estersalz verseift und in das Chlorid und weiter in das Pikrat verwandelt. In der Tat haben wir zwei verschiedene Pikrate bekommen, die durch ihr Löslichkeitsverhalten leicht von einander sich trennen liessen. Die Hauptmenge bestand aus Lysinpikrat. Es waren lange gelbe Prismen etwa 1.9g. mit genau denselben Schmelzpunkt und Krystallform wie das reine Lysin-Pikrat.

Das andere bestand aus hellgelben Prismen, die viel schwerer löslich in Wasser waren, als Lysin-Pikrat. Nach zweimaligem Umkrystallisiren aus heissem Wasser betrug es 0.8g. Für die Analyse wurde es in Vacuum bei 80° getrocknet :

[1] Noch zu bemerken ist, dass unsere Base eine starke dunkel rote Färbung mit Diazobenzolsulfosäure in alkalischer Lösung giebt, was von Pauly als eigentümliche Reaktion für Histidin und Tyrosin angegeben wurde. Wir sind aber sicher, dass unser Präparat nicht ein Gemisch von Histidin war.

0.1312g. Subst. 0.1726g. CO_2
+0.0403g. H_2O
0.119g. „ 20.2 c.c. N(13°, 766 mm.)
0.4237g. „ 0.3444g. Pikrinsäure.

		C	H	N	Pikrinsäure.
$(C_4H_{12}N_2)(C_6H_3N_3O_7)_2$	Ber.	35.17	3.30	20.52	83.88
$(C_5H_{14}N_2)(C_6H_3N_3O_7)_2$	Ber.	36.43	3.57	20.00	81.80
	Gef.	35.88	3.41	20.22	81.25

Das Pikrat bestand aus hellgelben Prismen, schwer löslich in kaltem Wasser. Im Capillarrohr erhitzt, fängt es von 200°C an allmählich braun zu werden, bei 230° dunkel braun und bei 260° (uncorr) zersetzt es sich plötzlich unter starkem Schäumen zu einer schwarzen Flüssigkeit.

Aus dem Pikrat wurde das Chlorid dargestellt. Es bildete farblose Prismen, spielend leicht löslich in Wasser, schwer in Alkohol und unlöslich in Aether. Im Capillarrohr erhitzt zersetzte es bis 290°C nicht. Erst bei höherer Temperatur zersetzt es sich unter Schäumen. Da die Menge des Chlorids zu wenig war, haben wir es nicht analysirt und unmittelbar in das Platindoppelsalz verwandelt, indem das Chlorid in wenig Wasser gelöst und mit einer wässerigen Lösung von Platin-Chlorid versetzt wurde. Es schieden sich dabei hellgelbe Krystalle eines Platindoppelsalz es, aus die selbst in heissem Wasser ziemlich schwer löslich waren. Sie wurden abgesaugt, mit wenig kaltem Wasser, dann mit absolutem Alkohol und Aether gewaschen und in Vacuum bei 80°C getrocknet und analysirt:

0.1939g. Subst. 0.0701g. CO_2
0.1939g. „ 0.0552g. H_2O
0.1351g. „ 0.0530g. Pt.

		C	H	Pt.
$C_4H_{12}N_2.2HCl\,PtCl_4$	Ber.	9.68	2.82	39.00
$C_5H_{14}N_2.2HCl\,PtCl_4$	Ber.	11.76	3.14	38.00
	Gef.	9.86	3.19	40.20

Das Platindoppelsalz bestand aus kleinen hellgelben Tafeln oder Prismen, die sich manchmal unregelmässig zusammen gruppiren. Im Capillarrohr erhitzt wurde es gegen 220°–230° schwarz, zersetzte sich aber bis 290°C nicht.

Die analytischen Zahlen stimmen also am besten mit der Formel $C_4H_{12}N_2$. Höchst wahrscheinlich handelt es sich hier um Tetramethylendiamin oder *Putrescin*, Da es kein Arginin in Schōyu vorhanden war, so kann man erwarten, dass das Arginin durch Enzyme oder Bakterien in Ornithin und Harnstoff und das Ornithin weiter in Tetramethylendiamin gespalten war. Dass es tatsächlich aus Arginin durch Bakterien-Wirkung Putrescin entsteht, hat Ellinger nachgewiesen.

B). Das Filtrat vom Quecksilberchlorid-Niederschlag A. wurde durch Schwefelwasserstoff vom Quecksilber befreit, im Vacuum eingeengt, um Schwefelwasserstoff auszutreiben. Als die Flüssigkeit bis auf $\frac{1}{2}$ Liter eingeengt war, wurde die vorhandene Salzsäure durch Silbernitrat gefällt. Zum Filtrat von Chlorsilber wurde ein Überschuss von Silbernitrat und Barythydrat zugegeben. Der dabei entstandene braune Niederschlag wurde abgesaugt, mit Wasser gewaschen, und dann in Wasser verteilt, durch Schwefelwasserstoff zerlegt, abfiltrirt und in Vacuum eingeengt. Von der conzentrirten Flüssigkeit wurde ein Pikrat dargestellt, das sich aus heissem Wasser erst ölig ausschied, und nach 24 Stunden zu nadelförmigen Krystallen umwandelte. Die Analyse des gereinigten Präparats gab folgendes Resultat.

C 15.21% H 3.22% N 18.50%

Höchst wahrscheinlich handelt es sich hier um ein anorganisches Pikrat. Arginin war also nicht vorhanden.

C). Das Filtrat von dem Niederschlag B. wurde durch Salzsäure vom Silber und durch Schwefelsäure vom Baryt befreit, und so viel Schwefelsäure zugegeben, bis die Flüssigkeit ca 5% derselben enthielt; hierauf wurde es mit Phosphowolframsäure-Lösung gefällt. Der Niederschlag wurde abgesaugt, mit 5% iger Schwefelsäure gewaschen und durch Barythydrat zerlegt. Das Filtrat, nach dem es vom Baryt befreit war, wurde im Vaccum stark eingeengt, und von der stark alkalisch reagirenden conzentrirten Lösung wurde unmittelbar in bekannter Weise das Pikrat dargestellt. Es hatte die charakteristische Krystallform von Lysin-Pikrat und betrug ca 5.5g. Das zweimal aus heissem Wasser umkrystallisirte Präparat fing bei 220°C an braunschwarz zu werden und gegen 245° zersetzte es sich unter Schäumen. Für die Analyse wurde es im Vacuum bei 80°C getrocknet:

0.767g. Subst. gab 0.4678g. Pikrinsäure.
0.1549g. „ 24.5 c.c. N (4°C, 762.5 mm.)

		N	Pikrinsäure.
$(C_6H_{14}N_2O_2)(C_6H_3N_3O_7)$.	Ber.	18.67	61.07
	Gef.	19.00	60.99

Aus dem Pitrat wurde das Chlorid und das Methylester-Salzsäuresalz dargestellt. Das Chlorid hatte dieselbe Krystallform und den Schmelzpunkt wie das active Lysinchlorid, und das Methylester-Salzsäuresalz krystallisirte in farblosen Prismen. Es schmolz gegen 195–196°C (uncorr.) unter Schäumen. Für die Analyse wurde es zweimal aus heissem Methylalkohol durch Zusatz von Aether ausgeschieden und im Vacuum bei 80°C getrocknet:

0.120g. Subst. gab 0.03615g. Cl = 30.13% Cl, auf
$C_7H_{16}N_2O_2$. 2HCl berechnet 30.47% Cl

Da wir aus der Fraktion A. etwa 1.9g. Lysin Pikrat gewonnen haben, so betrug die gesammte Ausbeute an Lysin-Pikrat 7.4g.

II. Monaminosäuren.

2 Liter Schōyu wurden im Vacuum eingeengt, von dem ausgeschiedenen Kochsalz befreit, und dann mit Wasser auf 4 Liter verdünnt, und ca 2 Liter einer 10% igen basischen Bleiacetatlösung zugesetzt. Das Filtrat vom Blei-Niederschlag wurde durch Schwefelwasserstoff von Blei befreit und im Vacuum stark eingeengt. Um das vorhandene Wasser vollständig auszutreiben, wurde der Syrup zweimal mit etwas absolutem Alkohol versetzt und in Vacuum eingedampft. Der Rückstand wurde jetzt mit 2 Liter absolutem Alkohol versetzt und trockenes Salzsäuregas bis zur Sättigung eingeleitet. Das dabei ausgeschiedene Kochsalz wurde abgesaugt und mit absolutem Alkohol gewaschen. Um die Veresterung möglichst zu vervollständigen wurde das Filtrat vom Kochsalz wieder im Vacuum eingedampft. Der zurückgebliebene dicke Syrup wurde nochmals mit 2 Liter absolutem Alkohol versetzt und trockenes Salzsäuregas bis zur Sättigung eingeleitet, im Vacuum bis zum Syrup eingedampft und zwei Tage in einem kalten Zimmer stehen gelassen. Da hierbei salzsaurer Glycocollester sich nicht ausgeschieden hatte, so wurden nach der bekannten Ester-Methode von E.

Fischer die freien Aminosäurenester dargestellt und bei niederem Druck fraktionirt. Nach der Verseifung der einzelnen Ester-Fraktionen haben wir folgende Aminosäuren isoliert: [1]

	Versuch 1.	Versuch 2.
Alanin	1.5	1.4
Alanin + Leucin	6.0	4.2
Leucin	4.0	6.0
Prolin	3.0	3.0
Asparaginsäure	Vorhanden.	Vorhanden.
Phenylalanin	Vorhanden?	?

Nach Glycocoll wurde mit besonderer Vorsicht gesucht, indem die erste Fraktion mit Salzsäuregas gesättigt und in der Kälte stehen gelassen wurde. Auch die Alanin und Leucinfraktion wurde ebenso behandelt um hier beigemengtes Glycocoll aufzufinden, aber in keinem Fall haben wir die Krystalle von salzsauren Glycocollester gefunden:

Analyse des Alanin Präparats.

0.1891g. Subst. 0.2956g. CO_2
+0.1334g. H_2O
0.1782g. „ 22.2 c.c. N(19°, 765 mm.)

		C	H	N
$C_3H_7NO_2$	Ber.	40.45	7.87	15.73
	Gef.	42.63	7.91	15.10

$[\alpha]_D^{20°} = +13.21°$

Leucin.

0.1780g. Subst. 0.3596g. CO_2
+0.1618g. H_2O
0.1475g. „ 13.5 c.c. N(9°, 762 mm.)

		C	H	N
$C_6H_{13}NO_2$	Ber.	54.96	9.93	10.68
	Gef.	55.04	10.10	11.02

$[\alpha]_D^{20°} = +14.84°$

[1] Die Operation wurde genau nach der von E. Fischer angegebenen Methode ausgeführt. Man vergleiche das Buch von E. Fischer "Untersuchungen über Aminosäuren, Polypeptide und Proteine."

Prolinkupfer (actives).

0.1280g. Subst. verlor bei 100°C in Vacuum getrocknet 0.0145g. = 11.31% H_2O

0.1105g. „ (wasserfrei) gab 0.029g. CuO od. 0.023159g. Cu = 20.96% Cu.

		H_2O	Ca
$C_{10}H_{16}N_2O_4 . Cu + 2H_2O$	Ber.	10.99	19.41
	Gef.	11.32	18.59

Da wir noch keine gute Vacuumpumpe in unserem Institut zur Verfügung hatten, konnten wir mit der Wasserstrahlpumpe höchstens 15–17 mm. Druck erreichen, was für die höher siedenden Ester noch zu hoher Druck war. Desshalb war das Vorhandensein von Glutaminsäure nicht leicht nachzuweisen, obgleich diese Säure in Schōyu sicher vorhanden sein wird. Die Ausbeuten an Asparaginsäure und Phenylalanin waren so gering, dass wir die beiden Aminosäuren nicht analysiren konnten.

Die Abwesenheit von Serin und Aminovaleriansäure ist ziemlich sicher. Nach Oxyprolin und Tryptophan wurde nicht gesucht.

Wir hoffen jedoch später nochmals mit besserem Vacuumapparate diese Versuche zu wiederholen.

Um das Vorhandensein von Tyrosin und Cystin nachzuweisen, wurden 200 c.c. Schōyu mit einer Lösung von basischem Bleiacetat versetzt. Die vom entstandenen Niederschlag abfiltrirte Flüssigkeit wurde nach Entbleiung durch Schwefelwasserstoffgas im Vacuum eingeengt. Um das Chlor zu entfernen, wurde die Flüssigkeit mit einen Überschuss von Silbernitrat gefällt. Das Filtrat von Chlorsilber wurde durch Schwefelwasserstoff vom Silber befreit, und in Vacuum eingeengt. Die so gewonnene Flüssigkeit gab schöne rote Färbung mit Millons Reagens. Da keine Eiweisskörper vorhanden waren, so ist diese Reaction auf Tyrosin zu beziehen. Vor Kurzem hat Pauly eine neue Reaktion auf Tyrosin empfohlen (Zeitschrift. f. physiol. Chem. 1904 s. 513) Dies beruht auf der Entstehung einer dunkelroten Färbung auf Zusatz von Diazobenzolsulfosäure zu einer soda-alkalischen Lösung von Tyrosin (Histidin giebt dieselbe Farbenreaktion). In unserem Fall haben wir hiemit ebenfalls eine schöne Farbenreaktion bekommen. Das Vorhandensein von Cystin wurde dadurch nachgewiesen, dass man die conc. Flüssigkeit mit conc. Kalilauge kurze Zeit kochte und basisches Bleiacetat

zugab. Der schwarze Niederschlag von Schwefelblei wird nur in Anwesenheit von Cystin hervorgerufen.

III. Organische Säuren.

100 c.c. Schōyu wurden mit 100 c.c. Wasser verdünnt, mit Schwelsäure angesäuert und im Vacuum destillirt. Das Destillat wurde in normal Natronlösung eingeleitet. Die flüchtige Säure im Destillat neutralisirte 0.24304g. NaOH. Als Essigsäure berechnet ergiebt sich 0.36456g. = 0.365%.

Für die Bestimmung der gesammten Acidität wurden 100 c.c. Schōyu mit Tierkohle entfärbt, mit Wasser verdünnt und unmittelbar durch normal Natronlösung titrirt. Als Indicator verwendeten wir Phenolphthalein. Es wurde 1.008g. NaOH zum Neutralisiren gebraucht. Wenn man nun 0.365g. (d.h. die Menge der Natronlauge die zum Neutralisiren der flüchtigen Säure nötig ist) davon abzieht, so bleibt 0.765g. NaOH für die nicht flüchtigen Säuren, welche als Milchsäure berechnet 1.721g. = 1.721% ergeben würden. Man darf aber nicht vergessen, dass diese Zahl auf keine Weise der totalen Menge der nicht flüchtigen Säuren entspricht, weil, wie wir schon angegeben haben, in Schōyu nicht nur freie organische Säure, sondern an Ammoniak, organische Basen und andere Körper gebundene vorkommt, was auf die Reaktion der Flüssigkeit grossen Einfluss ausübt.

Um die flüchtigen Säuren zu isoliren wurde 1 Liter Schōyu mit 13 c.c. conc. Schwefelsäure versetzt, und in Vacuum bei 50-60°C. destillirt. Das Destillat wurde in normal Natronlauge eingeleitet. Nachdem keine flüchtige Säure mehr im Destillat nachweisbar war, wurde die Natronlösung eingedampft, der Rückstand in wenig heissem Wasser gelöst, mit Schwefelsäure angesäuert und mit Aether wiederholt geschüttelt. Die ätherische Lösung hinterliess nach dem Verdampfen des Aethers einen nach Essigsäure riechenden Rückstand, der in wenig Wasser gelöst und mit Überschuss von Bleicarbonat am Rückflusskühler mehrere Stunden gekocht wurde. Es wurde dann abfiltrirt, stark eingeengt und mit viel absolutem Alkohol versetzt. Es entstand dabei ein weisser Niederschlag, der mit absolutem Alkohol und Aether gewaschen und bei 100°C getrocknet wurde. Die Ausbeute betrug etwa 0.15g. Die Bleibestimmung gab folgendes Resultat :

0.135g. Subst. gab 0.137g. $PbSO_4$ = 0.09357g. Pb

		Pb
$C_2H_2O_4Pb$	Ber.	69.70%
	Gef.	69.31%

Die Analyse stimmt also ziemlich gut mit ameisensaurem Blei. Ferner war die Krystallform mit dem reinen ameisensauren Salz identisch. Der eigentümliche Geruch der freien Säure und ihr Reduktions-Vermögen gaben uns keinen Grund zu zweifeln, dass es Ameisensäure war.

Das alkoholische Filtrat vom Bleiformiat hinterliess nach dem Einengen einen Rückstand, der etwa 0.77g. betrug. Dieser wurde aus wenig heissem Wasser umkrystallisirt. Die über Schwefelsäure getrocknete aus vierseitigen Prismen bestehende Krystallmasse wurde analysirt :

0.4495g. Subst. gab 0.3519g. $PbSO_4$

$(C_2H_3O_2)_2$ Pb + $3H_2O$	Ber.	54.50%
	Gef.	53.49%

Der mit Schwefelsäure angesäuerte und durch Abdestilliren von den flüchtigen Säuren befreite Rückstand wurde wiederholt mit Aether geschüttelt. Die ätherische Lösung hinterliess nach dem Verdampfen des Aethers einen gelbbraunen Syrup, aus dem nach mehreren Tagen Stehen eine kleine Menge Krystalle sich ausschied, die wahrscheinlich aus Bernsteinsäure bestanden. Leider reichte die Menge nicht aus für eine genauere Untersuchung. Der Syrup wurde jetzt in Wasser gelöst, mit Überschuss von Zinc carbonat mehrere Stunden gekocht, heiss abfiltrirt. Das Filtrat wurde stark eingeengt und erkalten lassen. Es schieden sich allmählich die Krystalle von Zinclactat in reichlicher Menge aus, die von der Mutterlauge getrennt und auf einer Tonplatte getrocknet wurden. Die Ausbeute an Rohprodukt betrug etwa 1.151g. Die Mutterlauge gab noch eine zweite und dritte Fraction, so dass die gesammte Ausbeute 2.517g. = 1.61g. freie Milchsäure betrug.

Das Rohprodukt wurde aus wenig heissem Wasser umkrystallisirt, über Schwefelsäure getrocknet und analysirt :

0.2620g. Subst. gab bei 110°C 0.227g. = 0.035g. H_2O

0.227g. wasserfreie Subst. gab 0.077g. ZnO

0.092g. wasserhaltige Subst. gab 0.0275g. ZnO

		Krystallwasser	Zn
$(C_3H_5O_3)_2Zu+2H_2O$	Ber.	12.9	23.41
	Gef. 1.	13.3	23.61
	Gef. 2.		23.57

Wie bekannt enthält das Zinksalz der activen Milchsäure zwei Molekule Krystallwasser, während das der inactiven Säure drei Molekule davon enthält, es war daher unser Präparat die active Säure. Die optische Drehung stimmte auch auf die active Säure ; es wurde gefunden :

$$[\alpha]_D^{20^\circ} = -6^\circ$$

Diese Zahl liegt ziemlich nah mit der Beobachtung von Y. Kozai, der mit den aus verschiedener Bakterien isolierten Säuren zwischen -5° bis -7° fand. Es sei hier bemerkt, dass das links drehende Zinksalz eine freie Säure gibt, die nach rechts dreht.

Zusammenfassung der Resultate.

Aus 2 Liter Schōyu wurden isolirt :—

Alanin	1.6g. + 5.0g.	unreines Alanin.
Leucin	6.0	
Prolin	3.0	
Lysin	2.6	
Neue Base $C_6H_9N_3$	1.0	
Base $C_4H_{12}N_2$	0.2	
Ammoniak	4.2	
Eiweissstoffe	5.4	(Nach der Berechnung).
Ameisensäure	0.10	
Essigsäure	0.40	
Milchsäure	3.20	

Vorhanden waren :—

Tyrosin
Asparaginsäure
Polypeptidartige Stoffe
Phenylalanin ?
Cystin

Nicht vorhanden waren :—

Glycocoll
Histidin
Arginin
Serin
Aminoisovaleriansäure ?
Glutaminsäure ?

Ueber die Verbreitung von "Anhydro-Oxy-Methylen-diphosphorsauren Salzen" oder "Phytin" in Pflanzen.

VON

U. Suzuki und K. Yoshimura.

Die von Palladin, Schulze, Winterstein, Posternak, Patten, und Hart u. a. untersuchten so-genannten "anhydro-oxy-methylen-diphosphorsauren Salze (von Ca und Mg) oder "Phytin," scheint im Pflanzenreich überall verbreitet zu sein. Besonders im Samen scheint diese Substanz als Reservestoff während der Keimung eine wichtige Rolle zu spielen, deshalb haben wir uns mit der Frage nach der Verbreitung und der physiologischen Function derselben beschäftigt. Wir haben zuerst unsere Aufmerksamkeit auf die Reiskleie gerichtet, die sehr reich an Phosphor ist.

In der Tat haben wir über 85% des gesammten Phosphors in der Kleie im Form des Phytins vorgefunden. Da die Darstellung desselben höchst einfach und das Präparat sehr leicht zu reinigen ist, bietet die Reiskleie ein ausgezeichnetes Material dar für die Gewinnung des Phytins in grösseren Mengen.

Auch aus anderen Pflanzensamen und aus anderen Organen haben wir das Phytin isoliert. Im Folgenden teilen wir das Resultat mit.

[1] W. Palladin; Beiträge zur Kenntniss pflanzlicher Eiweiss Stoffe Zeitschr. f. Biologie 1894, p. 199.

[2] Schulze und Winterstein; Über einen phosphorhaltigen Bestandteil der Pflanzensamen, Zeitsch. f. Physiol Chem. 22.90.

[3] Posternak; Revue generale de Botanique 12. 5 & 65 (1900).

„ Comptes rendus 137 { No. 3 (20. Iullet 1903). „ 5 (3. Aôut „). „ 8 (24. „ „).

[4] A. I. Patten and E. B. Hart. The Nature of the principal phosphorous compound in wheat bran. New York Agr.-Exp. Station. I. 1902-1904 Bull. No. 250.

1. Reiskleie.

Die sorgfältig gereinigte Reiskleie, die zu unserem Versuche diente, hatte folgende Zusammensetzung:

	In Trockensubstanz.	Gesammt Phosphor als 100 berechnet.
Gesammthos Pphor.....	2.27	100.00
Phosphor in Lecithin	0.02	0.86
Phosphor, löslich in 0.2% HCl	1.92	84.48
Davon { anorganischer Phosphor	0.13	5.89
Davon { organischer Phosphor	1.68	74.17

Für die Bestimmung des anorganischen Phosphors im 0.2% igen Salzsäure-Extracte wurde der Extract mit Ammoniak neutralisirt, mit Salpetersäure etwas angesäuert, und durch Molybdänlösung gefällt. Wir haben verschiedene Methoden versucht, und fast immer übereinstimmende Resultate bekommen.

Was hier als organischer Phosphor bezeichnet ist, wurde in der Weise bestimmt, indem wir den (0.2% tigen) Salzsäure-Extract unmittelbar mit Bariumchlorid versetzten und von dem Phosphor in diesem Niederschlag den organischen Phosphor subtrahierten. Die Differenz wird annähernd den Phosphor des Phytins repräsentieren.

Für die Darstellung des Phytins wurden 100g Reiskleie mit Aether extrahirt und dann zweimal mit 95% tigem Alkohol gekocht. Der Rückstand wurde in 40 c.c. von 0.2% iger Salzsäure suspendirt, öfters geschüttelt, und bei gewöhnlicher Temperatur stehen gelassen. Nach sechs Stunden wurde abfiltrirt, und das Filtrat mit absolutem Alkohol vesetzt, wobei ein weisser flockiger Niederschlag in reichlicher Menge entstand, der sich allmählich am Boden absetzte. Nach 24 Stunden wurde der Niederschlag gesammelt, mit 50% tigem Alkohol, dann mit absolutem Alkohol und zuletzt mit Aether gewaschen, und über Schwefelsäure getrocknet. Das so gewonnene Produkt war schon ziemlich rein, und die Ausbeute betrug etwa 7–8g, je nach Umständen. Diese Menge entspricht über 80% des gesammten Phosphors.

Für die Analyse wurde das Rohprodukt noch zweimal in 0.2% iger Salzsäure gelöst, durch absolutem Alkohol gefällt, und bei 100° c. getrocknet.

Analyse des Phytin-Präparats aus Reisklei.

Verlust beim Glühen	27.31 %
Phosphor	23.48 „
Magnessium	17.48 „
Calcium	5.18 „
Kalium	spur
Natrium	—
Chlor	—
Stickstoff	—

Dieses Präparat ist ein weisses Pulver, nicht hygroscopisch, löst sich in kaltem Wasser, die wässerige Lösung reagirt schwach sauer, beim Kochen entsteht ein weisser flockiger Niederschlag, der beim Erkalten wieder verschwindet. Es löst sich sehr leicht in verdünnter Salzsäure, Schwefelsäure und Salpetersäure, nicht aber in Essigsäure. Durch Zusatz von verdünnter Kali oder Natronlauge wird es gallertartig, löst sich aber nicht.

In Methyl- und Aethylalkohol ist es unlöslich. Mit Molybdän-Lösung erwärmt giebt es eine weisse Fällung. Die wässerige Lösung wird durch neutrales Bleiacetat, Kupferacetat und Bariumchlorid gefällt. Silbernitrat giebt ebenfalls einen weissen Niederschlag, der durch Zusatz von Salptersäure verschwindet.

1.5g. Phytin wurden mit 15 c.c. einer 30% igen Schwefelsäure auf 130° C. 14 Stunden erhitzt. Nach dem Erkalten wurde mit Wasser verdünnt, die Schwefelsäure durch Barytwasser quantitativ entfernt, und das Filtrat stark eingeengt, und mit viel absolutem Alkohol versetzt. Der dabei entstandene Niederschlag wurde schnell abfiltrirt, und das Filtrat stark eingeengt, mit einem Überschuss von absolutem Alkohol und Aether gefällt. Der weisse Niederschlag verwandelte sich allmählich in farblose Krystalle. Nach der Reinigung bestand es aus glänzenden rhombischen Tafeln, die bei 220° schmolzen, Scherer's Reaction gaben, und vollständig das Verhalten des Inosits zeigten.

2. Weizen-Kleie.

Die Weizen-Kleie hatte folgende Zusammensetzung:

	In Trockensubstanz.	In 100 Teilen des Gesammt Phosphors.
Gesammt Phosphor	1.114	100.00
Phosphor in Lecithin	0.010	0.81
Phosphor, löslich in 0.2% HCl	0.638	57.24
Davon { anorganischer Phosphor	0.050	4.49
Davon { organischer Phosphor	0.579	52.00

Die Darstellung des Phytins war genau dieselbe wie aus Reiskleie, die Ausbeute war etwa 2% der luftrockenen Weizen-Kleie. Das Produkt enthielt 16.81% Phosphor. Patten und Hart haben aus 1 Kilo Weizen-Kleie 7g Phytin isoliert, das 16.38% Phosphor enthielt, somit war bei uns die Ausbeute etwa dreimal so hoch wie bei jenen Autoren.

3. Samen von Sesamum indicum.

	In Trockensubstanz.	In 100 Teilen des Gesammt Phosphors.
Gesammt Phosphor	0.772	100.00
Phosphor in Lecithin	0.030	3.91
Phosphor, löslich in 0.2% HCl	0.144	18.61
Davon { anorganischer Phosphor	spur	spur
Davon { organischer Phosphor	0.125	16.24

4. Samen von Ricinus communis.

	In Trockensubstanz.	In 100 Teilen des Gesammt Phosphors.
Gesammt Phosphor	0.261	100.00
Phosphor in Lecithin	0.013	5.13
Phosphor, löslich in 0.2% HCl	0.110	42.29
Davon { anorganischer Phosphor	spur	spur
Davon { organiseher Phosphor	0.109	41.61

5. Oel Kuchen von Brassica Napus.

	In Trockensubstanz.	In 100 Teilen des Gesammt Phosphors.
Gesammt Phosphor	1.195	100.00
Phosphor in Lecithin	0.034	2.88
Phosphor, löslich in 0.2% HCl	0.592	49.52
Davon { anorganischer Phosphor	spur	spur
Davon { organicher Phosphor	0.532	44.46

6. Kleie aus den Samen von Hordeum Vulgare (Nackte Gerste).

	In Trockensubstanz.	In 100 Teilen des Gesammt Phosphors.
Gesammt Phosphor	0.541	100.00
Phosphor in Lecithin	0.010	1.85
Phosphor, löslich in 0.2 HCl	0.327	60.44
Davon { anorganischer Phosphor	0.089	16.45
Davon { organischer Phosphor	0.238	44.00

7. Kleie aus den Samen von Panicum frumentaceum.

	In Trockensubstanz.	In 100 Teilen des Gesammt Phosphors.
Gesammt Phosphor	0.765	100.00
Phosphor in Lecithin	0.026	3.40
Phosphor, löslich in 0.2% HCl	0.363	47.45
Davon { anorganischer Phosphor	spur	spur
Davon { organischer Phosphor	0.344	44.97

In verschiedenen Wurzeln und in Obst-Arten war verhältnissmässig viel Phosphorsäure vorhanden. Die frischen Materialien wurden abgepresst und die Säfte geprüft. Je 100 c.c. der so gewonnenen Säfte gaben folgende Zalen:

		Raphanus wurzel.	Brassica wurzel.	Apfel.	Birnen.
Gesammt Phosphor		0.0187 g.	0.0232 g.	0.0075	0.0071
anorganischer Phosphor		0.0156	0.0197	0.0039	0.0038
In 100 Theilen des Gesammt Phosphors	anorganischer P.	84.87	84.94	51.66	53.85
	organischer P.	15.13	15.06	48.14	46.15

Tierische Knochen enthalten bekanntlich hauptsächlich anorganische Phosphate, die in 0.2% Salzsäure schwer, in 1–2% iger Salzsäure aber leicht löslich sind.

a). Gedämpftes Knochen-Mehl.

		In Lufttrockensubstanz.	In 100 Teilen des Gesammt Phosphors.
Gesammt Phosphor		10.077	100.00
Phosphor in Lecithin		0.009	0.09
Phosphor, löslich in 2% HCl		10.066	99.89
Davon	anorganischer Phosphor	8.919	88.51
	organischer Phosphor	0.267	2.65

6. Frischer Knochen von einem jungen Hahn.

		In Trockensubstanz.	In 100 Teilen des Gesammt Phosphors.
Gesammt Phosphor		9.186	100.00
Phosphor, löslich in 1% HCl		7.168	78.03
Davon	anorganischer Phosphor	6.514	70.91
	organischer Phosphor (?)	0.167	1.82

Aus den oben angegebenen Fällen ersieht man, dass in manchen Pflanzen-Samen die Menge des in 0.2% iger Salzsäure löslichen Phosphors nicht die Hälfte des gesammten Phosphors erreicht. Wir haben gefunden, dass das Phytin sich aus denjenigen Samen, die sehr reich an Kohlehydraten und Eiweisstoffen sind durch 0.2% ige Salzsäure nur unvollständig extra-

hiren lässt, so das man bei sorgfältigem Zerkleinern des Materials, oder durch Extraktion mit stärkerer Salzsäure als 0.2%, manchmal doppelt so viel Phosphor in die Lösung bringen kann. Wir haben mit polirtem Reismehl einen Versuch angestellt. Da das Reismehal sehr reich an Stärke und arm an Phosphor ist, findet man aft nur schwache Trübung, wenn man zum 0.2%-igem salzsauren Extracte, Alkohol, oder Bariumchlorid-Lösung zugiebt. Wenn man aber das Reismehl vorher verkleistert, durch Diastase (phosphorfreies Diastasepräparat!) verzuckert, klar abfiltrirt, und den Rückstand mit 0.5% iger Salzsäure extrahirt, so kann man den grössten Teil des Phosphors in Lösung bringen. In dieser Weise haben wir aus 100g. luft-trockenem Reismehl mehr als 90% des gesammten Phosphors in Lösung gebracht und in dem ganzen Rückstand nur 0.0162g. P.=0.0162% Phosphor gefunden, während im Ausgangsmaterial etwa 0.2% Phosphor vorhanden war.

Über die Bestimmung der Phosphorsäure im Pflanzen-Extracte. Es ist mehrfach von verschiedenen Autoren untersucht worden, ob die gewöhnliche Molybdänmethode für die Bestimmung der Phosphorsäure im Pflanzen-Extracte ein zuverlässiges Resultat giebt.

Hart und Andrew behaupten, dass beim Erwärmen mit der gewöhnlichen, Salpetersäure haltigen, Molybdän-Lösung ein Teil der organischen Phosphor-Verbindungen eine Abspaltung von Phosphorsäure erleidet, und sich dadurch ein zu hohes Resultat für anorganischen Phosphor ergiebt. So haben deise beiden Autoren die Menge der Salpetersäure in der Molybdänlösung auf ein Minimum vermindert, und behaupten, dass sie so ein befriedigendes Resultat bekommen haben. E. Schulze und N. Castoro haben eine andere Methode vorgeschlagen. Sie beruht auf der Tatsache, dass das frisch gefällte Di-oder Tri-Calcium-Phosphat sich in neutraler Ammoncitratlösung löst, und aus dieser Lösung die Phosphorsäure unmittelbar durch Magnesiamischung gefällt wird.

Wir haben diese beiden Methoden mit der gewöhnlichen Molybdän-Methode verglichen, konnten aber keinen wesentlichen Unterschied finden, wie die folgenden Resultate zeigen. Frische Keimlinge von *Brassica Napus* wurden mit 0.2% iger Salzsäure extrahirt, je 50 c.c. des Extractes diente für die Bestimmung. Es wurde gefunden :

	$Mg_2P_2O_7$.	P.
Gewöhnliche Molybdän-Methode.....	0.0179	0.0049
Hart und Andrew „	0.0175	0.0049
Schulze und Castoro „	0.0168	0.0047

2. Gersten-Keimlinge, 50 c.c. vom 0.2% igem Salzsäure-Extracte.

	$Mg_2O_2P_7$.	P.
Gewöhnliche Molybdän-Methode.....	0.0179	0.0048
Hart und Andrew „	0.0173	0.0048
Schulze und Gastoro „	0.0170	0.0047

3. 50 c.c. einer Lösung, welche 0.0046g. Phosphor als Reis-Phytin, und noch 0.0046g. Phosphor als Natrium-Phosphat enthielt, ergaben.

	$Mg_2P_2O_7$.	P.
Gewöhnliche Molybdän-Methode.....	0.0161	0.0045
Hart und Andrew „	0.0162	0.0045
Schluze und Castoro „	0.0164	0.0046

Aus diesem Resultate ersieht man, dass die drei Methoden fast immer übereinstimmende Zahlen gaben, desshalb muss man schliessen, dass die von Anderen beobachteten Abweichungen bei verschiedenen Bestimmungsarten nicht durch die Unvollkommenheit der Methode verursacht ist. Wir nehmen vielmehr an, dass die Autoren eine wichtige Tatsache übersehen haben, d. h. sie wussten nicht, dass in Pflanzen ein Phosphorsäure abspaltendes Enzym existirt. Während der Extraction und Verarbeitung wird das Enzym schon seine Tätigkeit entfalten, und je nach den Bedingungen wird mehr oder weniger Phosphorsäure abgespalten werden, so dass die Abweichung der Resultate auf die Tätigkeit des Enzyms zurückzuführen sein wird.

Für die Methode von Schulze und Castoro ist die gewöhnliche Magnesia-Mischung nicht geeignet. Die Mischung muss 24% iges Amononiak enthalten.

Ueber ein Enzym „Phytase," das „Anhydro-oxy-methylen diphosphorsäure" spaltet.

VON

U. Suzuki, K. Yoshimura und M. Takaishi.

Wenn man Reiskleie in Wasser suspendirt und bei gewöhnlicher Temperatur einige Tage stehen lässt, so beobachtet man, dass die Hauptmenge des organischen Phosphors gelöst wird, und gleichzeitig die Bildung von Phosphorsäure auf Kosten der organischen Phosphorverbindung energisch vor sich geht. Wir haben im ersten Versuche vier Erlenmeyerkolben mit je 5 gramm Reiskleie und 200 c.c. Wasser gefüllt, und bei einer Temperatur von 20-25°C stehen gelassen. Nach 18 Stunden, 4, 7 und 15 Tagen wurden Proben herausgenommen, und analysirt, mit folgendem Resultat. Gesammt-Phosphor in der trockenen Kleie betrug 2.04% :

Nach.

	18 Stunden.	4 Tagen.	7 Tagen.	15 Tagen.
Gesammt-Phosphor gelöst	0.930%	1.932	1.937	1.971
Anorganischer Phosphor	0.145	0.523	0.629	0.707
Organischer Phosphor.....	0.640	1.281	1.180	1.164

Gesammt-Phosphor in der Kleie als 100 berechnet, nach.

	18 Stunden.	4 Tagen.	7 Tagen.	15 Tagen.
Gesammt-Phosphor	45.59	94.71	94.94	96.61
Anorganischer Phosphor.....	7.11	25.63	30.83	34.66
Organischer Phosphor.....	31.37	62.76	57.84	57.06

Derselbe Versuch wurde auch mit dem Oelkuchen von Brassica napus wiederholt. Der Oelkuchen enthielt 1.099% Phosphor:

	Nach 24 Studen.	10 Tagen.	19 Tagen.
Gesammt-Phosphor gelöst	0.434	0.932	0.969
Anorganischer Phosphor.....	Spur.	0.287	0.521
Organischer Phosphor.....	0.384	0.615	0.327

Gesammt-Phosphor im Oelkuchen als 100 berechnet.

	Nach 24 Stunden.	10 Tagen.	19 Tagen.
Gesammt-Phosphor gelöst	39.52	84.78	88.14
Anorganischer Phosphor	Spur.	26.14	47.72
Organischer Phosphor	34.96	55.91	29.79

Aus diesen beiden Versuchen sieht man deutlich, dass aus der organischen Phosphorverbindung Phosphorsäure abgespalten wird. Wir haben anfangs angenommen, dass diese Erscheinung einem Fäulnissprozess zuzuschreiben sei. Die folgenden Versuche bewiesen uns aber, dass der Fäulnissprozess keine befördernde Wirkung auf die Phosphorsäureabspaltung hat, sondern im Gegenteil etwas hindernd darauf einwirkt.

Versuch 1. In diesem Versuch wurde die Fäulniss ausgeschlossen. Zwei Erlemneyerkolben wurden mit je 10 gram Reiskleie (nicht entfettet) und 100 c.c. Wasser gefüllt, mit Watte verschlossen. Der Inhalt des Kolben *a*) wurde 10 Minuten lang lebhaft gekocht, während der Inhalt des anderen *b*) nur mit 10 c.c. Toluol versetzt wurde. Nachdem sie eine Woche bei einer Temperatur von 30–35°C gestanden waren, wurde abfiltrirt. Die Phosphorsäurebestimmung wurde nach der gewöhnlichen Molybdänmethode ausgeführt.

Phosphor in Phosphorsäure.

a) Gekocht .....0.31% der lufttrockenen Kleie.
b) Nicht gekocht, Toluol zugesetzt .....1.28% „
Gesammt-Phosphor der Kleie 2.04%

Versuch 2. Vier mit Wattepfropfen versehene Kolben wurden mit je 5 gramm entfetteter Reiskleie und 100 c.c. Wasser gefüllt und in folgender Weise behandelt:

a) Gekocht.
b) Nicht gekocht.
c) Gekocht und Toluol zugesetzt.
d) Nicht gekocht und Toluol zugesetzt.

Sie wurden bei einer Temperatur von 30–35°C stehen gelassen. Nach 4 Tagen wurde filtrirt und analysirt:

In 100 Theilen der Trockensubstanz.

	Gesammt-Phosphor, gelöst.	Der gebildete Phosphorsäure-Phosphor.
a)	—	0.19
b)	—	0.78
c)	1.19	0.19
d)	1.49	1.36

Versuch 3. Zwei Erlemmeyer-Kolben wurden mit je 0.5g. Phytin und 45 c.c. Wasser gefüllt, gekocht und abgekühlt. Ferner wurden 5 gramm Reiskleie mit 50 c.c. Wasser verrieben und abfiltrirt. 5 c.c. von diesem Filtrate wurde fünf Minuten gekocht und zum Kolben *a*) zugegeben. Zum Kolben *b*) wurden ebenfalls 5c.c. des Filtrats zugesetzt, aber ohne diese vorher zu erhitzen. Die beiden Kolben nun wurden mit je 10 c.c. Toluol versetzt und bei 30–35°C stehen gelassen. Für die abgespaltene Phosphorsäure ergab sich:

Phosphor in Phosphorsäure.

	Nach 4.	5.	6 Tagen.
a)	0.002g.	0.005	0.005
b)	0.010	0.014	0.016

Man sieht hieraus, dass das Phytin nur gespalten wird, wenn der zugesetzte Reiskleie-Extract nicht vorher gekocht wor.

Versuch 4. 5 gramm Weizenkleie wurde mit 50 c.c. Wasser bei 30–35°C vier Tage stehen gelassen.

	Der gebildete Phosphorsäure Phosphor.	In Prozent der lufttrockenen Kleie.
a) Gekocht und Toluol zugesetzt.....	0.0040g.	0.08%
b) Nicht gekocht und Toluol zugesetzt..	0.0205g.	0.41%

Aus diesen Versuchen kann man schliessen, dass in der Reis- und Weizenkleie ein Enzym existirt, das die organische Phosphorverbindung bzw. Anhydro-oxy-methylen-diphosphorsäure spaltet.

Wir haben ferner beobachtet, dass bei Reis-, Gerste-, Brassica-, und anderen Samen, während der Keimung ein grosser Theil des organisch-gebundenen Phosphors als Phosphorsäure abgespalten wird, was wieder am einfachsten durch Enzymwirkung erklärt wird.

E. Schulze und N. Castoro haben ferner berichtet, dass in etiolirten Keimlingen verschiedener Pflanzen Phosphorsäure sich anhäuft, doch haben sie sich nicht speziell über die Beziehung zwischen der Anhydro-oxy-methylen-diphosphorssäure zur abgespaltenen Phosphorsäure geäussert, und auch über eine Enzymwirkung nichts erwähnt. Wir wollen nun die Resultate einiger Versuche mittheilen, die Bildung der Phosphorsäure auf Kosten des organischen Phosphors bzw. des Phytins beim Keimungsvorgang deutlich zeigen :

Versuch 1. Am 12ten August wurden Reissamen in feuchte Sägespähne gesät und bis 1sten September in einem hellen Zimmer gehalten Als die jungen Blätter etwa 3–4 cm. erreichten, wurden sie getrocknet und analysirt.

Resultat.

	500 Samen.	500 Keimlinge.	(+) Zunahme. (−) Abnahme.	
Trockensubstanz	11.50g.	7.50	(−)	4.00
Gesammt-Phosphor	0.0341	0.0326	(−)	0.0015
Lecithin-Phosphor	0.0005	0.0014	(+)	0.0009
In 0.2% HCl löslicher Phosphor ...	0.0151	0.0259	(+)	0.0108
Davon. Anorg.-Phosphor	Spur.	0.0086	(+)	0.0086
Davon. Org.-Phosphor	0.0142	0.0145	(+)	0.0003

Gesammt-Phosphor der Samen als 100 berechnet.

	Samen.	Keimlinge.	(+)	(−)
Gesammt-Phosphor	100.00	95.60	(−)	4.40
Lecithin-Phosphor	1.47	4.10	(+)	2.63
In 0.2% HCl löslich Phosphor	44.28	76.00	(+)	31.72
Davon. Anorg.-Phosphor	Spur.	25.22	(+)	25.22
Davon. Org.-Phosphor	41.64	42.52	(+)	0.88

Versuch 2. Am 9ten October wurden 200 von *Brassica Napus* in feuchten Quarzsand gesät und keimen glassen. Am 18ten als die Keimlinge etwa 3 cm. erreichten, wurden sie getrocknet und analysirt:

	200 Samen.	200 Keimlinge.	(+)	(−)
Trocken-Substanz	5.90	4.20	(−)	1.70
Gesammt-Phosphor	0.0417	0.0403	(−)	0.0014
Lecithin-Phosphor	0.0029	0.0041	(+)	0.0012
In 0.2% HCl löslicher Phosphor	0.0269	0.0326	(+)	0.0057
Davon. Anorg.-Phosphor	Spur.	0.0292	(+)	0.0292
Davon. Org.-Phosphor	0.0259	0.0012	(−)	0.0248

Gesammt-Phosphor der Samen als 100 berechnet.

	Samen.	Kleimlinge.	(+)	(−)
Gesammt-Phosphor	100.00	96.64	(−)	3.36
Lecithin-Phosphor	6.95	9.83	(+)	2.88
In 0.2% HCl löslicher Phosphor	64.51	78.18	(+)	13.67
Davon. Anorg.-Phosphor	Spur.	70 02	(+)	70.02
Davon. Org.-Phosphor	62.11	2.88	(−)	59.23

Versuch 3. Am 5ten October wurde Gerstensamen in feuchten

Quarzsand gesät, und am 18ten, als die Keimlinge etwa 9 cm. erreicht hatten, wurden sie getrocknet und analysirt:

	300 Samen.	300 Keimlinge.	(+)	(−)
Trocken-Substanz	12.00	9.10	(−)	2.90
Gesammt-Phosphor	0.0435	0.0404	(−)	0.0031
Lecithin-Phosphor	0.0018	0.0035	(+)	0.0017
In 0.2% HCl löslicher Phosphor	0.0255	0.0319	(+)	0.0064
Davon. Anorg.-Phosphor	Spur.	0.0287	(+)	0.0287
Davon. Org.-Phosphor	0.0246	0.0030	(−)	0.0216

Gesammt-Phosphor der Samen als 100 berechnet.

	Samen.	Kleimlinge.	(+)	(−)
Gesammt-Phosphor	100.00	92.89	(−)	7.11
Lecithin-Phosphor	4.14	8.05	(+)	3.91
In 0.2% HCl löslicher Phosphor	58.62	73.34	(+)	14.72
Davon. Anorg.-Phosphor	Spur.	65.98	(+)	65.98
Davon. Org.-Phosphor	56.55	6.89	(−)	49.66

Versuch 4. Weizensamen wurden am 5ten October in feuchten Quarzsand gesät und am 18ten October, als die Keimlinge etwa bis zu 13 cm. gewachsen waren, wurden sie getrocknet und analysirt:

	300 Samen.	300 Keimlinge.	(+)	(−)
Trocken Substanz	12.000g.	9.35	(−)	2.65g.
Gesammt-Phosphor	0.0373	0.0352	(−)	0.0021
Lecithin-Phosphor	0.0012	0.0026	(+)	0.0014
In 0.2 HCl löslicher Phosphor	0.0211	0.0277	(+)	0.0066
Davon. Anorg.-Phosphor	Spur.	0.0236	(+)	0.0236
Davon. Org.-Phosphor	0.0207	0.0040	(−)	0.0167

Gesammt-Phosphor der Samen als 100 berechnet.

	Samen.	Kleimlinge.	(+)	(−)
Gesammt-Phosphor	100.00	94.37	(−)	5.63
Lecithin-Phosphor	3.32	6.97	(+)	3.65
In 0.2% HCl löslicher Phosphor ...	56.56	74.26	(+)	17 70
Davon. Anorg.-Phosphor	Spur.	63.27	(+)	63.27
Davon. Org.-Phosphor	55.50	10.72	(−)	44.78

Wie man sieht, geht die Bildung der Phosphorsäure während des Keimungs-Prozesses manchmal auffallend rasch vor sich, bei Brassica sogar bis zu 70 prozent des gesammten Phosphors; und dieser Vorgang geht im hellen Licht ebenso schneil wie im Dunkeln vor sich.

Darstellung des Enzyms.

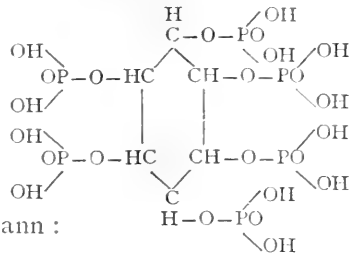
65 gramm entfetteter Reiskleie wurden in einem Porzellan-Mörser mit Wasser verrieben. Nach 4–5 Stunden wurde abfiltrirt und zum klaren Filtrat so viel Barytwasser und Bariumchlorid zugesetzt bis kein Niederschlag mehr entstand. Die meisten Unreinigkeiten, besonders organische so wie anorganische Phosphorverbindungen wurden dadurch entfernt. Das Filtrat von dem Niederschlag wurde dann mit einem Gemisch von 85% igem Alkohol und Aether versetzt, wobei ein weisser flockiger Niederschlag entstand, der das meiste Enzym enthielt. Dieser Niederschlag wurde in wenig Wasser gelöst, nochmals mit Bariumchlorid versetzt, um noch darin vorhandene Phosphate und andere Stoffe möglichst vollständig wegzuschaffen. Dem Filtrate wurde dann ein Ueberschuss von absolutem Alkohol und Aether zugegeben. Der weisse amorphe Niederschlag setzte sich allmählich auf den Boden ab. Nach 24 Stunden wurde er gesammelt, mit absolutem Alkohol und Aether gewaschen, und über Schwefelsäure getrocknet. Das so gewonnene Produkt war ein weisses Pulver und betrug etwa 0.15g. Es löst sich sehr leicht in kaltem Wasser, enthält keinen Phosphor und zeigte weder diastatische, peptische noch tryptische Wirkung. Wir schlagen für dieses Enzym den Namen *Phytase* vor.

0.5 gramm Phytin wurde in 50 c.c. Wasser gelöst, 0.02g. dieses Phytase-Präparat zugegeben, und bei einer Temperatur von 35--40°C stehen gelassen. Nach vier Stunden konnte man schon die Abspaltung von Phosphorsäure durch Molybbän-Lösung nachweisen, und nach zwei Tagen waren etwa 10 Prozent des angewandten Phytins gespalten.

Analoge Versuche wurden auch mit anderen bekannten Enzymen ausgeführt, um zu sehen ob sie auch Phytin spalten können. In keinem Fall aber wurde Phosphorsäure abgespalten. Blos Emulsin schien etwas Phosphorsäure gebildet zu haben. Ob das Emulsin-Präparat ein einheitliches Enzym war, oder ob es ein Gemisch mit Phytase war, können wir zur Zeit nicht sagen. Doch halten wir es für wahrscheinlich, dass es sich bei der Phytase um ein neues Enzym handelt. Wir beabsichtigen später noch weiteres über die Natur der Phytase zu berichten.

Es ist uns endlich auch gelungen, *aus den enzymatischen Spaltungs-Produkten des Phytins Inosit zu isolieren.* Zu diesem Zwecke wurden 5 gramm Phytin in 100 c.c. Wasser gelöst, 0.1g. Phytase zugesetzt und das Gemisch unter Toluolzusatz bei 30–35°C 4 Tage stehen gelassen. Die Flüssigkeit wurde jetzt mit Bariumchlorid-Lösung gefällt, um das unverändert gebliebene Phytin und auch gebildete Phosphorsäure möglichst vollständig zu entfernen. Das Filtrat wurde stark eingeengt, der dabei ausgeschiedene Niederschlag abfiltrirt, weiter stark eingeengt, und mit wenig absolutem Alkohol versetzt. Der sofort gebildete Niederschlag wurde schnell abfiltrirt und zum Filtrat ein Ueberschuss von absolutem Alkohol und Aether zugegeben. Die weisse Trübung verwandelte sich allmählich in charakteristische Krystalle von Inosit. Leider genügte die Menge nicht für eine Analyse, wesshalb wir uns mit der Schmelzpunkt-bestimmung und einigen Farbenreaktionen begnügen mussten, wodurch aber jeder Zweifel entfernt wurde, es lag unbedingt Inosit vor. Da bis jetzt Inosit aus Phytin nur bei Anwendung von starker Salzsäure und hoher Temperatur erhalten worden war, wobei Umlagerungen und Kondensationen nicht ausgeschlossen waren, so spricht dieser enzymätische Inositspaltung sehr für das *ursprüngliche Vorhandensein von Inosit im Phytin* und desshalb müsste diese Substanz als Inosit-hexa-phosphorsäure (ein Analogon der Mellithsäure) aufgefasst werden. Zudem ist es uns

weder gelungen, Formaldehyd aus Phytin abzuspalten, noch Formaldehyd mittelst starker Salzsäure in Inosit umzuwandeln. Die Formel der Phytinsäure wäre dann:



womit die analytischen Resultate Posternaks ebenfalls stimmen würden.

Aus der Reiskleie haben wir ebenfalls Inosit isoliren können. 100g. Reiskleie wurde mit Wasser versetzt. 4 Tage bei 30 35°C stehen gelassen, abfiltrirt, das Filtrat durch Bariumchlorid gefällt, nochmals abfiltrirt, und zum Filtrate basisches Bleiacetat zugegeben. Der dadurch entstandene Niederschlag wurde durch Schwefelwasserstoff zerlegt, vom Schwefelblei abfiltrirt, stark eingeengt, und mit Alkohol und Aether gefällt. Der so ausgeschiedene Inosit zeigte die charakteristische Krystallform so wie die eigentümlichen Reaktionen des Inosit.

Bekanntlich ist Inosit auch im tierischen Muskel gefunden worden. Es dürfte hier die Vermutung berechtigt sein, dass dieser Inosit aus dem Phytin der vegetabilischen Nahrung stammt.

Zussammenfassung der Resultate.

1. Der grösste Theil des Phosphors in Pflanzensamen besteht aus der in Wasser und in verdünnten Mineralsäuren löslichen organischen Phosphorverbindung, die schon von Schulze, Palladin, Winterstein, Posternak, Patten, u. A. erhalten wurde und als „Anhydro-oxy-methylen-diphosphorsäure“ oder „Phytin“ bezeichnet wurde.

Aus der Reiskleie haben wir etwa 8% und aus der Weizenkleie etwa 2% Phytin isolirt.

2. In Wurzeln, Zwiebeln, und Obst herrscht jedoch die anorganisch gebundene Phosphorsäure vor.

3. In den Knochen ist das Vorhandensein des Phytins zweifelhaft.

4. Während der Keimung der Pflanzensamen, entweder im Lichte oder im Dunkeln nimmt die anorganisch gebundene Phosphorsäure beträchtlich zu.

Auch wenn man Reis- oder Weizenkleie oder verschiedene Samen zerreibt und in Wasser suspendirt und einige Tage stehen lässt, bildet sich Phosphorsäure in grösseren Mengen auf Kosten des Phytins.

5. Aus Reis- und Weizenkleie wurde ein Enzym isolirt, das Phytin in Phosphorsäure und Inosit spaltet. Es ist wahrscheinlich ein neues Enzym und scheint im Pflanzenreich weit verbreitet zu sein.

Studies on Humus formation, III.

BY

Shigehiro Suzuki.

Altho the humus substance is most widely spread and as a soil constituent closely related to the fertility of the soil, its chemical constituents have not yet been fully studied. It is generally believed that a greater part of soil nitrogen is contained in the humus substance, but the question as to the nature of the nitrogen content of humus has not been definitively settled. Mulder[1] believed that the nitrogen in humus is present in the form of ammonium humate, while Detmer[2] denied this and concluded that it is present in organic combination, observing that the humic acid when treated by caustic potassa did not liberate any ammonia and only a small part of nitrogenous compounds was decomposed by bromated alkali. But he also infered from his further experiments, that humic acid after purification does not contain nitrogen as an essential constituent. G. Loges[3] observed that the hydrochloric acid extract of soils contains a nitrogen holding humus body and yields a precipitate with phosphotungstic acid. He found the ratio of nitrogen to carbon in this precipitate to be about 1 : 6.2. Baumann[4] states that when soils are boiled for two hours with very dilute hydrochloric acid ammonia is produced, the quantity exceeding by ten or twenty times

[1] Chemie der Ackerkrume. Deutsch von Grimm. 1862. Bd II. p. 256. Mulder has classified the humus substance according to the degree of decomposition and the solubility to caustic alkali into six groups: Ulmin, ulmic acid, humin, humic acid, apocrenic acid and crenic acid. But this distinction is generally held as not justified.

[2] Landw. Versuchsst. Bd XIV. 1871. p. 248.

[3] Landw. Versuchsst. Bd XXXII. 1886. p. 201.

[4] Forschungen auf den Gebiete der Agr. physik, 1886. p. 283.—or Landw. Versuchsst. 1886. XXXII. p. 247.

that preexisting in the soil and he suggested this nitrogenous compounds were perhaps originally present as amino-compounds. Berthelot and Andrée[1] have studied the action of hydrochloric acid on soil far more thoroly.

They found that it splits up the nitrogenous matter, producing ammonia and soluble nitrogenous compounds. The action goes further the greater the strength of the acid, the longer its contact with the soil and the higher the temperature. A soil containing 0.174% of nitrogen, boiled for two hours with a solution containing 20% by volume of the ordinary liquid hydrochloric acid gave up 31.8% of its nitrogen; of this amount 7.1 was ammonia, produced by the action of the acid. The proportion of the nitrogen liberated as ammonia, to that remaining in organic combination dissolved by the acid, was about 1 : 3. From these results Berthelot and Andrée, conclude that *the nitrogenous matter of soil consists chiefly of insoluble amides.* By the action of acids, alkalies, or even water, these are split up into ammonia and soluble amides. Also, R. Warington[2] ascertained the presence of a minute quantity of soluble amide in the soil, by treating this with hypobromite and with nitrous acid. From these experiments it seems to be highly probable that at least a part of soil nitrogen is present as amino-compounds, but the question as to the connection of these with the humus, was not yet perfectly clear, and indeed, sometimes the humus substance consists chiefly, as has been pointed out by P. E. Müller[3], H. von Post[4] and P. Kostytscheff[5], of bodies of bacteria, of mycelia of fungi, of chitin parts of insects or of excrement of lower animals. Therefore the writer had confined himself to study the nature of the precipitated humic acid, the so called Grandeau's "Matiere noire."

Since Grandeau[6] had stated that the amount of mineral matter, especially of phosphoric acid, lime and potassa, contained in the precipitated humic acid is in close relation to the soil fertility, many authors had endeavored to

1 Compt. rend. T. 103, 1886. p. 1101.

2 Chem. News. Vol. LV. 1887. p. 27.

3 Studien über die natürlichen Humusformen. Berlin. 1887.

4 Landw. Jahrbücher. 1888. p. 405-420.

5 Annales agronomiques. T. XVII. 1891. p. 17-38.

6 Handbuch für agric. chem. Analyse. Berlin. 1879. p. 111.

study the chemical nature of this substance. As for the nitrogen contents of this substance, C. G. Eggertz[1] analysed 13 samples of mull (he called the humic acid prepared from the natural humus "Mullbody" and that of artificial as "humusbody") and found that the nitrogen content varied from 0.37 to 10.47% and that it was present in intimate combination and not as ammonium salt, because the humic acid can not be freed from nitrogen originally present in it by repeatedly treating with alkali and acid, while the ammonium nitrogen absorbed by the humic acid entirely separated by the said process. Hilgard and Jaffa[2] found that the humus of soils in an arid climate contains a higher proportion of nitrogen than is found in the humus of a humid climate and Hilgard[3] stated further that the amount of nitrogen in humic acid would permit a conclusion as to the requirement of nitrogen of soils. H. Snyder[4] mixed various materials with soils and, extracting after one year the humus matters formed, with 3% caustic potash he found that the humus derived from a substance rich in nitrogen (clover, flesh and cow-dung) contained more nitrogen than that obtained from saw-dust, straw or sugar and the same was the case with humus from virgin and cultivated soil, respectively. F. Sestini[5] has shown that at least a part of nitrogen of the humic acid is present in the form of amino-acid, as free nitrogen is devolped when it is treated with nitrous acid. Dojarenko[6] made quantitative determinations of the different forms of nitrogen contained in the seven samples which were prepared from Russian black earths, by extracting them with ammonia or sodium carbonate, with the following result :

Total nitrogen.....	2.64–4.58%	
Ammonia-nitrogen	trace.	
Amino-acid-nitrogen	1.01–2.34 „	(determined by Böhmer's method)
Amino-nitrogen	0.22–0.48 „	(determined by Sachsse's method)

[1] Meddelanden fran konigl. Landbruks-Akademiens-Experimentalfält, Nr. 3. Stockholm. 1888. p. 1–66.—Biedermann's Centralbl. f. Agrik. Chem. 1889. p. 75.

[2] Agric. Science 8. 165.—Centr. Bl. Agrik. 1895. 24. 218.

[3] D. landw. Presse, 1895. 490; ref. Centr.-Bl. Agrik. 1896. 25. 271.

[4] Exper. Stat. Rec. 1898. 9. 632 (Minnesota Stat. Bul. 53. 12).

[5] Landw. Versuchsst. 1899. 51. p. 153.

[6] Landw. Versuchsst. 1902. 56. p. 311.

All experiments seem to show that a portion of nitrogen contained in the soil or especially in the humic acid is of the nature of an amide or amino-acid. But it is not yet clearly examined what kinds of amino-compounds or amino-acids are contained in the humic acid, or are formed as decomposition products when the humus is boiled with hydrochloric acid. In order to study these questions more clearly, the writer has examined three samples of humus, one was imported from Germany (A), Acid. humic., pur., von E. Merck-Darmstadt, while the second (B) was prepared from a soil not manured for seven years. The third (C) was derived from a kind of compost heap. In the two latter cases the humus was extracted by caustic soda[1] (2%) (the soil being at first repeatedly washed on a funnel with 1% hydrochloric acid to remove lime compounds) and precipitated by a weak solution of hydrochloric acid and after well washing dried.[2] Unfortunately, the origin and the method of preparation of the sample A was quite unknown to the writer, but according to its chemical behavior it is highly probable that it was derived from peat. These three samples were crushed in a mortar and after sifting with a sieve of 0.5 mm. meshes served for the following tests. On examination they showed the following nitrogen content :

	In per cent of Original dry matter.	In per cent of ash-free substance.
A	3.73	3.90
B	3.14	3.76
C	3.02	3.53

The chemical behavior of these samples was as follows : They were but little soluble in cold water, but on boiling partly decomposed with a dark brown color and had an acid reaction. When they were heated in a test-tube a strong development of ammonia was observed and more energetically so when mixed with soda-lime. At the ordinary temperature dilute hydrochloric acid attackes them a little, but on boiling with concentrated hydrochloric acid they were decomposed. They dissolved com-

[1] According to the investigation of Charles Rimbach (Report of the Agri. Ex. Stat. of the Univ. of California. 1898–1901. p. 43) the humic acid prepared by the ammonia extraction method is widely different, from that obtained by the caustic soda extract. The latter contained more nitrogen than the former.

[2] The amount of yield was in the case B 3.92% of dry fine earth and in C 3.34%.

pletely in a caustic alkali solution and reappeared on neutralisation with acids as a flocculent brown precipitate. Dilute solution of sodium nitrite upon addition of a few drops of hydrochloric acid develops only a few bubbles of nitric oxid gas, but on addition of some humic acid a most energetic development of gas was observed, perhaps owing to the presence of NH_2 groups in humic acid. The work of the writer which clears up the nature of the nitrogen compounds present, is described in the following lines :

Five g. of humic acid were boiled for 10 hours with 50 c.c. concentrated hydrochloric acid in connection with reverted cooler. The extraction was repeated three times and after well washing the residue served for the elementary analysis. In the extract was determined besides total nitrogen (by Kjelhahl's method) also ammonia-nitrogen (ammonia was expelled by an excess of magnesia, using a vacuum distillation apparatus) and the nitrogen in phosphotungstic acid precipitate. (For this purpose 50% solution served for precipitation and in the precipitate washed with a 5% sulfuric acid the nitrogen was determined by Kjeldahl's method). The results were as follows :—

a). Residue.

In per cent of dry matter.

	Total residue	N		C		H		Ash	
		in original	in residue	in original	in residue	in original	in residue	in original	in residue
Humic acid A	63.20	3.73	1.78	58.36	60.92	5.21	5.00	4.40	3.34
„ „ B	53.76	3.14	1.45	44.54	63.18	3.49	2.98	16.53	5.63
„ „ C	58.86	3.02	1.79	45.58	66.15	4.16	3.58	14.34	9.50

In per cent of ash-free dry matter.

	N		C		H	
	in original	in residue	in original	in residue	in original	in residue
Humic acid A	3.90	1.83	61.05	63.03	5.45	5.17
„ „ B	3.76	1.53	53.36	66.96	4.18	3.15
„ „ C	3.53	1.97	53.21	73.10	4.86	3.95

Hence, from 100g. of original dry matter, the following amounts of C, H and N were dissolved by boiling with concentrated hydrochloric acid.

	C	H	N
Humic acid A	19.86g.	2.05g.	2.61g.
„ „ B	10.57	1.89	2.36
„ „ C	6.64	2.05	1.97

b). Extract.

Humic acid A.

	In 100g. original sample	Total N calculated as 100
Total N	3.729g.	100.00
N, not dissolved in HCl (N in residue) ...	1.118	29.98
N, dissolved in HCl	2.611	70.02
N in phosphotungstic precipitate (ammonia excluded)	0.045	1.20
N in ammonia	0.312	8.37
N in other forms	2.254	60.45

Humic acid B.

	In 100g. original sample	Total N calculated as 100
Total N	3.137g.	100.00
N, not dissolved in HCl (N in residue) ...	0.777	24.77
N, dissolved in HCl	2.360	75.23
N in phosphotungstic precipitate (ammonia excluded)	0.322	10.26
N in ammonia	0.093	2.97
N in other forms	1.945	62.00

Humic acid C.

	In 100g. original sample	Total N calculated as 100
Total N	3.021g.	100.00
N, not dissolved tn HCl (N in residue)	1.051	34.79
N, dissolved in HCl	1.970	65.21
N in phosphotungstic precipitate (ammonia excluded)	0.539	17.84
N in ammonia	0.181	5.99
N in other forms	1.250	41.38

These results show that the humic acids were decomposed by the concentrated hydrochloric acid and lost about half of their weight and also the greater part (65–75‰ of total nitrogen) of nitrogen; the residue became poorer in N[1], H and ash, but relatively richer in C. Moreover, there was found in the extract the larger portion of nitrogen (41–62%) present in forms *not precipitable by phosphotungstic* acid. The writer has further made qualitative tests for amino compounds by boiling 10g. of humic acid (A and C, separately) with 200 c.c. conc. HCl (the details of the method will be described below) and confirmed the presence of several kinds of amino-acids in the hydrochloric acid extract.

Hereupon, the quantitative separation of amino-acids was made after Fischer's method.[2] 600g. of the sample A (dry matter = 535g) was boiled with 1.5L. strong hydrochloric acid for six hours with reverted cooler. After filtration, the residue was again treated as before. The total extract was evaporated in vacuo to a syrupy mass, mixed with some absolute alcohol

[1] The residue of sample A was treated with conc. HCl two times more and analysed. The result was:

		ash free
C	63.97	64.11 %
H	3.34	3.35 „
N	0.80	0.80 „
Ash	0.23	—

Altho the nitrogen content of residue by and by decreases, it is very difficult to remove it completely.

[2] Zeitschr. f. physiol. Chem. 33. p. 151. and 412 (1901).

and passed dry hydrochloric acid gas to saturation, the precipitate[1] formed was filtered off. The filtrate was again evaporated, mixed with absolute alcohol and saturated with hydrochloric acid gas and then the excess of hydrochloric acid removed by boiling away in vacuo. The syrupy residue was treated with caustic soda, with continuous cooling by ice, and anhydrous potassium carbonate was added to a consistency of a semi-fluid and the free ester thus formed extracted with ether, shaking out several times, until the extract did no more show alkaline reaction to test paper. Then, after evaporation of the ether, the residue was subjected to the fractional distillation with the following result :

Fractions	Temperature	Weight of distillate (g.)	
1	till 60°C	5.0	(colorless)
2	60-100 „	9.0	(slightly green)
3	100-150 „	12.1	(light yellow)
4	150-200 „	3.5	(„ „)
		Sum 29.6	

The first two fractions were decomposed by boiling with water for 7-8 hours, while the third and fourth fractions by baryta water heating at 70-80° C on the water bath for 2 hours. Thus it was easy to decide that various amino-compounds were present and evidently were formed from the protein or some related substances attached to the humic acid. After decomposition, the solutions of free amino-acids were evaporated to a small volume and treated with absolute alcohol and the following products obtained :

I). From first fraction (at 60°C) :

0.26g.....(Substance I)

[1] This precipitate was easily soluble in water, and the solution showed the presence of much ammonium salt by Nessler's reagent. The precipitate contained 54.8% of crude ash which had the following composition. In per cent of crude ash :

Per cent	SiO_2 ..... 32.36	Mn_3O_4 ..... 0.63		K_2O 1.17	
	Fe_2O_3 ..... 13.31	CaO 12.02		Na_2O 11.77	
	Al_2O_3 ..... 0.29	MgO 0.20		P_2O_5 ..... 0.48	
				SO_3 ..... 16.12	

II). From second fraction (60–100°C) :

1). First crude product.....0.54g.

Purified by the recrystallisation.

i) 0.28g.....(Sub. II)

ii) 0.14g.....(Sub. III)

2). Second crude product.....3.99g.

By the fractional crystallisation, 3 samples are obtained.

i) 0.47g..... .....(Sub. IV)

ii) 0.12g..... .....(Sub. V)

iii) 2.55g.....(Sub. VI)

III). From third fraction (100–150°C) :

a) Water solution.

i) Insoluble in alcohol.....1.51g.....(Sub. VII)

li) Soluble ,, ,,

α) Copper salt, insol. in alcohol...0.53g. (Sub. VIII)

β) ,, ,, , sol. ,, ,, ...0.71g. (Sub. IX)

b) Ether extract.

2.03g.....(Sub. X)

IV). From fourth fraction (150–200)°C :

i) Insol. in alcohol.....0.59g.....(Sub. XI)

ii) Sol. in ,,

Copper salt .....0.8g. .....(Sub. XII)

V). From residual part. Residue was treated by an excess of baryta and the filtrate therefrom just neutralized with sulfuric acid and the precipitate filtered while hot and the filtrate evaporated to a small volume and heated to boiling with copper hydroxyd to make copper salt and filtered. After evaporation copper salt was precipitated by absolute alcohol. The weight of product was 6.06g. (Sub. XIII).

After purifying by the recrystallisation method, the identification of these products was carried out in the following manner :—

Sub. I. Result : This product is not glycocoll. Water solution did not yield on evaporation characteristic needle crystals of glycocoll and also the preparation of ethylesterhydrochlorid gave negative result.

Sub. II. Result: Leucin. (yield=0.28g.)

i) Melting point.....291°C

ii) Elementary analysis.

0.1277g. Sub. gave .....0.2570g. CO_2+0.1060g. H_2O

0.1396g. „ „ .....13.5 c.c. N (15°, 762 mm.)

		C	H	N
Leucin $C_6H_{13}NO_2$	Calculated:	55.80	10.07	10.85
	Observed:	54.89	9.31	11.47

The flocculent crystaline mass was difficulty moistened with water and formed an insoluble copper-salt.

Sub. III. Result: Aminovalerianic acid (yield=0.14g.)

i) Elementary analysis.

0.1326g. Sub. gave .. ..13.3 c.c. N (14°, 765 mm.)

		N
Aminovalerianic acid $C_5H_{11}NO_2$	Calculated:	11.97
	Observed:	11.98

Sub. IV. Result: Aminovalerianic acid (yield=0.47g.)

i) Melting point.....290°C

ii) Elementary analysis.

0.1944g. Sub. gave.....19.8 c.c. N (13°, 766 mm.)

0.1808g. „ „ .....0.3412g. CO_2+0.1546g. H_2O

		C	H	N
Aminovalerianic acid $C_5H_{11}NO_2$	Calculated:	51.28	9.40	11.97
	Observed:	51.47	9.59	12.26

Sub. V. Result: Aminovalerianic acid+alanin (yield=0.12g.)

Elementary analysis.

0.1204g. Sub. gave.....13.4 c.c. N (11°, 760 mm.)

			N
Aminovalerianic acid	$C_5H_{11}NO_2$	Calculated:	11.97
Alanin	$C_3H_7NO_2$	„ :	15.73
		Observed:	13.40

Sub. VI. Result: Alanin (yield=2.55g.)

i) Melting point.....253°C

ii) Elementary analysis.

a) 0.2009g. Sub. gave.....25.7 c.c. N (20°, 755 mm.)
b) 0.1996g. „ „ .....25.2 „ „ (19°, 758 „)
c) 0.1912g. „ „ .....24.3 „ „ (13°, 766 „)
d) 0.1977g. „ „ .....0.2974g. CO_2+0.1360g. H_2O

		C	H	N
Alanin $C_3H_7NO_2$	Calculated :	40.45	7.86	15.73
	Observed : *a*)	—	—	14.68
	„ : *b*)	—	—	14.62
	„ : *c*)	—	—	15.29
	„ : *d*)	41.03	7.71	—

Sub. VII. Result : Impure aspartic acid (?) (yield=1.51g.

i) Melting point.....191–193°C

ii) Elementary analysis of free acid.

0.1890g. Sub. gave.....19.5 c.c. N (13°, 762 mm.)
0.1934g. „ „ .....0.2620g. CO_2+0.1170g. H_2O

		C	H	N
Aspartic acid $C_4H_7NO_4$	Calculated :	36.09	5.26	10.53
	Observed :	38.05	6.98	12.35

iii) Analysis of copper salt.

0.2319g. Sub. (dried at 100°C in vacuo) gave 17.6c.c. N(17°, 762 mm.)
0.1870g. „ („) „ 0.0676g. CuO

		N	Cu
Copper aspartate $C_4H_5NO_4Cu$,	Calculated :	7.21	32.67
	Observed :	8.83	28.88

Sub. VIII. Result : Impure copper salt of inactive prolin (yield=0.53g.)

0.1216g. Sub. (dried at 100°C in vacuo) gave 0.0396g. CuO

		Cu
Prolin copper salt	Calculated :	21.80
	Observed :	25.99

Sub. IX. Copper salt of active prolin (yield=0.71g.)

No test was made.

Sub. X. Result : Leucin (yield=2.03g.)

i) Melting point.....291°C

ii) Elementary analysis.

a) 0.1966g. Sub. gave 18.4 c.c. N(13°, 761 mm.)
b) 0.2062g. „ „ 0.4129g. CO_2+0.1906g. H_2O
c) 0.1958g. „ „ 0.3888g. CO_2+0.1764g. H_2O
d) 0.1267g. „ „ 0.2562g. CO_2+0.1080g. H_2O

		C	H	N
Leucin $C_6H_{13}NO_2$	Calculated	55.80	10.07	10.85
	Observed : *a*)	—	—	11.08
	„ : *b*)	54.61	10.36	—
	„ : *c*)	54.16	10.10	—
	„ : *d*)	55.15	9.56	—

iii) Determination of the rotation power.
0.1845g. Sub. dissolved in 10 c.c. 20% HCl. In 10 c.m. tube observed, the angle of rotation was +0.34°.

$$\therefore \quad [\alpha]_D^{20°} = +18.43°$$

Sub. XI. Result: Impure aspartic acid (yield=0.59g.)
i) Elementary analysis of free acid.
0.1682g. Sub. gave 12.3 c.c. N (13°, 761 mm.)
0.1699g. „ „ 0.2350g. CO_2+0.0900g. H_2O

		C	H	N
Aspartic acid $C_4H_7NO_4$	Calculated :	36.09	5.26	10.53
	Observed :	37.72	5.94	8.89

ii) Analysis of copper salt.
0.1473g. Sub. (dried at 100°C in vacuo) gave 0.0580g. CuO
Observed 31.43 Cu
Aspartate of copper $C_4H_5NO_4Cu$ Calculated : 32.67

Sub. XII. Result: Copper salts of impure acids. (yield=0.8g.)
0.1496g. Sub. (dried at 100°C in vacuo) gave 0.0502g. CuO
% of Cu=26.81.

Sub. XIII. Result: Copper salt of impure acids. (yield=6.06g.)
i) Determination of copper.
Dry substance (dried at 100°C in vacuo) contained 24.20% Cu.
ii) Other qualitative tests were made as follows:
The sample dissolved in water and the copper was precipitated by H_2S and the filtrate evaporated to a small volume. The solution

had an acid reaction. Biuret reaction negative. Mercuric chlorid and tannin gave no precipitate, Millon's reaction distinctly.

Phosphotungstic acid gave voluminous white precipitate, soluble in excess on warming. The remaining portion was boiled with five times of its volume conc. HCl for 6 hours. The filtrate did not show the Millon's reaction and on evaporation long needle crystals appeared (perhaps hydrochlorid of glutamic acid). The yield was too little to study its nature.

For the detection of tyrosin, another portion of humic acid A (20g.) was boiled with concentrated hydrochloric acid (300 c.c.) for five hours. The extract evaporated to a small volume in vacuo to remove an excess hydrochloric acid, and after neutralisation by NaOH tested for tyrosin with Millon's reagent. A faint red coloration showed a presence of a minute quantity of tyrosin.

From these results we can conclude that the nitrogen in humus is chiefly due to protein or some related compounds which are split up into several kinds of amino-acids when boiled with concentrated hydrochloric acid. But the true nature of that compound originally present in humic acid was not yet fully determined. And also the questions remained unsolved what would be the result when the humic acid was boiled with dilute hydrochloric acid or water instead of concentrated hydrochloric acid, and whether organic bases would be produced as an decomposition product of humus. To answer these questions, further experiments were carried out.

10g. of the sample A was boiled with 200 c.c. of water for one hour and the extract filled up to 250 c.c. and the determination of total nitrogen, ammonia nitrogen and the nitrogen in phosphotungstic acid precipitate were made in the same way as mentioned above with the following result :—

	In 100g. original sample	Total N calculated as 100
Total N	3.729g.	100.00
N, not dissolved in H_2O (N in residue)	3.113	83.48
N, dissolved in H_2O	0.616	16.52
N in phosphotungstic precipitate (ammonia excluded)	0.315	8.45
N in ammonia	0.047	1.26
N in other forms	0.254	6.81

Fresh portions of the sample A (150g.) were again split with 1.5L of 10% hydrochloric acid for one hour, and with a part of the extract determinations of nitrogen in different forms were made. The result was as follows :

	In 100g. original sample	Total N calculated as 100
Total N	3.729g.	100.00
N, not dissolved in HCl (N in residue)	1.423	38.16
N, dissolved in HCl	2.306	61.84
N in phosphotungetic acid precipitate (ammonia excluded)	0.398	10.66
N in ammonia	0.309	8.30
N in other forms	1.599	42.88

The main extract was treated with phosphotungstic acid, then this precipitate with baryta, and the excess of baryta removed in the filtrate by sulfuric acid and thus obtained a solution of a strong basic character. With a portion of the solution some qualitative tests were carried out.

i) Phosphotungstic acid. After acidifying the solution, this reagent gave a strong white precipitate, which dissolved on warming or in excess of the reagent, but was insoluble in sulfuric acid.

ii) Mercuric chlorid or nitrate, basic lead acetate, tannin and a mixed solution of KI and $HgCl_2$ in acetic acid produced a white precipitate.

iii) Distinctly showed Biuret and Millon's reaction.

iv) White precipitate formed by a mixture of ether and absolute alcohol, but the filtrate therefrom did no more show Biuret reaction.

v) Nessler's solution showed a slight turbidity but almost no precipitate.

vi) Xanthoprotein reaction was ambigious and no picrate was formed.

The remaining solution was evaporated in vacuo to a small volume and put into a vacuum desiccator and let it stand to dry at ordinary temperature. After three weeks a yield of 3.67g. was obtained.

2.5g. of this sample was again boiled with 50 c.c. of 20% HCl for 6 hours; and a portion of the extract served for the determination of the different forms of nitrogen with the following result:

	In total extract	Total N calculated as 100
Total N	0.348g.	100.00
N, in phosphotungstic precipitate	0.186	53.45
N, in ammonia	0.000	0.00
N in other forms	0.162	46.55

Of the remaining portion, arginin, lysin and histidin were tested after Kossel's method, but no organic bases were found, except only a trace of histidin. From these results we can say that the humic acid was also decomposed by dilute hydrochloric acid of 10% and more than half of its nitrogen dissolved. The nitrogen compounds contained in extract and precipitable by the phosphotungstic acid seems to be of the compounds akin to protein substance, but they do not exactly coincinde. Altho in many cases they showed the same reactions, the former had a strong basic character and was partly soluble in absolute alcohol, and when boiled with more concentrated hydrochloric acid decomposes partly into a form not precipitable by phosphotungstic acid, but produces no ammonia. Perhaps, it has a simpler constitution than the ordinary protein matter.

A further experiment was made in regard to the ash content with the following result:

100g. of original dry matter contained.

	Humic acid A	B	C
Crude ash:	4.40g.	16.53g.	14.34g.

100 parts of dry ash contained:

	Insoluble residue	Soluble SiO_2	CaO	MgO	Fe_2O_3	Al_2O_3	K_2O	Na_2O	P_2O_5	SO_3	C, CO_2 and Cl.
Humic acid A	33.92	0.90	12.07	1.87	11.62	7.94	1.82	6.92	7.32	10.96	4.67
„ B	19.82	1.08	0.71	0.51	50.36	7.00	0.57	8.34	7.93	1.67	2.02
„ C	41.95	0.42	0.74	0.90	13.84	26.15	0.37	3.20	8.09	1.25	3.12

This result shows that various mineral compounds accompany the humus or partly remain in intimate connection with the humus when it is dissolved and precipitated.

Conclusion.

This investigations show that the nitrogen in the humus is not present as amino compounds, but chiefly as a kind of protein which may be connected more or less intimately with the black substances. In my former communication[1] the observation was mentioned that not only starch but also proteins are blackened by the humification process. This, however, does not exclude that some of the protein is derived from soil bacteria, while another part from the decaying roots.—It seems that during the humification process certain atomic groups in the protein molecule are considerably changed or also oxydized away and this becomes thus less suited as food for bacteria and mold fungi.

According to this result, the writer is inclined to believe that Udransky's artificial nitrogenous humic acid[2] would naturally differ from that of the natural one, because the protein-like substance or several kinds of amino-acids would not be formed when a mixture of glucose and urea is treated with boiling hydrochloric acid.

It was further shown that of amino-acids as such only traces are present, and that such compounds are only obtainable after treating with hot concentrated hydrochloric acid ; 500g. of dry humic acid yielded the following decomposition products :—

[1] These Bull. Vol. VII. No. 3. p. 419.

[2] Zeitsch. f. physiol. Chemie. Bd. XII. p. 42. (1888).

Alanin	2.39g.[1]
Leucin	2.16
Alanin + aminovalerianic acid	0.11
Aminovalerianic acid	0.57
Prolin { Copper salt of active prolin	0.67
Prolin { „ „ „ inactive „ (?)	0.50
Aspartic acid	0.06
Impure aspartic acid (?)	2.16
Glutamic acid	present.
Tyrosin	trace.
Histidin	trace.
Ammonia	1.90
Copper salts of unknown acids	30.30

[1] Of course, these quantities show the minimum amount of the yield, as a part of these substances are lost during the purification process.

The writer expresses his sincere thanks to Prof. U. Suzuki for his valuable advice thruout the progress of the work.

Studies on Diseases of Saké.

BY

T. Takahashi.

In a former report[1] the writer described a new variety of Mycoderma yeast causing a kind of Saké disease, but there remain still many questions regarding Saké diseases to be solved, altho various observers have made investigations on this subject.

The samples of the altered "Saké," generally called here Hyochi-Saké, which were investigated by the writer amounted to fifty[2] in number, while the 48 factories of these samples were distributed over 17 prefectures. These samples could be classified by their flavor and taste as follows.

1. Characteristic "Hyochi" flavor and somewhat strong acidic taste.
2. Taste merely sour.
3. Flavor of sea weed.
4. Odor putrefactive and fish-like.
5. Strong volatile acid flavor.
6. Flavor after acetic acid and taste strongly sour.
7. Very strongly acid and bitter taste.
8. Characteristic "Hyochi" flavor but destitute of any distinguishable taste.
9. No distinguishable flavor; only sweet and sour taste.
10. Trace of Characteristic "Hyochi" flavor and sweet taste.
11. Odor of acetic acid and acetic-ester emitted; taste sour and bitter.

[1] These Bulletins. Vol. 7. No. 1. 1906.

[2] Two of these samples had altered already during their fermentation and must be distinguished from "Hyochi," which name is generally applied to the altered Saké after the fermentation, clarification and pastorisation.

12. Flavor of "Hyochi" Saké and of caramel.

As to turbidity several cases can be distinguished.

1. A sediment forms easily, the liquid becoming clear.
2. The turbidity continues very long, sometimes even for several months.
3. The sediment shows a brown color.[1]
4. The formation of turbidity is succeeded by "Hyochi" flavor.[2]

By direct microspical observation it was observed that in the majority of cases a long non-motile bacillus was present and only in nine cases a motile bacillus[3] and in further forteen, those microbs were accompanied by yeast cells.

Method of isolation.

As culture medium for the isolation of the bacteria in these samples was used sterilised "Saké"-agar[4] in glas tubes. The dilution was made in three tubes and after producing an inclined surface in glas tubes sterilised "Saké" was poured in, whereupon the mouth of the tubes was sealed with a parafinized cotton.

The microbes thus isolated were :—

1. Mycoderma yeast from three samples.
2. Acetic bacillus accompanied with lactic bacillus from four samples.
3. Acetic bacillus from four samples.
4. Mycoderma yeast and Saprogenes (Hyochi) bacillus from 3 samples.
5. Lactic bacillus from six samples.
6. Mould and nothing else from one sample.
7. Saprogenic (to Saké) bacillus from 35 samples.

[1] The white deposit is common with the "Hyochi" Saké.

[2] It is common that the "Hyochi" flavor developes before a marked turbidity is produced.

[3] This kind of bacillus was unfortunately not always found on the agar-culture plate prepared from these samples.

[4] The original "Saké" for this preparation contained 16.4% Alcohol, 0.18% ext. matters. After sterilisation of the culture medium there was a certain amounts of alcohol retained.

I.) Bacillus saprogenes Saké ("Hyochi" bacillus).

For this bacillus it is characteristic that it makes a growth almost exclucively in Saké, and that it produces a peculiar flavor[1] of the "Saké" attacked by this bacillus. We can distinguish two chief varieties :[2]

Bacillus saprogenes Saké. I.

This variety implies those forms which make a growth in yeast-water as a nutrient. We must distinguish[3] further here motile and non-motile sub-varieties, which all are sporeless, and occur in 7 forms.

I. Sub-variety.

The bacillus of this sub-variety makes a growth only in Saké 14% and less alcohol,[4] but not in stronger ; in Saké-agar it developes a colony composed of disks attached to each other in 3 directions, as will be seen from drawing or plate produced from the photograph.[5] But such a form is not quite constant.

1. Form and size : Very long bacillus, 4–7μ in Saké, sometimes filanmentous, attaining to 15–20μ. (with a width of .5μ). Frequently a long chain of cells or two cells combined. Non-motile. Colored well by common anilin colors, and *Gram's staining*.

2. Growth. *a*) Solid nutriment : Stab-culture on Saké-gelatine ; no growth after two months (at 16°C), but grows well as round or rather deformed pea shaped colonies in hydrogen atmosphere. Saké-agar streak culture : grows slowly along the track, but more quickly when covered with "Saké" or yeast-water. (5 days at 22–28° C).

b) Fluid nutriment[6] : Yeast-water : Only traces of developement

[1] This flavor is called, in Japan, "Hyochi Ka."

[2] A former observer, Torii, has reported only one variety of "Hyochi" bacillus which from his description must belong to the first variety observed by myself.

[3] The forth sub-variety is motile.

[4] Alcohol 14.4%. Total acidity 0.137% as succinic acid.

[5] The colonies of such a form are frequently observed with yeast, especially with mycoderma yeast on agar culture, but the growth is quicker in the case of yeast.

[6] All the nutriments mentioned here were employed to all other bacilli isolated.

(a month at 28–30.5° C). No growth in tyrosin solution,[1] "koji"-extract, or "moto"-mash. More or less in yeast-water-galactose or yeast-water-glucose. Almost no growth in alcoholic-yeast-water,[2] or yeast-water-maltose. No growth in protein-free-saké.[3]

2. Behavior to carbohydrate: Galactose is assimilated but neither glucose nor maltose and from galactose a non-volatile acid is formed.

3. Behavior to "Saké": Duration of turbidity of "Saké"[4] is short (5–6 days) in general. In diluted "Saké" 0.0413% of non-volatile acid and 0.0156% of volatile acid[5] are formed after 55 days (summer time). Bitter taste is formed also.

4. Behavior to temperature: Delopes at 16–31° C favorably at 20–28° C very slowly at 10–12° C. Death will result at 55–56° C during 15 minutes in diluted "Saké."[6]

5. Behavior to an anticeptic. 0.2131%[7] of salicylic acid in diluted Saké does not prevent the growth.

Second sub-variety.

The bacillus of this sub-variety grows easily in "Saké" with even above 14% alcohol[8] compaired to the first sub-variety. The form of the colony is not constant, sometimes round and flat[9] in another case resembling the first variety and still in another case it appears as two plates in cross position.

1. Form and size: Very similar to the first one but in some cases[10] somewhat longer; 5–7–10μ. larely 25μ. in "Saké." Involution

[1] Tyrosin 0.025g. glucose 8.0g. alcohol 48 c.c. maltose 2.0g. magnesium-sulphate 0.2g. K-phosphate 2.0g. water 400 c.c.

[2] Alcohol 160 c.c. yeast-water 300 c.c. sugar 2.0g. water 650 c.c.

[3] After the evaporation of the volatile part of "Saké" the excess of basic-lead-acetate is added to precipitate protein matters and the filtrate is passed enough H_2S gas and the filtrate is made to the original volume.

[4] and [6] Alcohol 12.4%. Total acidity 0.1713%.

[5] The non-volatile acid was calculated as succinic acid and the volatile acid as acetic acid.

[7] 10.5 "momme" in one "koku" (180.39. L).

16.4%. But no growth in "Saké" containing 17.5% of alcohol, and by this character we can distinguish this from 4th and 5th sub-varieties.

[9] The bacillus of such colony assimilate non-albuminoid nitrogenous compound in "Saké."

[10] Round flat colony.

form[1] is found in "Saké" (20 days) culture especially in round flat colony. *Stains by Gram's method* in the case of round flat colony.

2. Growth: *a*) Solid nutriment: Saké-gelatine stab-culture; (16–20°C); spheric colony appears along the stab canal. Better growth in hydrogen atmosphere. Sake-agar stab-culture:[2] grows like the first sub-variety.

b) Fluid nutriment: No growth in tyrosin solution, koji-extract, "moto"-mash[3] and only traces in yeast-water-, and in yeast-water-glucose.[4] Negative in alcoholic-yeast-water, yeast-water-maltose:[5] Generally negative in protein-free-"Saké."[6]

3. Behavior to carbohydrate: The assimilation of sugars and acid formation not quite constant, according to the origin of the bacillus. i. e.

Origin.[7]	Assimilation of		Acid formation from	
	galactose.	glucose.	galactose.	glucose.
α.	+++	−	+	−
β.	++	+	++	+
γ.	−	−	−	−
δ.	++	+	±	−
η.	−	++ ++	−	++ ++

4. Behavior to Saké: Turbidity increases for 7–10 days. The acid production is also not constant,[8] but the fixed acid is surely lactic acid (Uffelmann's test).

[1] As shown Fig. 6.

[2] In agar culture the death of the bacillus will be observed after 6 months; while in gelatine culture it lives still longer.

[3]; [4]; [6] Some of this sub-variety (round flat colony bacillus) show a good growth as an exception

[5] With an exception.

[7] Perhaps it will be good to make a further sub-division of this sub-variety according to their origin, but the above description is more simpler.

[8]

	Duration of cult.	Non-vol. acid.	Volatile acid.
α	40 days.	+0.13806%.	−0.0015%.
β.	22 „	+0.00708%.	−0.033%.
δ.	55 „	+0.13098%.	+0.018%.
η.	22 „	+0.0755%.	−0.0134%.

5. Behavior to temperature: Developes at 16–31° C, favorably at 28–29° C[1] or 20–21.5° C.[2] Death temperature is the same as that of the first sub-variety.

6. Behavior to salicylic acid. Some (η. β) grow in diluted Saké containing 0.2131% of salicylic acid, but others not.

Third sub-variety.

The bacillus of this sub-variety grows very well in "Saké" containing 16.4% of alcohol (coincide with second sub-variety) but differs by not forming acid in fluid containing galactose in spite of the assimilation being possible. The form of the colony is the same as that of the first sub-variety.

1. Form and size: Very similar to the second sub-variety. Staining by *Gram's method is negative*, but cellulose reaction observed.[3]

2. Growth: Solid nutriment: Not distinguishable from the above varieties.[4] Fluid nutriment: In all the above liquids negative, except in yeast-water and alcoholic-yeast-water.

3. Behavior to carbohydrate: Neither glucose nor maltose are assimilated but only galactose, but no acid is formed.

4. Behavior to Saké: Fixed and volatile acid are produced[5], beside "Hoychi" flavor and an unpleasant taste accompanied by a bitter taste.

5. Behavior to temperature and salicylic acid: Grows at 16–32° C; optimum 25–26° C not at 10–12° C. Dies at 55–56° C (15 m.). A still smaller quantity (0.2101%) of salicylic acid is sufficient to prevent the growth

Forth sub-variety.

The bacillus of this sub-variety is capable to make a growth in "Saké" with 17.5% of alcohol. This bacillus is *motile and grows well in protein-free-"saké,"* containing some amido-compound. The form of the colony in agar culture is round and flat. The duration of turbidity of "Saké" is longer sustained than with other bacillus.

[1] δ and η.

[2] α, β, γ.

[3] Jod and concent. H_2SO_4. (Blue coloration)

[4] This sub-variety grows unfavorably on "Saké"-agar

[5] During 40 days at 26–29°C, the fixed acid was increased by 0.1770% and the volatile acid by 0.0108% of the Saké.

1. Form and size: Very long in "Saké" culture: 7–9μ. seldom 17.5μ. or 4–5μ. Similar form with above varieties. Involution forms in old "Saké" culture: Club shaped, curved, etc. *Gram's staining is negative.*

2. Growth: Saké-gelatin: Same as the former varieties. Trace in yeast-water, yeast-water-galactose. Negative in tyrosin-solution, "koji"-extract, mato-mash. Good in alcoholic yeast-water or protein-free-Saké.

3. Behavior to carbohydrate and "Saké." Assimilate glucose but not galactose or maltose. In diluted[1] "Saké" forms fixed acid and destroys the volatile acid[2] and alcohol. The duration of turbidity of "Saké" continues from 20–58 days according to the nature of "Saké."[3]

4. Behavior to temperature and salicylic acid. Grows 16–31, optimum 23–28° C, not at 10–12° C. Dies at 55–56° (15 minutes). 0.2101% of salicylic acid is sufficient to prevent the growth.

Fifth sub-variety.

The bacillus of this sub-variety grows in "Saké" with 17.5% alcohol as the third sub-variety. The form of the colony in "Saké"-agar is round and flat. In general properties this bacillus has many similarities to the third sub-variety, but in regard to the assimilability of sugars distinction exists.

1. Form and size: Similar form as the first sub-variety: 2.5–4μ. *Gram's staining is negative.*

2. Growth: White (spheric) colony in "Saké"-gelatine, better in H-atmosphere. Very energetic growth in "Saké"-agar.[4] Trace in yeast-water, better in yeast-water-galactose. Not in the other nutrient fluids above mentioned.

3. Behavior to carbohydrate and "Saké." Like the third sub-variety

[1] Alcohol 12.4%.

[2] After 40 days (at 26–29° C): +0.10856% of fixed acid and −0.0036% of volatile acid, −1.67% of alcohol.

[3] Composition of Saké. Duration of turbidity.

a). Alcohol 16.4%. Ext. m. 1.8%. 25–29° C..... 58 days.

b). „ 14.4%. „ 1.6%. „ „ ..... 52 „

c). „ 12.4%. „ 1.4%. „ „ ..... 20 „

[4] Galactan, perhaps may be assimilated by this bacillus.

assimilates galactose, but not glucose or maltose. Trace of acid is produced from galactose. Destroys "Saké."[1]

4. Behavior to temperature and salicylic acid. Grows at 16–32° C; optimum 27–28° C, not at 10–12° C. Dies at 55–56 after 15 minutes.

Sixth sub-variety.

The bacillus of this sub-variety grows as well in normal "Saké" as the fifth sub-variety, but on regard to the assimilation of sugars and to acid formation just the reverse is observed. The form of the colony in "Saké" agar is the same as that of the first sub-variety.

1. Form and size: Similar to the fifth sub-variety; commonly 2.5μ., 3–5μ, forming very long chains. Involution form not observed. *Gram's staining failed.*

2. Growth: Glolular in "Sake"-gelatine; resembling a mouldy growth in the "Saké" of the "Saké"+"Saké"-agar culture. No growth in all the fluid nutriments above mentioned except in yeast-water.

3. Behavior to carbohydrate and "Saké." Assimilates glucose and forms acid from it, but not so with galactose. Fixed and volatile acid are increased in Saké.[2]

4. Behavior to temperature and salicylic acid. Grows at 16–32° C, optimum 29–30° C, not at 10–12° C. 55–56° C (15 minutes) and 0.2101% of salicylic acid in diluted Saké are sufficient to cause the death of the cells.

Seventh sub-variety.

The bacillus of this sub-variety grows well in Saké[3] not diluted. The colony in "Sake"-agar appears generally in two types; one of them has likeness to the first sub-variety and the other one appears as two round plates intersecting each other vertically. This bacillus assimilates glucose, galactose, and maltose and thus is distinguished from the sixth-sub-variety.

1. Form and size: Long bacillus: 7.5, 12μ. *Gram's staining is*

[1] During 17 days at 26–29° C the fixed acid was increased by 0.1298% of the Saké while the volatile acid was decreased by 0.0237% of the Saké.

[2] 0.2548% of fixed acid and 0.0465% of volatile acid were formed during 36 days at 26–29° C.

[3] Alcohol: = 17.5%.

positive. Involution forms are observed in "Saké" culture.

2. Growth: White, globular colony in Saké-gelatine, better in H.-atmosphere. Grows well in yeast-water, moto-mash, and protein-free-Saké, but not in the other fluid nutriments mentioned.

3. Behavior to carbohydrate and Saké. Assimilates the three sugars mentioned and forms fixed acid from them. *Alcohol is produced from glucose*. The increase of acid in Saké somewhat large.[1]

4. Behavior to temperature and salicylic acid: Grows at 10-30° C,[2] dies at 55–56° C during 15 minutes. 0.2101% of salicylic acid in diluted Saké is sufficient to prevent the growth.

Bacillus saprogenes Saké. II.

The bacillus belonging to this variety can not grow in yeast-water and by this character we can distinguish this from the first variety. Moreover, none of the sub-varieties show any growth in protein-free-Saké, except the first sub-variety. All die at 55–56°C in 15 minutes; 0.2101% of salicylic acid in diluted Saké[3] is sufficient to prevent their growth. In H.-atmosphere grow better than in the common medium.

First sub-variety.

The characteristic property which distinguishes this sub-variety from the following, is its faculty of growing in protein-free-Saké. The form of the colony in Saké-agar is generally a combination of three discs developing in three directions.

1. Form and size: Short bacillus: 2–5μ. in Saké, often in long chain of cells. *Gram's staining negative*.

2. Growth: White spheric or kidney-bean shaped colony in Saké-gelatine. No growth in all the fluid nutrients mentioned above, except in protein-free-Saké.

[1] After 27 days at 26-29° C, the increase of fixed acid was 0.2383% and of the volatile acid 0.018%.

[2] At 15–17° C it grows almost equally well as at 30° C.

[3] Alcohol 12.4%. Total acid 0.1713%.

3. Behavior to carbohydrate and Saké: Galactose was assimilated and oxidized to acid. In Saké, fixed and volatile acid were increased, but alcohol[1] was destroyed.

4. Favorably grows at 16–30° C; optimum 23–25; but not at 10–12° C.

Second sub-variety.

This bacillus grows well in Saké containing 16.4% of alcohol, and the developement in Saké-agar was very energetic; the forms of the colony in this medium are either the form of the first sub-variety or two plates intersecting vertically with each other.

1. Form and size: Either long (5–7.5μ) or short (2.5–4–5μ) cell. Two cells in combination are common. Involution forms were observed in old Saké-culture. (30 days culture). *Stains well by Gram's method.*

2. Growth: A white spheric or bean shaped colony in Saké-gelatine. An energetic growth in Saké-agar led me suppose that this bacillus has perhaps, the power to assimilate galactan. No growth in all fluid nutrients mentioned above.

3. Behavior to carbohydrate and Saké: Galactose is oxidized into acid. The acid was increased in Saké;[2] and the flavor of caramel was observed at the same time.

4. Temperature limit for the growth lays from 16–32° C; optimum 23–25° C, but not at 10–12° C.

Third sub-variety.

The bacillus of this sub-variety grows well in Saké containing 16.4% of alcohol. The colony in Saké-agar is composed of five discs; three of them starting from a common line, the other two growing in other way forming certain angles to the former discs. (compare plate).

1. Form and size: Long bacillus; commonly 8μ, sometimes very

[1] After 25 days at 26–29° C, there was the increase of the fixed acid by 0.0566% and of the volatile acid by 0.2024%; the decrease of alcohol by 2.95%.

[2] After 33 days (at 26–29° C); +0.0233% of fixed acid and +0.0084% of volatile acid.

long filaments. The involution forms appear easily.[1] *Stains well by Gram's method.*[2]

2. Growth: A spheric colony in Saké-gelatine. All fluid cultures failed, except in yeast-water-galactose.

3. Behavior to carbohydrate and Saké: Galactose was oxidized to acid. In Saké an increase of fixed acid and a decrease of volatile acid[3] was found.

4. Temperature limit for the growth lays 10–31° C but the optimum lays between 23–25° C. No growth at 10–12° C.

Fourth sub-variety.

As the former sub-variety, this bacillus grows well in Saké containing below 16.4% of alcohol; while assimilation of galactose did not take place in this case and by this character we can distinguish it from the third sub-variety. The colony in Saké-agar has the same shape of the first sub-variety.

1. Form and size: Either short or long bacillus: generally 5–7μ or sometimes 10μ. Involution forms were observed. *Stains well by Gram's method.*

2. Growth: A white spheric colony in Saké-gelatine.[4] The fluid cultures mentioned above, all failed as nutriment.

3. Behavior to carbohydrate and Saké: Galactose was not assimilated. Fixed and volatile acid were increased in Saké.[5]

4. The optimum temperature for the growth was 29–30° C, though it can grow at 16–32. No growth at 10–12° C.

A bacillus was isolated which behaves very simillary in many respects to this bacillus, but the differences consisted in the size (3–5μ, chiefly), in its

[1] It appears in 18 days culture already.

[2] By this character we can distinguish this from the second sub-variety of B. saprog. s. II.

[3] After 37 days at 26–29° C, there was an increase of fixed acid by 0.03068% and a decrease of volatile acid by 0.02373%.

[4] A bacillus very similar to this bacillus, differing by its formation of round flat disc formed colony in Saké-agar, dies off more quickly, in agar-culture, than this bacillus.

[5] After 2 months at 22–29° C there was an increases of the acid.

Fixed acid ..... by 0.2147%.
Volatile acid ..... „ 0.0108%.

failure by Gram's staining and in a scarcely noticeable growth in yeast-water containing a trace of alcohol.

Fifth sub-variety.

This bacillus can grow in Saké containing alcohol up to 14.4%. Further, the faculty of developing in yeast-water containing a small quantity of alcohol is a characteristic property, which distinguish this from other sub-varieties.

1. Form and size: Long bacillus; 5–8μ. or very long chains of cells. Involution forms were observed (Fig. 24) and these forms, when stained, show colored stripes. *Gram's staining failed.*

2. Growth: A white spheric colony in Saké-gelatine. All the fluid nutriments mentioned above failed as nutrient media, except yeast-water containing a small quantity of alcohol, or galactose.

3. Behavior to carbohydrate and Saké. Galactose was oxidized to acid. In Saké fixed acid was increased but volatile acid was destroyed.[1]

4. The optimum temperature lays near 30° C, though it can grow at 16–33° C. No growth at 10–12° C.

Sixth sub-variety.

This bacillus stands very nearly in its general properties to that of the fifth sub-variety. We can distinguish it from this by:—

a) Size: generally shorter than the former one; 2.5–4–5μ.

b) Staining well by Gram's method.

c) The colony in Saké-gelatine having *spines on its surface.*

d) The surface[2] growth in H-atmosphere, when Saké-gelatine was used as nutrient medium.

e) The weak growth in yeast-water containing a small quantity of alcohol.

[1] After 66 days culture, there was an increase of fixed acid by 0.0799% and a decrease of volatile acid by 0.003%.

[2] Such a property have not been met with in any other case.

Seventh sub-variety.

This bacillus has closely related properties to the sixth sub-variety, but the differences consist in :

a) The shorter size ; 2.5–5μ.

b) The cellulose reaction. (Blue color made by Jod-Jodpotasium and strong H_2SO_4).

c) The white spheric colony with smooth surface in Saké-gelatine.

d) The volatile acid in Saké was destroyed.

e) Involution forms were not observed.

Eighth sub-variety.

This bacillus has almost equal properties with that of the seventh sub-variety, but may be distinguished by the following points :—

a) The longer size : 7–8μ. commonly.

b) Negative behavior to Gram's staining method.

c) The colony in Saké-gelatine was semi-transparent and a spleen shaped.

Ninth sub-variety.

This bacillus can grow in Saké containing less than 12.4% of alcohol (diluted Saké). The colony in Saké-agar consisted of three combined discs.

1. Form and size : Either short or long bacillus ; 2.5–3–5–7μ. sometimes in long chains of cells. Involution forms were observed. *Stains well by Gram's method.*

2. Growth : A white spheric or kidney-shaped colony in Saké-gelatine. A weak growth in yeast-water-galactose, but no growth in all other untrients mentioned above.

3. Behavior to carbohydrate and Saké. Galactose was assimilated, but no acid was found in the culture. In Saké an increase of fixed and volatile acid was observed.[1]

[1] After 2 months at 20-29° C, there was found an increase of the fixed acid by 0.1911 6% and that of the volatile acid by 0.0204%.

A caramel flavor was found in the culture in Saké.

II.) Lactic acid bacillus group.

The bacilli belonging to this group form lactic acid[1] in Saké or other nutrients but we can not fined any trace of the so-called characteristic "Hyochi"-flavor. Six varieties must be mentioned. All of these are non-motile except one variety and sporless. All grow well in a H-atmosphere. All die at 50–52° C after 15 minutes.

I. Bacillus panis fermentati. var Saké.

1. Form and size: Short bacillus and forms long chains of cells in diluted Saké.[2] In "koji"-extract a combination of two cells was found chiefly, or long irregularly curved cells are seldom found.

2. Growth: In Saké-agar plate culture there was found chiefly imbedded in medium, a white, round and flat colony, with smooth surface. The surface, when observed under microscope, seems granular. *Stab-culture:* A white spheric colony was found in the inner part of Saké-gelatine. (16° C after 2 months). An energetic growth was observed along the stab-canal of koji-extract-agar; when old there was found a pin-head like growth at the mouth of the canal accompanying gas production. A still more energetic growth was found in case of Saké-agar, better after pouring some Saké on the solid medium.

Fluid culture: In yeast-water, bouillon, koji-extract, *protein-free-Saké* makes a good growth, especially in koji-extract; but there was no growth in "moto"-mash, Hayduck's solution or tyrosin solution.

3. Behavior to carbohydrate and Saké. In the culture of diluted Saké, there was an increase of both acids (fixed and volatile)[3]; while the alcohol of it was destroyed. This bacillus could not develope in Saké

[1] The acid was isolated by Uffelmann's method from the culture of these bacilli in yeast-water-galactose (10%).

[2] Alcohol 12.4%, total acid 0.1713%.

[3] In Saké containing 12.4% of alcohol and 0.1713% of total acid, there was an increase of the fixed acid by 0.0413% and the volatile acid by 0.025%, where the decrease of alcohol was by 0.27% after 27 days at 26–29° C.

containing 17.55% of alcohol and 0.177% of total acid; while at 16.4%, it shows an energetic growth[1]. The acid formation from various sugars may be shown by the following table :—

Substance.	Bacillus panis fermentati.[2]	Bacillus panis ferm. var Saké.
Arabinose	±	± + + +
Xylose	/	+ + + +
Galactose	±	+ + +
Glucose	+	+ + +
Fructose	±	+ +
Rhamnose	−	±
Maltose	+ ±	+ +
Saccharose	±	± + + +
Lactose	−	−
Raffinose	−	+ +
Dextrine	−	± +
Starch	/	±
Inulin	−	−
Mannite	−	−
α-Methyl-glucoside	−	+ + + +

From the above table, it will be seen that this bacillus coinsides with B. pan. fermentati in forming acid from arabinose, fructose, maltose and saccharose; but in failing of doing so from lactose, inulin, mannite, while in regard to the formation of acid from galactose or glucose, raffinose, dextrine, α-methyl-glucoside and also from rhamnose (altho only little acid results) it differs from the latter variety. Alcohol was formed from galactose or maltose.

4. Behavior to temperature and salicylic acid. Grows at 16-31° C; optimum being 22-23° C, but no growth at 10-12° C. 0.2131% of salicylic acid was insufficient to prevent the growth in Saké.[3]

[1] Saccharobacillus past v. b. makes no growth in a solution containing 8% of alcohol and may be distinguished from this bacillus.

[2] In the table, +, indicate production ; −, not ; ±, trace.

[3] Alcohol 12.4%.

II. Bacillus Aderhordi. var Saké.

1. Form and size: In Saké or other nutrient it appears as a short bacillus: 1.25×0.5; 1.3×0.7 sometimes in long chains of cells. Involution form was not observed in Saké. A very long filament in protein-free-Saké.

2. Growth: A round creamy colony was formed on the surface of koji-extract-agar, while in the inner part of the same medium a lens-shaped one was observed. An irregular mass in Saké-agar at an addition of Saké above it. Yeast-water, bouillon, moto-mash,[1] protein-free-Saké, koji-extract are very good nutriment, especially the latter one, but no growth was found in Hayduck's solution or tyrosin-solution. *Stab-culture:* A brown spheric colony with spine on the surface in the inner part of the canal, but no growth at the mouth. Any gelatine disolving power was not found by this culture.[2] When cultured in koji-agar, there was observed a developement of CO_2 gas.

3. Behavior to carbohydrate and Saké. There was no growth in Saké containing 15.7% of alcohol and 0.1593% of total acid, but when diluted to 14.87%[3] of alcohol and 0.1228% of total acid an energetic growth was observed. There was an increase of fixed acid by 0.00236% and volatile acid by 0.00324% after one month at 26–29° C. The acid formation is shown by the following table:—

Substance.	Bacillus Aderhordi.	Bacillus Aderhordi. var Saké.
Arabinose	−	− ±
Xylose	/	+ − − −
Galactose	− −	− − −
Glucose	− −	− − ±
Fructose	− − ±	±
Rhamnose	/	−
Maltose	± ±	± ±

[1] The capability of growing moto-mash has an important fact from the practical stand point.

[2] The absence of liquefying power for gelatine was observed in all five varieties described below.

[3] The dulability to such a high % of alcohol distinguishes this from saccharobacillus pasteurianum, var berolinensis, which is prevented its growth in 8% of alcohol.

Substance.	Bacillus Aderhordi.	Bacillus Aderhordi. var Saké.
Saccharose	+ +	+ + + +
Lactose	+ +	+ + + +
Raffinose	+ +	+ + + +
Dextrine	+	±
Starch	/	−
Inulin	−	−
Mannite	−	−
α-Methyl-glucoside	−	−

Thus we can easily find that this bacillus behaves very similarly, to sugars, to Bacillus Aderhordi. var Saké.

4. Behavior to temperature and salicylic acid. Grows at 16–32° C; optimum 28–29° C, but not at 10–12° C, maximum 32° C. 0.2101% of salicylic acid is sufficient to prevent the growth.

III. Bacillus Delbrücki. var Saké.

1. Form and size: A long bacillus: 7.5–10.5μ sometimes 3μ. The involution forms were observed in old culture, therefore the cells in Saké culture have almost the same form as that of B. Saprogenes Saké. When stained with a common anilin color, the both ends are colored stronger than the other part.[1]

2. Growth: A white creamy and round colony developes on the surface of the plate culture of Saké-agar, while at the inner part it appears as a white round disc, moreover when old, the surface of the disc is covered by a hairly growth. Yeast-water, bouillon, koji-extract[2] and protein-free-Saké are good nutriments for this bacillus, but no growth in Hayduck's solution, moto-mash or tyrosin-solution. *Stab-culture*. A white spheric colony was formed along the canal in Saké-gelatine, a brownish growth in koji-extract-agar and most energetic growth in Saké-agar[3]. But no growth on the surface part.

[1] This property was observed well, especially in yeast-water-glucose.

[2] It is highly probable to suppose that it has a faculty to assimilate galactan.

[3] When old, the growth alter into a flocculent mass.

3. Behavior to carbohydrate and Saké: It grows well in Saké containing up to 14.4% of alcohol and 0.137% of total acid, where a decrease of fixed[1] acid and alcohol and an increase of volatile acid were observed. Further, bitter taste and an unpleasant taste were formed in Saké. The acid formation was observed as following table shows:—

Substance.	Bacillus Delbrücki.	Bacillus Delbrücki. var Saké.
Arabinose	−	++++
Xylose	-	++++
Galactose	+	++++
Glucose	+++	++++
Fructose	+−+	++
Rhamnose	−	−
Maltose	+−−	++++
Saccharose	+	++++
Lactose	−	−
Raffinose	−	++
Dextrine	+−	++
Starch	−	+
Inulin	−	−
Mannite	−	−
α-Methyl-glucoside	−	++++

Thus the property of the forming acid from arabinose, xylose, starch, α-methyl-glucoside and raffinose forms a decisive difference from B. Delbrücki. Further, a trace of alcohol was formed from galactose and maltose.

4. Behavior to temperature and salicylic acid. Grows at 16–32° C; optimum 28–29, but not at 10–12° C. 0.2131% of salicylic acid in Saké prevent the growth, but not 0.2101%.

[1] After one month, there was an increase of volatile acid by 0.066% and a decrease of fixed acid by 0.0047%.

IV. Bacillus lactis acidi. var Saké.

1. Form and size: Either short or long bacillus: 3-4μ-7.5μ. Involution forms were observed in old culture. Revolving motion was observed.

2. Growth: A white pasty colony on the surface of koji-agar plate culture; while in the inner part grows as white round disc[1], which observed under microscope was found a granular surface. In yeast-water, bouillon, koji-extract grows very well especially in the latter one, but no growth in Hayduck's solution, moto-mash, tyrosin-solution or protein-free-Saké. *Stab-culture.* A white spheric colony with spines on the surface was found in Saké-gelatin. Grows only along the canal in Saké-agar or koji-extract-agar but not on the surface.

3. Behavior to carbohydrate and Saké: It grows in Saké containing up to 14.04% of alcohol[2] and 0.1416% of total acid, whereby an increase of fixed[3] and volatile acid and decrease of alcohol were observed. Further, unpleasant flavor and bitter taste developed after the growth of the bacillus. The acid formation from sugars is shown in the following table:—

Substance.	Bacillus lactis acidi.	Bacillus lactis acidi. var Saké.
Arabinose	−	+ + + +
Xylose	−	+
Galactose	+	+ + +
Glucose	+ + +	+ + +
Fructose	+ + +	+ + +
Rhamnose	−	±
Maltose	+ + +	+ + + +
Saccharose	+ + +	+ + + +
Lactose	±	+ + + −

[1] Or sometimes appears a second disc growing vertically to the first one.

[2] B. lactis acidi loss its acid forming power and its propagating energy in 3% of alcohol.

[3] After one month at 26–29° C, there was an increase of fixed acid by 0.0212% and volatile acid by 0.0273%.

Substance.	Bacillus lactis acidi.	Bacillus lactis acidi. var Saké.
Raffinose	± ±	+ +
Dextrine	– –	±
Starch	–	±
Inulin	– ?	–
Manuite	–	–
α-methyl-glucoside	–	+ + +

Thus the acid forming property of this bacillus from arabinose, xylose, rhamnose, starch, α-methyl-glucoside identifies this from B. lactis acidi. Further, alcohol was formed from galactose and maltose.

4. Behavior to temperature and salicylic acid: Grows well at 16–32° C; optimum 23–24° C, but no growth at 10–12° C. Very weak growth in Saké containing 0.2101% of salicylic acid but not in 0.2131%.

V. Bacillus wortomani. var Saké.

1. Form and size: Very long bacillus: 2–2.5–3μ commonly but in old culture: 20μ as a filament. A flocculent or mouldy mass was formed in old Saké culture.

2. Growth: The form of the colony in Saké-agar was almost the same as Bacillus lactis acidi. var Saké. A mouldy mass was found also in Saké over Saké-agar culture, but when it grows in the inner part it was almost equal to that of B. Delbrücki. var Saké. In yeast-water, bouillon, protein-free-Saké, koji-extract it grows well especially in the latter one, and a trace in moto-mash but not in Hayduck's solution or tyrosin solution. *Stab-culture.* A white spheric colony in Saké-gelatine but not on the surface. Only along the stab canal in Saké-agar, koji-extract-agar.

3. Behavior to carbohydrate and Saké. A very weak growth in Saké containing 16.4% of alcohol and 0.15% of total acid, but an energetic growth was observed at lower parentage of alcohol (13.163%) and acid (0.1228%). In Saké an increase of fixed acid and decrease of volatile acid

were found.[1] The acid formation from carbohydrate will be shown in the following table:—

Substances.	Bacillus Maerkeri.	Bacillus cucumeris fermentati.	Bacillus Wortomani.	Bacillus Wortomani var Saké.
Arabinose	+	+ + +	+ + +	+ + + +
Xylose	/	/	/	− + + +
Galactose	+ + +	+ + +	+	− − +
Glucose	+ + +	+ + +	+ + +	−
Fructose	+ + +	+ + +	+ +	+ + + −
Rhamnose	/	/	/	+
Maltose	+ + +	+ + +	+ + +	− + + +
Saccharose	+ + +	− + −	+ + +	+ + − +
Lactose	+ +	+ + +	− +	− + − −
Raffinose	+ + +	+ + +	+ +	− +
Dextrine	+	+	+ +	+ − − −
Starch	/	/	/	+ +
Inulin	−	−	−	−
Mannite	+ +	− +	+ +	− − +
α-methyl-glucoside	−	−	+	− + +

Thus, this bacillus do not form acid from glucose and is thereby distinguishable from the three closely related varieties. Further, the acid forming property from α-methyl-glucoside may be mentioned as a distinguishing point from B. Maerkeri and B. cucumeris fermentati. Moreover, a trace of alcohol was formed from maltose, saccharose, lactose, galactose and α-methyl-glucoside.

4. Behavior to temperature and salicylic acid. 0.2101% of salicylic acid in Saké is sufficient to prevent the growth. Grows at 16–32°; optimum, 28–29°C, but no growth at 10–12°C.

VI. A new lactic bacillus.

1. Form and size: Short bacillus: 2.5 × 1.25μ; 4 × 2μ, rarely 7μ, or

[1] After 21 days at 26–29° C, there was an increase of fixed acid by 0.04248% and a decrease of volatile acid by 0.018%.

in long chains of cells. Both ends of the cell have larger refractive power of light than other part.

2. Growth: A white disc in Saké-agar, which under microscope seems granular. Yeast-water, bouillon, koji-extract[1] are good untriment but Hayduck's solution or tyros in solution not. *Stub-culture:* A white spheric colony was found in Saké gelatine and a pin-head like growth was found at the month of the stab-canal. A good developement was observed in Saké-agar or koji-extract-agar.

3. Behavior to carbohydrate and Saké. A weak growth was found in Saké containing 16.4% of alcohol and 0.153% of total acid, whereby an increase of acid was observed. The acid forming property from carbohydrat will be shown in the following table:—

Substance.	This bacillus.
Arabinose	±
Xylose	++++
Galactose	−
Glucose	+
Fructose	−
Rhamnose	−
Maltose	−
Saccharose	±
Lactose	+
Raffinose	±
Dextrine	±
Starch	/
Inulin	−
Mannite	±
α-methyl-glucoside	±

Thus, this bacillus behaves very similar, to sugars, as B. Wortomani. var Saké; the difference between them is generally a weaker production of acid and the absence of this property in regard to fructose, rhamnose, maltose and galactose in the case of this bacillus.

[1] Acidic fluid is better than neutral one.

4. Behavior to temperature and salicylic acid: It grows well at 16–33°C; optimum 28–29°C, but not 10–12°C. 0.2101% of salicylic acid in Saké is sufficient to prevent the growth.

III. Acetic acid bacillus group.

The bacillus of this group appeared in Hyochi Saké in two types. One of its type was a bacillus, which shows a surface growth on Saké without causing turbidity in Saké. The rather smooth film, with grey white coloration, was very brittle; so that it sanks down to the bottom of the flask as soon as it reached to its maximum point of growth. Further, it was killed soon by its own product, so unfortunately the pure culture failed.

The other type was a bacillus which must be divided into five varieties, all of them belonging to a species of Bacillus küttingianum.

I. α.–Variety.

1. Form and size: A short bacillus: $2.5 \times .5\mu$. $3 \times .5\mu$. Involution form was not found in Saké even when old. Non-motile.

2. Growth: A grey round creamy colony on the surface of the plate culture of koji-extract gelatine. A grey white disc formed colony on the surface of the plate culture of Saké-agar, whereas in the inner part it appears as a combined three discs growing in three directions. *Stab-culture:* A smooth light-refracting growth at the mouth of the stab-canal, and a redish-yellow color at the most elevated part of the growth. A ring-like growth along the side of the tube of wort-gelatine. Chiefly a surface light refracting growth on *Saké-agar* culture, where the central elevation colored purplish-grey, moreover fine streams were found on its surface. Of such culture, after four months, a test with guaiac tincture and H_2O_2 was made but no positive result. Further, such old culture contained large (even 27.5μ) involution forms of various shape.

Chiefly upper part of the stab-canal in *Saké-gelatine*, where no sign of liquefying gelatine was observable. *Surface-culture.* A grey[1] white light

[1] The grey color was most dense among the five varieties.

refracting creamy coating after 10 days. (at 27-30°C). *Fluid culture:* A ring-like growth along the side of the test tube with certain turbidity and sediment. (10 days at 28–30°C). This culture medium after one month loss its power of reducing Fehling's solution. In koji-extract containg 5% of alcohol, it does not form the film causing a turbidity and acetic acid flavor after three days, but after 4–5 weeks there was formed a grey white smooth film on the surface of the fluid and *this fim stained blue by iodine KI solution.*[1] There was no growth in koji-extract which contained more than 10% of alcohol. A thin film was formed on the surface of a beer. In Saké[2], a turbidity after 5 days (at 27–28°C) and after 15 days the fluid changed to the clear fluid with some sediment.

3. Behavior to carbohydrate[3] and temperature.

Substance.	After 5 days.	Acid production.
Glucose	Fluid clear, a trace of sediment.	−
Fructose	Little turbidity „	+
Galactose	„ „	−
Arabinose	Ring on side of the tube, fluid clear.	−
Saccharose	Fluid clear, little sediment.	−
Maltose	Little turbidity, little sediment.	−
Lactose	Fluid clear, „ „	−
Raffinose	Little turbidity.	−
Dextrine	„ „	−
Inulin	Ring on side of the tube, fluid clear.	−
Starch	Thick film, little turbidity.	−
Mannite	Little turbidity, little sediment.	+
Glycerine[4]	Fluid clear, „ „	−

The optimum temperature lay near 27–28°C. and grows difficultly above at 31°C. 55–56°C in 15 minutes kills the cell.

[1] All other varieties described below gave the same reaction.

[2] Alcohol 16.4%, and 14.4%, and these Saké were used to all varieties described below.

[3] Carbohydrates were added into bouillon in another five experiments mentioned below.

[4] In all cases used, glycerin was added instead of saccharose in Hayduck's solution.

II. β.-Variety.

The different points from the α.-variety are:

1. Form and size: Short bacillus: 2μ in common; $4-5\mu$ rarely.

2. Growth: A white round creamy colony on Saké-gelatine plate culture. A yellowish round colony but sometimes a rhizoidal growth was found. *Stab-culture.* A smooth flat[1] growth with a yellowish-red coloration on beer-agar. A filmy growth ascending along the side of the tube on wort-gelatine. A flat and creamy growth with more or less purple coloration, on Saké-agar. A redish greyey white growth on Saké-gelatine. *Surface-culture.* A creamy but lighter grey than α.-variety on Saké-agar. *Fluid-culture.* A very thin film was formed on yeast-water, but a thick film on koji-extract containing 5% of alcohol.

3. Behavior to carbohydrate and temperature.

Substance.	Growth after 5 days.	Acid formation.
Glucose	Thin film, turbid, sediment.	−
Fructose	„ dense turbidity, sediment.	+
Galactose	Turbid sediment.	−
Arabinose	Thin film, fluid clear.	−
Saccharose	„ turbid, sediment.	+ +
Maltose	„ „ „	−
Lactose	„ „ „	−
Raffinose	„ fluid clear.	−
Dextrine	„ „	−
Inulin	„ „	−
Starch	Thick film, „	−
Mannite	Turbid, sediment.	−
Glycerine	Thin film, turbid, sediment.	−

4. In koji-extract forms butyric acid and ethyl-alcohol in addition to a flavor of altered vingar, but not acetic—, formic acid, methyl-lactate or

[1] This is a distinguishing point from α.-variety, and the three mentioned varieties below coinside in this point.

fusel-oil. (after 3 days at 28°C).

It grows at common temperature and optimum lays at 30-32°C. Heating 15 minutes at 55–56°C is not sufficient to kill the cells.

III. γ.-Variety.

This variety may be distinguished from two above varieties by the following properties.

1. Form and size: The shortest bacillus among five varieties: 1–1.5 × 1μ, but when in the film 5 × 1μ.

2. Growth: A white round creamy colony with the radiated streams around the periphery on the surface of Saké-gelatin plate culture. A white hemispheric waxy colony on the surface of the Saké-agar plate culture. *Stab-culture.* A very light brownish-white flat growth around the mouth of the stab-canal in beer-agar. A similar growth with α.-variety was observed on wort-gelatine. On Saké-agar makes a similar growth to that of α.-variety, but large cells were not observed in this culture, even 4 months old, whereas the blue, guaiac reaction for peroxidase was observed. *All other four varieties failed to give this reaction,* which was observed B. küttingianum already known. A redish growth was found on Saké-gelatine.

Fluid culture: In yeast-water (10 days at 28-30.5°C), a ring formation and an island-like growth were found. This culture, after one month reduced Fehling's reagent,[1] differing thus from other four varieties. In koji-extract containg 5% of alcohol emits the flavor of acetic acid. On Saké it forms *a film and a ring along the side of the tube after* 16 days culture. In koji-extract (6 days at 26–27°C) there were found[2] isopropyl alcohol and methyl-lactate, but no methyl alcohol or aceton.

3. Behavior to carbohydrate and temperature.

Substance.	After 5 days.	Acid formation.
Glucose.....	——	——
Fructose	Turbid sediment.	–
Galactose	„ „	±

[1] B. rancens has this reducing power.

[2] In the distillate.

Substances.	After 5 days.	Acid formation.
Arabinose	Ring, fluid clear.	±
Saccharose	Fluid clear, sediment.	–
Maltose	Thin film, turbid, sediment.	–
Lactose	Fluid clear, sediment.	–
Raffinose	Ringly growth, fluid clear.	–
Dextrine	,, ,, ,,	–
Inulin	,, ,, ,,	–
Starch	Thick film turbid.	–
Mannite	Turbid, sediment.	±
Glycerine	,, ,,	–

Thus the acid forming character almost equal to that of B. oxydans.

Optimum at 30–32°C. 10 minutes heating at 55–56°C was not sufficient to kill the cells.

IV. δ.–Variety.

1. Form and size: Short bacillus: $1.5 \times 0.5\mu$, $2.5 \times 0.5\mu$, on Saké-agar. An involution form was not observed in old Saké-culture.

2. Growth: A similar colony to that of α.–variety on the surface of the koji-extract gelatine plate culture. A round somewhat elevated brownish-yellow colony was formed on the surface of Saké-gelatine plate culture. *Stab-culture.* A similar growth with α.–variety on beer-agar. A flat growth was observed around the mouth of the stab-canal in wort-gelatine. Chiefly grows around the mouth of the canal, with an elevation of its central part, which was colored yellowish greyey-white but not purplish. (difference from α.–and γ.–varieties). In this culture the involution forms were observed after 4 months, but not large cells as in the case of α.–variety. Further, the cells were colored *purplish* when a cellulose reaction[1] was made and this property was not found in former described three varieties. The guaiac reaction for peroxidase failed. Very similar growth

[1] Jod Jodpotassium + H_2SO_4.

as γ,–variety, in Saké-gelatine. *Surface culture.* A greyey white creamy coating on Saké-agar. *Fluid-culture:* A similar growth with α.–variety after one month in yeast-water. This culture lost reducing power of Fehling's solution. In koji-extract containg 5% of alcohol, there was a turbidity after 3 days at 29°C, while after one month a thick grey-white film, breaking easily, was formed; whereby the flavor of acetic acid and acetic ester were emitted. In beer: formation of a ring along the side of the tube, in addition to the film formation. In Saké: very similar to α.–variety.

3. Behavior to carbohydrate and temperature.

Substance.	After 5 days.	Acid formation.
Glucose.....	Ring, fluid clear.	+
Fructose	Turbid, sediment.	+
Galactose	„ „	–
Arabinose.....	Fluid clear.	–
Saccharose	Ring, fluid clear, sediment.	–
Maltose.....	Turbid, sediment.	+
Lactose.....	Fluid clear, sediment.	±
Raffinose	Fluid clear.	–
Dextrine	„	–
Inulin	„	–
Starch	Thin film, fluid clear.	–
Mannite	Turbid, sediment.	+
Glycerine.....	„ „	–

Thus, the acid forming faculty from fructose, maltose, mannitol distinguishes this from B. küttingianum. This bacillus grows at common temperature, but its growth becomes difficult when the temperature rises above 30°C. Heating 10 minutes to 55–56°C was not sufficient to kill the cells.

V. η.–Variety.

1. Form and size: Short bacillus: 2.5–3μ. in Saké, 1–2.5 in Saké-agar.

2. Growth: Very similar growth as γ.–variety on Saké-gelatine plate culture. A white disc in Saké-agar plate culture. *Stab-culture.* A light yellowish white flat growth on beer-agar. A similar growth on beer-gelatine. Almost equal growth as δ.–variety on Saké-agar, whereby the *cell gave purplish coloration by the cellulose reaction*, but no peroxidase reaction. The involution form in this culture appeared like a leaf of the reed. (l=20-27.5; b=2μ). On Saké-gelatine a redish growth, which produced a feathery growth around it after one month, was found. *Surface culture.* Same growth as δ.–variety on Saké-agar. *Fluid culture.* In yeast-water, the growth was very similar to that of δ.–variety. In koji-extract containing 5% or 10%[1] of alcohol it makes a growth. In Saké (in case of 14.4% of alcohol) after 20 days, there was formed a thin film. Same growth as other varieties on beer. In koji-extract culture was found trace of isopropyl alcohol and methyl-lactate, acetic and butyric acid but not formic acid or aceton.

3. Behavior to carbohydrate and temperature.

Substance.	After 5 days.	Acid formation.
Glucose	Thin film, turbid, sediment.	++
Fructose	Ringly growth, „ „	++
Galactose	„ „ „	++
Arabinose	Thin film, turbid.	++
Saccharose	Ringly growth, turbid, sediment.	++
Maltose	„ „ „	++
Lactose	„ „ „	±
Raffinose	Thin film, turbid.	–
Dextrine	„ „	–
Inulin	„ „	–

[1] The former 4 varieties could not.

Substance.	After 5 days.	Acid formation.
Starch	Fluid clear.	–
Mannite	Thin film, turbid, sediment.	–
Glycerine	Fluid turbid, „	–

Thus the acid forming property has likeness rather to that of B. oxidans.[1]

Optimum temperature: 30–32°C. Heating 10 minutes to 55–56°C was not sufficient to kill the cells.

IV). "Kahm-hefe" group.

The "Kahm-hefe"-group found in Hyochi-Saké may be classified into two divisions:

1. Willia anomala (sacch. anomalus.) with hat-like spores and emiting a flavor of acetic ester.

2. Mycoderma group; with no spores but emit a flavor of altered vinegar.

THE EXPERIMENT ABOUT THE BEHAVIOR OF KNOWN BACTERIA AGENT SAKE.

Since a former observer Ōtani had reported that the microbs of "Hyochi" belong to a certain group of well known bacteria, the writer has also infected steritised Saké (12.4% of alcohol) with several kinds of known bacteria and observed the following result:—

Bacteria.	After 5 days.	After 19 days.
Sarcina citrina	Sediment.	Sediment increased.
„ alba	Turbid, sediment.	„
„ fusca	No growth.	No growth.
„ aurantiaca.....	Sediment.	Sediment increased.

[1] B. oxidans forms acid from mannitol and glycerine.

Bacteria.	After 5 days.	After 19 days.
Sarcina liquefaciens	Fluid clear, sediment.	Sediment increased.
Micrococcus candidus	Turbid, sediment.	Fluid clear, many sediment.
B. Megaterium	No growth.	No growth.
„ proteus mirablis	„	„
„ „ vulgaris.....	„	„
„ „ zenkeri	„	„
„ lactis niger	Turbid, sediment.	Fluid clear, many sediment.
„ „ saponacei	No growth.	No growth.
„ acid lactici Hüppe.....	Sediment.	Sediment.
„ fluorescens albus	Turbid (trace).	„
„ lactis III. Flügge	No growth.	No growth.
„ subtilis	„	—
„ mesentericus ruber	„	—
„ „ vulgatus	„	—
„ „ fuscus	„	—
„ Zopfii	„	—
„ butyricus Hüppe	Fluid clear, many sediment.	—

Further infection, to Saké containing still higher % of alcohol (16.5%), was made with bacteria which showed any growth in the above trial, and the result was that the five bacteria mentionèd below were capable to develope moderately:

1. Sarcina alba.
2. „ aurantiaca.
3. „ citrina.
4. „ liquefaciens.
5. Micrococcus candidus.

Thus, we learn, that the most resistant hay-bacillus did not show any growth in even diluted Saké.

Summaries.

1. In Sake showing a peculiar disease known in Japan under the name "Hyochi" a peculiar kind of bacillus was found, named by the writher B. saprogenes Sake which produced the characteristic "Hyochi" flavor and further lactic acid. But, in such diseased Sake there are freqentry found also other kinds of microbes, and sometimes Mycoderma, which may favor indirectry the growth of the B. saprogenes Sake, especially an acetic bacillus. There can be distinguished /7 subvarieties belonging to 2 chief varieties. Some of them reqire protein matters, other assimilate simpler N-compound, they are further distinguished by their resistant power to alcohol and their different behavior to sugars. Their growth in Sake is due to some, protein-matters and amido-compound, present.

2. Also common lactic acid bacilli were found in Hyochi Saké, they showed a stronger resistant power to alcohol than the known varieties. (/3./63% – /6.4% of alcohol in Saké.) All six varieties observed have the faculty of forming acid from xylose and, with exception of one variety, from starch or α-methyl-glucoside. A fact of some technical interest is the faculty of these six varieties to grow well in koji-extract. Further, some of these varieties grow well in "moto"*-mash, inspite of its acidity which explains very well a kind of injury of the moto-mash.

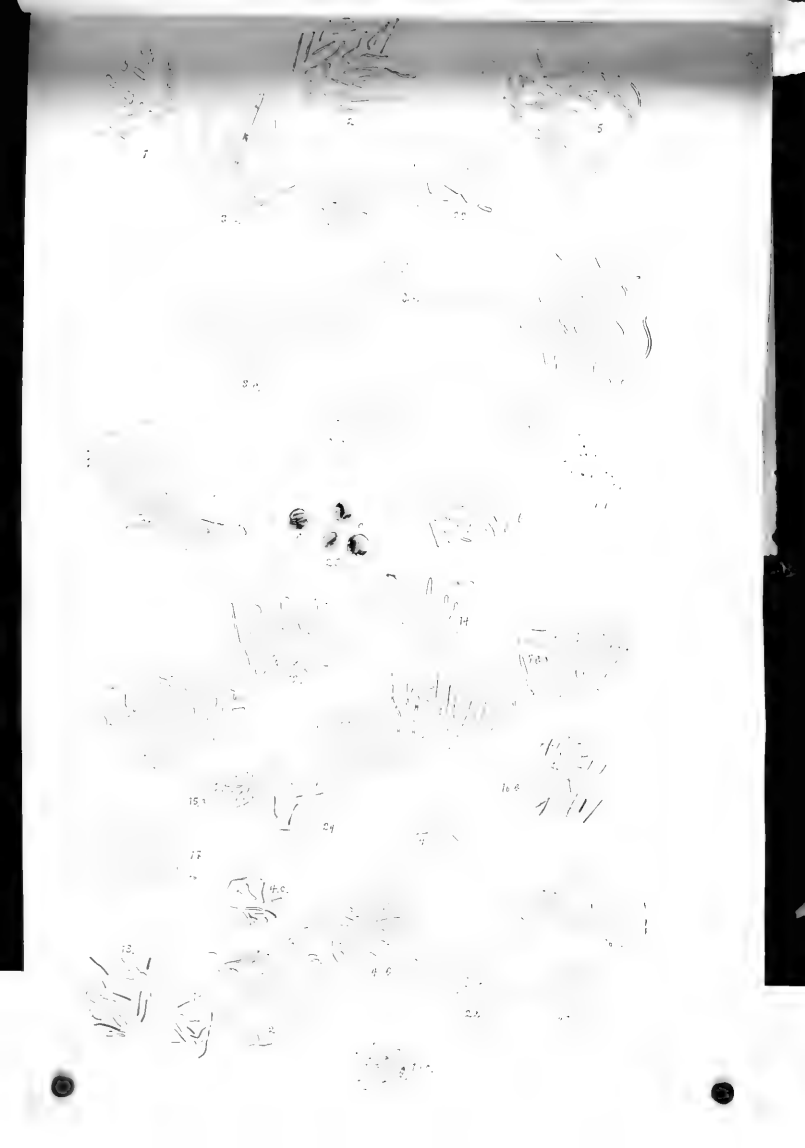
3. The group of acetic acid bacilli found in Hyochi Saké belong to the variety of B. Küttingianum; The resistant power of the group observed, to alcohol of /4.4% is considerable and can develope in Saké of this strength. Even /6.4% of alcohol did not kill yet the microbes of this group in Saké. In the faculty of forming acids from sugars this group excels that of B. Küttingianum.

* This word applies to a koji-mash in which yeast and some microbs have grown.

Explanation to the plate.

1. Bacillus saprogenes Saké I. subvar. 6; 5 days in diluted Saké (1500/I). 2. B. sapr. Saké II. subv. 3; 40 days in diluted Saké (1500/I). 3, a: B. sapr. Saké I. subv. 4; old culture in Saké, stained by methylen blue. (1500/I). 3. b: B, sapr. Saké I; subv. 4; 30 days culture in diluted Saké. 3. c: B, sapr. S. I, subv. 4; 43 days. in diluted Saké. Involution forms. (1500/I.) 4. a: B. sap. S. I. subv. 2. In diluted Saké. (770/I.). 4. b: The same. 2 months culture. (1500/I.). 5. B. sap. S. I. subv. 7: 40 days in diluted Saké. (1000/I). 6. B. sap. S. I. subv. 2. η: 44 days in diluted Saké. (1500/I). 7. a. The same. α: 3 months on Saké agar. (1500/I.). 7. b. The same. : 3 months in diluted Saké (1500/I). 8. a. B. sap. S. II. subv. 3. β: 40 days in diluted Saké (1500/I.). 8. b: The same. (1500/I.). 9. B. sap. S. II. subv. 2: 35 days in diluted Saké (1500/I). 10. B. Aderhordi var Saké: a. Old culture in yeast-water-glucose. (1500/I.). b. In koji-extract. (1500/I.). c. The same 4 months culture (1500/I.). 11. B. Delbrücki. var Saké: In yeast-water-galactose, stained with methylen blue. (1500/I.) 12. B. sap. S. I. subv. 5: 4 months in diluted Saké. (1500/I). 13. B. sap. S. II. subv. 3: 50 days in diluted Saké. (1500/I.) 14. B. sap. S. I. subv. 1: 2 months in diluted Saké. (1500/I.) 15. B. sap. S. II. subv. 8. a: 5 months culture in diluted Saké (1500/I.). b: 5 months culture on Saké-agar. (1500/I.). 16. B. sap. S. II. subv. 9. a: 2 months in diluted Saké. (1500/I.). b: The same; 2 months culture. (625/I.). c: The same; 70 days culture (1500/I.). 17. B. sap. S. II. subv. 1; 50 days in diluted Saké. (1500/I.). 18. B. sap. S. II. subv. 4; 3 months in diluted Saké, stained with methylenblue. (770/I.). 19. B. sap. S. II. subv. 4; 3 months in diluted Saké, stained by methylenblue. 20. B. sap. S. I. subv. 3; 35 days in diluted Saké. 21. A new lactic bacillus 4 months in koji-extract (1500/I.). 22. B. Wortomani. var Saké. 4 months in koji-extract (1500/I.). 23. B. panis fermentati. var Saké. 26 days in diluted Saké. (1500/I.). 24. B. sap. S. II. subv. 5; 2 months in diluted Saké, stained with methylenblue (1500/I.). 25. Colonies of B. sap. Saké. a: Three discs combined. b: Four discs combined.


13.
2
15. e.
16. a.
5
15. a.
24.
16. e.
4. a.
17
4. c.
4. e.
16. c.
18.
19.
20.
22.
21.
10. c.



On the detection of Methyl-lactate.

BY

T. Takahashi.

In a former communication* the writer has described a mode of detection and determination of fusel-oil by means of anisaldehyd and sulphuric acid. The fusel-oil, however, is accompanied in general by certain kinds of esters which fact induced me to apply the same test to the following esters.

Compound.	Coloration at the surface of contact.
Methyl-acetate	Dirty yellow.
Buthyl-acetate	Yellowish to a dirty crimson.
Propyl-acetate	Intensely yellow.
Amyl-acetate	Clear purple-red; below this a yellow stratum.
Amyl-ether	Opaque blood red.
Ethyl-aceto-succinate	Intensely yellow.
Ethyl-succinate	Dirty crimson red.
Propyl-tartrate	Bluish-green layer, below crimson red.
Ethyl-tartrate	Clear yellow.
Methyl-lactate	Intensely bluish-green, blow this a grey-yellow.
Ethyl-lactate	Milky white; below which a yellowwish to crimson red stratum.

* These Bull. Vol. 6. No. 4. p. 437.

Thus the coloration produced by amyl-acetate and methyl-lactate is characteristic, especially that of the latter; hence in order to define the delicacy of the reaction, methyl-lactate in certain dilutions was tested, 3 c.c. of these solutions serving for the test[1].

% of methyl-lactate.

0./%. After 20 minutes a weak but decidedly bluish layer formed.

/.0%. After /–2 minutes a bluish-green stratum.

Methyl-lactate in higher concentration yields on account of the opaque turbidity produced, a less delicate reaction.

Further tests were made with whisky[2] and the distillate of Saké. The result was positive, hence an effort was made to prove the presense of lactate also in another way. A sufficient quantity of $\frac{1}{2}$ normal sodium-hyroxide solution was added to these liquids and after saponification by heating the mixture for 4 hours, methyl-alcohol was found in the destillate, while in the residue lactic acid was identified after Ueffelmann's method. It can therefore safely been concluded that anisaldehyd in conjunction with concentrated sulphuric acid can be applied as a test for methyl-lactate, a bluish green[3] colored stratum being the characteristic reaction.[4]

[1] To the solution contained in the test tube 3 to 4 drops of alcoholic solution of anisaldehyd were added and after shaking well, 3 c.c. of strong H_2SO_4 was run carefully along the side of the test tube forming then the lower stratum.

[2] Sco'ch whisky and Gleviven' whisky; on account of the brown color these liquids were distilled before the test was applied.

[3] The cause of this colorreaction is of course the lactic acid, resp. the group CHOH in it. Indeed free lactic acid gives also a coloration, but this is purplish. It appears that methyl-lactate is more easily split than ethyl-lactate since this latter gives no bluish coloration.

[4] The coloration always occurs above the purple-blue layer (fusel oil layer) so that the observer can distinguish very clearly both substances.

On Changes of Availability of Nitrogen in Soils, II.

BY

O. Loew and K. Aso.

The writers have, in their first communication on this subject[1] called attention to the existence of *bacteriolytic enzyms which probably play a rôle in the soils when the nitrogen of bacteria is rendered available for the roots.* It was desirable to observe whether common soil-bacteria produce such enzyms, as the *B. pyocyaneus* does, the specific enzym of which had been thus far alone be studied to some extent. Sterilised bouillon was inoculated with *B. mycoides, B. megatherium, B. subtilis, B. fluorescens liquefaciens* and *Proteus vulgaris.* The cultures were kept in a thermostate at 20° and were gently shaken almost daily. The development was most rapid with B. fluorescens and after 7 days the bacterial sediment agglutinated to a compact ropy mass while the liquid itself turned very slimy. Only a small residue remained insoluble after several weeks; the slimy character of the liquid did not change further even after 6 weeks. The slowest development was observed with *B. subtilis,* but after 3 weeks a considerable mass of flocculi had developed, which two weeks later were dissolved again, leaving only an insignificant sediment, showing that also this microb produces a bacteriolytic enzym. The solution did *not* become so slimy as with *Fluorescens,* but somewhat less slimy.

B. mycoides and *B. megatherium* formed voluminous flocculent masses, which even after 7 weeks did not decrease in volum; hence bacteriolytic enzyms are not produced by these microbs *when cultivated in bouillon.*

[1] Bul. College of Agriculture, Tokyo, vol. VII. No. 3.

Proteus vulgaris produced no flocculi but an intense turbidity which decreased gradually after five weeks but had not entirely disappeared after six weeks.

The microscopical examination revealed among amorphous particles also bacterial forms in all these cases and the inoculation into sterile bouillon proved that there were still living microbs also in the slimy sediment of Fluorescens and Subtilis. Perhaps they had been protected by the slime from the attack of the enzym which gradually increased in concentration in the measure as the dissolution of the microbs had proceeded.

The dissolving process of bacteria is but the first step which will lead to a production of amidocompounds either by the same enzyms or by other proteolytic enzyms secreted by microbs. If, furthermore, the conditions for the growth of the microbs of putrefaction (*Proteus* and others) are favorable, a further decomposition with production of ammonia can result. When bacteria die off in the soil and are gradually dissolved with a further splitting of their proteins, the roots will no doubt reap a certain benefit from this process but a part of the available nitrogen produced will again be eagerly absorbed by new microbs. Thus probably the bacterial flora changes back and fro with the conditions changing from favorable to unfavorable and back. Moldfungi and certain microbs will also by oxidation destroy the products of dissolved bacteria, but it appears that a certain fraction of the nucleoproteidmolecule offers considerable resistance (perhaps after assuming an acid character by partial oxidation?) and remains unchanged for a long time associated with the humus[2] in the soil.

We have further endeavored to obtain a sufficiently large quantity of bacteriolytic enzym for some special experiments. For this purpose served *B. mycoides* and *B. fluorescens liquefacieus*. Our hope that *B. mycoides*, found unable to produce bacteriolytic enzym in *bouillon*, might form such an enzym in certain other solutions, was realised.

[2] Cf. the communication of S. Suzuki on humusformation, in these Bulletins VII No. 3 and 4, and VIII, No 1.

Three liters of the following culture solution were distributed in six flasks, each of one liter capacity, sterilised as usual and after infection kept in a thermostate at 24°.

The solution first infected contained:

Glycerol	5	%
$NaNO_3$	0.5	„
K_2HPO_4	0.2	„
$MgSO_4$	0.02	„
NaCl	0.2	„
$FeSO_4$	trace.	

Since after one week no growth was observed, 5g. pepton and 0.5g. Na_2CO_3 were added to each flash and after sterilisation again infected. Gradully films developed which were distributed through the liquid daily by shaking. After six weeks two of the flasks developed not further any new film and the liquid assumed a lightbrown color, while the sediment was reduced to a minimum. It was evident that the great masses of bacteria that had grown were mostly dissolved again.[3] The liquid had not assumed any slimy consistency, showed still a weak alkaline reaction and had a weak not unpleasant odor The larger part of the two flasks in which further growth had stopped was now evaporated in vacuo to one fourth of its original volume and to 100cc., placed in a sterilised conical flask, added one gramm of soil from the depth of about one foot of a field that had received organic manures about 4 months previously. Another similar quantity of the concentrated bacterial culture liquid was boiled for a few minutes and after cooling also mixed with one gramm of soil from the same spot.

After one week a very striking difference was noticed: the not boiled liquid was clear, *neither film nor turbidity had developed* and an inoculation in bouillon yielded after 5-6 days only some growth of Bac. subtilis, perhaps originating from spores that resisted the dissolving action of the enzym—while the portion that had been

[3] The sediment contained amorphous and crystalline particles, baterial remnants and but very rarely also barterial rods.

boiled, hence where the enzym was destroyed, developed a *luxuriant film* of microbs and the *liquid became very turbid*. After further four days a development of gas commenced, while even after four weeks there was no change noticed with the *not boiled liquid*[4].

This result leaves no doubt that also soil bacteria can produce a bacteriolytic enzym which gradually renders new bacterial growth difficult. Since this may happen also in the soil, some explanation can be furnished for the fact that bacterial life does not increase infinitely in organically manured soils.

Under certain conditions the nitrogen of the microbs absorbing free nitrogen[5] seems to become rapidly available for the crops, as the *cultivation of rye* on an experimental field near Halle showed. *T. Kühn* observed on that field no decrease of harvest for twenty years during which no nitrogenous manure had been applied. It was calculated that 25—30 kilo nitrogen per ha was annually gained by the azomicrobs. Similar observations were made at the Experiment-Station of Rothamsted (by Hall) and recently also at Cracow (by S. and H. Krzemieniewski). *How important would this be for the entire agriculture if the necessary conditions could be produced in every soil!*

Beijerinck[6] believes that "the protoplasm of *Azotobacter* is easily changed to ammonia." But this change can hardly be due to any thing else but to the action of an enzym, which perhaps also causes the involution of the Azotobacter cells.

An interesting experiment was recently carried out by Heinze.[7] *Mustard* was successfully grown with a mass of Azotobacter as the only nitrogenous manure. This author recommends to work and cultivate the soil very well in order to render the conditions very favorable for

[4] A portion of the not boiled liquid was exposed to air for some time, but no microbs, only a small red Torula, developed.

[5] It might be suggested to call the group of "*microbs assimilating free nitrogen*" simply "*azomicrobs*" for the sake of abbreviation. This name does of course not imply any botanical connection.

[6] Centr. Bl. Bakt. II. Abt. 9, p. 43.

[7] Landw. Jahrb. 1907 p. 910.

an abundant growth of *algae* which can furnish carbohydrates as carbonaceous food for *Azotobacter*.

But it appears that not in every soil the conditions are favorable for a very abundant growth of Azotobacter. Gerlach and Vogel[8] report at least that a special inoculation showed no influence.

Since Azotobacter loses gradually its power of assimilating free nitrogen, when cultivated in media containing assimilable nitrogen compounds it is not surprising that it shows on fallow fields more power of assimilating free nitrogen than on richly manured fields. Thus it may be explained why Azotobacter isolated from certain fields yields not very satisfactory results. A pure culture of Azotobacter derived from an experimental field near Cracow assimilated in three weeks only 1.33 milligrams free nitrogen,[9] in a solution of 4g. mannitol in 200cc. water. In 200cc. of a 1.2% glycose solution was assimilated only 3.2—4.9 milligrams nitrogen. *Crude* cultures assimilated more nitrogen than *pure* cultures, but in the former case always the bulyric bacillus (Clostridium pastorianum) was present, to judge from the odor emitted.

Our own observations agree very well with those made in Cracow. The *microb developing butyric acid can however be avoided* by using *sodium malate* as organic nutrient for the crude culture. Our solution contained:

Sodium malate	0.5 %
K_2HPO_4	0.3 „
$MgSO_4$	0.04
$CaCl_2$	0.02
$FeSO_4$	trace.

After adding 10g of earth from 30cm. depth of a fallow field, a rich development was gradually taking place of Azotobacter but later on also a very fine mycelium developed. For *pure* culture of Azotobacter, however, sodium malate seems not very favorable. We have tried a variety of

[8] Centr. Bl. Bakt. II. Abt. 9, p. 887, Cf. their publications ibid. 8, p. 669 and 10, p. 636.

[9] S. and H. Krzemieniewski, Bull. de l'etcad. Sc. Cracow, July, 1906.

solutions but will mention only, that no growth at all was obtained when potassium oxalate (0.5%) was used as organic substance. This salt had the peculiar effect of dissolving some humus[10] from the earth, probably by mutual decomposition with calcium humate. The dark brown solution thus formed containing potassium humate did not favor the growth of Azotobacter.[11]

In regard to Azotobacter it is a most remarkable fact that it requires lime, as *Gerlach* and *Vogal* had observed. We have made several observations confirming this observation. A mannitol culture solution as well as a sodium malate culture solution without nitrogenous compound were prepared, with and without lime, but growth was only observed where a few drops of a diluted solution of calcium chlorid had been added. This is a remarkable fact since it is an *exceptional case with lower fungi and algae;* should there exist a relation between this fact and the power of assimilating free nitrogen? *Christensen*[12] proposes even to test with Azotobacter the presence of $CaCO_3$ in soils. He observed that that the occurence in different soils stands in close relation to the presence of calcium carbonate and to the basicity of soils.

Whether Azotobacter prepares also a bacteriolytic enzym is not yet decided, but as to the nodule-forming B. radicicola it is quite evident that this does so, especially when cultured in pea leaves extract. After a maximal development a gradual decrease sets in and finally, after 6 weeks at 24° C the liquid is perfectly clear and only a small sediment at the bottom. It is well known that the microbs in the nodules of leguminous plants dissolve gradually; this is due evidently to their own bacteriolytic enzym, after this attains a certain concentration in the surrounding fluid.

Views on the chemistry of assimilation of free nitrogen. The question as to the first product of transformation of the free nitrogen

[10] The fallow field contained still 7% humus.

[11] Since Heinze (l.c., page 909) mentions that humus can be utilised as food by azotobacter it may be that in the above case the exalate acted as a poison.

[12] Centr. Bl. f. Bakt. II Abt. 17 (1906).

in the living cells of the specific microbs is doubtless of profound interest. Altho there exists a great difference in the opinions and no safe ground has thus far been reached, so much is sure that the *chemical energy* of the living matter of those cells is absolutely necessary for the process. But since chemical energy is present in every living cell, there must still, in addition, exist a *special condition* in those microbs whereby the gaseous nitrogen is forced to yield a soluble compound.

Winogradski, Reinke and Stoklasa[13] assume transformation of free nitrogen into ammonia by nascent hydrogen, which would be at once utilized in the process of protein-or asparagin formation. But the supposed development of hydrogen cannot be noticed in pure cultures of *Azotobacter*[14], altho it is observed with those of *Clostridium*.

Gautier and Drouin hold that the free nitrogen is oxidised by the microbs and the nitrous acid or nitric acid thus formed is rapidly changed to other nitrogenous compounds. It may be mentioned that nitrate reaction is sometimes—by no means always—obtained with the nodules of Leguminosæ but this may be due to absorption from the soil when nitrification had been going on. For the oxidation of nitrogen in the laboratory, however, a very high temperature or electric sparks are by no means absolutely necessary, for Ilosway has observed the formation of nitric oxid, when a mixture of nitrogen and oxygen was passed over platinum black at 180C°.

Gerlech and Vogel[15] assume that the free nitrogen combines directly with carbon-compound in the living cells, pointing out the absorption of free nitrogen by calcium carbid as an analogy. But the example seems to us not well suited, as the absorption in this case is more due to the calcium than to the carbon. Heinze[16] entertains the

[13] Stoklasa mentions the occurrence of small amounts of asparagin in the nodules of leguminous plants at a certain stage of development.

[14] S. and H. Krzemieniewski, Bull. Acad. Sc. Cracow, July 1906.

[15] Centr. Bl. Bakt. 9, p. 817 and 881.

[16] Landw. Jahrb. 1906, p. 907. This author also holds that the formation of carbamic acid may result from the assimilation of nitrogen, but this view seems to be identic with that of the formation of ammonia.

same view as those authors and mentions in support of it the formation of hydrocarbons of the acetylene series he observed in the *crude* but not the pure cultures of Azotobacter. Such a process, e.g., might be expressed by the following equations:

$$\begin{matrix} CH \\ \| \\ CH \end{matrix} \quad \begin{matrix} N \\ \| \\ N \end{matrix} = 2CNH$$

Acetylene Freenitrogen Hydrocyanic acid[17]

$$CNH + 2H_2O = HCOONH_4$$

Ammonium formate.

Hydrocyanic acid can of course not be assumed to exist longer than for a moment in the cells on account of its poisonous action.

Our own view holds as most probable the formation of ammonium nitrite, as represented by the following equation:

$$N_2 + 2H_2O = NO_2NH_4$$

which formation is rapidly followed by the reduction of the nitrous acid to ammonia. Indeed nitrous acid is found sometimes in the nodules of Leguminous plants, while we did not succeed yet to show the presence of ammonia in them. However it may be objected that traces of nitrous acid are formed by oxidation process. This view can be supported by the possibility of realising this process at the ordinary temperature by the action of very energetic platinum black upon free nitrogen in the presence of some alkali[18].

As diffrent as these four views are, in one point they agree, the formation of ammonia before the proteinsynthesis commences.

[17] Berthelot has observed the formation of hydrocyanic acid from acetylen and nitrogen under influence of the induction current.

[18] This view was discussed by one of us (L) in the Ber. D. Chem. Ges. 23, p. 1443 (1890) where this action of platinum black was described, which was confirmed by L. Woehler, ibid. 36, p. 3879. The nodules of leguminous plants show often traces of ammonia.

On Observation of the Continuous Growth of Pea on the Same Soil.

BY

Shigehiro Suzuki.

Many observations have been made on the continuous cultivation of clover and pea on the same soil and in this case very often a surprising decrease of yield was obyersed from year to year. This phenomenon called "Kleemüdigkeit" and "Erbsenmüdigkeit" have been very differently explained. K. K. Gedroiz[1] who has made extensive investigation with clover in this direction has especially determined the phosphoric acid content of clover in relation to its development and in comparing the requirement of clover for easily soluble phosphoric acid with that of other plants, he reached the conclusion that at least in certain cases it is the decrease of easily soluble phosphoric acid which causes the above phenomenon, but he admits that there may exist cases in which the phenomenon can be produced by the exhaustion of the soil in regard to potassa. There exist, however, no doubt also cases in which the phenomenon is caused by nematodes[2] and since they increase immensely with each year of culture of pea or clover on the same soil it becomes intelligible why even full and rich manure may often not prevent the extension of the calamity[3]. Since that phenomenon with pea has been very often observed in Japan, the writer has also undertaken to study that phenomenon. For four years peas have been grown in the same soil pots holding 8 kilo of humy loam soil which had not been manured for six years. The manuring and sowing was done between October 22 and December 3. In each pot were grown in the first year 7, in the second 5, in the third 3 and in the fourth 4 pea plants. The result was as follows:—

[1] Russisches Journal für Exp. Landwirtschaft, 1907, Heft. 1, p. 61.

[2] Salfeld: Die Boden-Impfung, 1896, p. 99.

[3] The writer has observed here in Tokyo similar phenomenon with the egg-plant and the examination made it evident that an increase of nematodes was the cause.

	General manure, per pot in g.	No. of pots and treatment of the soil.	Average length of plants.	Weight of harvest, per pot in g.			Remarks.
				Total weight	Fruits.	Seeds.	
First year (1903—1904)	Double superphos....20 Compost 50	All four pots contained a frech soil.	c.m. 120	135.0	72.4	66.2	In air-dry state, average of 4 pots.
Second year (1904—1905)	Potassium sulfate....8.5 Double superphos. ...18 Ammo. sulfate..... 8 Sodium nitrate. 6	I (Fresh soil.)	131	321.5	167.2	—	weighed in the fresh state.
		II („)	137	325.9	169.4	—	
		Average.	134	323.7	168.3	—	
		III (Second years soil.)	135	381.5	188.4	—	
		IV („)	147.5	373.5	192.5	—	
		V („)	147.5	343.5	181.5	—	
		Average.	143.3	366.2	187.5		
		VI (Second years soil, sterilized by steam at 100° C. for 4 hours on consecutive 3 days.)	135	337	180.0	—	
		VII (Second years soil, well washed with water.)	145	370.9	176.9	—	
Third year (1905—1906)	Same as the 2nd year.	I (Fresh soil.)	—	75.0	29.4	26.6	in the air-dry state.
		II („)	—	80.9	32.8	30.1	
		Average.		78.0	31.1	28.4	
		III (Second years soil.)	—	94.0	34.7	31.4	
		IV („)	—	96.9	39.2	35.8	
		Average.		95.5	37.0	33.4	
		V (Third years soil.)	—	107.4	42.4	39.0	
		VI („)	—	103.7	40.7	37.0	
		VII („)	—	111.4	43.4	39.6	
		Average.		107.5	42.4	38.5	
Fourth year (1906—1907)	Manured with Potassium sulfate ...8.5 Superphosph.....45.0 Sodium nitrate 6.0	I (Fresh soil, manured.)	—	57.8	33.8	30.7	in the air-dry state.
		II („)	—	63.2	34.5	32.0	
		Average.		60.5	34.2	31.4	
		III (Third years soil, manured)	—	74.2	40.5	37.2	
		IV (Fourth years soil, manured)	—	86.5	50.5	46.5	
		V („)	—	78.0	46.0	42.0	
		Average.		82.3	48.3	44.3	
	No manure.	VI (Fresh soil, unmanured.)	—	2.7	1.0	0.8	
		VII („)	—	3.8	1.9	1.8	
		Average.		3.3	1.5	1.3	
		VIII (Third years soil, unmanured.)	—	57.0	39.0	35.0	
		IX (Fourth years soil, unmanured.)	—	47.7	31.5	28.7	

Relative harvest: Total weight of yield on the fresh soil=100.

	Fresh soil	Second years soil	Third years soil	Furth years soil
1903—1904	100.0	—	—	—
1904—1905	100.0	untreated —113.1 steriliged —114.1 well washed with water—114.6	— — —	— — —
1905—1906	100.0	122.4	137.8	—
1906—1907	manured =100 unmanured=100	— —	122.6 1757.6	136.0 1445.1

This result shows that by very rich manuring no trace of "Müdigkeit" appears and what is especially surprising that in the third and fourth year an increase of yield was obtained on the used soil. This may be due to the excess of manure that had not been consumed the year previously. Carefully examination of the rootlets showed that bacteria nodules were very few, while nematodes had not been observd at all. The writer is therefore inclined to agree with the explanations of Gedroiz, Salfeld, Liebscher and other authors.

On the Absorption of Varying Amounts of Lime and Magnesia by Plants.

BY

T. Takeuchi.

It has been observed in numerous experiments carried out at this college that a certain ratio of lime to magnesia is very important for a maximum crop. It seemed of some interest to determine, now, under this condition, lime and magnesia are distributed in roots, leaves and seeds. The leaves of course are always richer in lime than in magnesia, while the seeds in most cases richer in magnesia than in lime; stalks and roots hold the middle between these two extremes. The question arises also how these ratios are changed in leaves, roots and seeds when the ratio of these two bases in the soil changes considerably. In the absorption of nutrients by the roots not only osmotic laws play a rôle, but also the current of transpiration which forces into the root not only more of each nutrient than needed, but also compounds which are not absolutely necessary, as silica, manganese &c. However, an undue increase of lime in the soil will not show, in the same measure, an undue increase of lime in the leaves. It will above all retard the growth of the root and this condition will lead to a retarded growth of the whole plant. It may be that the chief injurious effect of an excess of lime or magnesia tells at first with regard to the roots.

Josef Seissl[1] has investigated the ratio of lime to magnesia in the leaves of various plants in different states of development and has found that e.g. for Gramineæ in two successive years the same ratio of lime to magnesia viz. 1.71:1 and 1.72:1 when the ratio in the soil was 2.13:1

[1] Zeitschr. f. d. landw. Versuchswesen in Oesterreich, 1907, p. 88-101.

and 1.85:1. O. Loew and K. Aso[2] have increased the lime content of a soil from 0.5% to 3.3% (1:6.6) and observed in the stalk of the barley plants only an increase of 1.036 to 1.827 (=1:1.76). The lime had been applied in the form of $CaCO_3$. The relative increase of lime in the stalk was therefore only about $^1/_3$ of that in the soil.

In order to collect further data in regard to this question oats were grown in a soil to which was added so much $CaCO_3$ that the ratio of lime to magnesia was 10:1, while in the control pot the ratio did not differ much from 1:1[3]. The plants were cut when the ears commenced to show. The roots were carefully washed and dried while also the leaves separated from the stem were dried and served also for determination of total ash and further of the lime and magnesia content. The difference in the fresh weight of 10 plants grown per pot of 10 kilo soil was very great[3] viz. as follows:

Lime factor, in the Soil	10 : 1	1.2 : 1
Greatest height cm.	92	108
Number of shoots	32	42
Fresh wt. of stalks and leaves, g.....	106.3	301.0
Dry wt. of roots, g.	4.4	13.5

The great difference in the weight of the roots will account to a considerable extent for the lesser development of the shoots.

		Root.		Leaf.	
Ratio CaO : MgO in the Soil.		10 : 1	1.2 : 1	10 : 1	1.2 : 1
CaO		2.64	1.25	2.26	1.36
MgO		0.71	0.62	0 58	0.55
Crude ash		8.17	9.45	11.97	12.43
In % of the crude ash.	CaO	32.31	13.22	18.82	10.94
	MgO	8.69	6.66	4.85	4.42

[2] These Bulletins, vol. VI. No. 4.

[3] See the article of Kanomata on this question in this Bulletin.

Hence the ratios in the plant are changed as follows:

Ratio of $\frac{CaO}{MgO}$

Soil.	Root.	Leaf.
$\frac{CaO}{MgO} = \frac{1.2}{1}$	$\frac{2}{1}$	$\frac{2.5}{1}$
$\frac{CaO}{MgO} = \frac{10}{1}$	$\frac{3.72}{1}$	$\frac{4}{1}$

It will be seen that the increase of lime from 0.6% to 5% in the soil has led to the relative increase of lime from 1 to 2.1 in the root, and to that from 1 to 1.7 in the leaves.

Further it will be noticed that at the ratio in the soil of CaO: MgO=1.2:1, the root absorbed double as much lime as magnesia while the leaf contained 2.5 times as much lime as magnesia.

By changing that ratio in the soil to $^{10}/_{1}$, the ratio in the root increased only by 1.7/1 in the leaf to 1.5/1. Nevertheless this relative small increase in the organs of the plant sufficed to cause a considerable depression in development.

Gypsum as a Manure.

BY

T. Takeuchi.

Gypsum is generally considered to be only of subordinate value as a *direct* mineral food for plants and comes as such more into consideration as a source of sulphur than as a source of lime, since soils may sometimes contain mere traces of sulphate, while in regard to lime this is but rarely the case. As a source of lime, however, gypsum can occasionally also become important for instance when on poor sandy soils bonedust is applied as phosphatic manure, since calcium carbonate would in this case, depress the availability of phosphoric acid, while gypsum will not[1].

The action of gypsum is according to various authors chiefly an *indirect* one and consists in unlocking in the soil of certain nutrients as magnesia, soda, potassa and probably ammonia. It acts in this way more decisively than burnt lime or carbonate of lime do.

In various reports further has been stated that the harvested plants manured with gypsum showed a relative increase of water and proteïn, and in one case also of fat; however, an absolute increase of the total harvest was not always observed[2].

It is remarkable that certain plants are favorably affected by gypsum on the same soil on which other plants are injured by it. A favorable action has been observed with potatoes by different authors, while for

[1] Cf. Bul. College of Agr., Tokio. Vol. VI. p. 353. Katayama:—Is the availability of phosphoric acid in bonedust modified by the presence of gypsum?

[2] Thus Heiden and Brunner observed with clover a decrease of harvest but an improvement of quality. (Heiden, Düngerlehre, p. 762.)

clover contradictory reports exist. Schneidwind and Ringleben[3] observed on the same soil a very favorable action on potatoes while an unfavorable one on clover and grass. Fleischer[4] who had applied on a muck soil (Hochmoorboden) 8000 kg. lime per ha. with good effect, observed that on the same soil gypsum (647—5180 kg. per ha.) exerted an injurious action upon pea and clover, while potatoes, rye and oats were favorably influenced. Heinrich observed a decrease of the crop of the yellow lupin of 36 per cent after adding as much as 0.5 per cent gypsum to the soil and Ulbricht by adding only 0.011 per cent gypsum observed a gain of 21.5 per cent of buckwheat, while with red clover and with timothy a gain of only 1.5 per cent was noted.

The question why gypsum can prove very beneficially on one soil, while on another soil not, has generally been explained by assuming that in the latter case nutrients were not present in such a state that they could be unlocked by the action of gypsum. *But it is by no means certain that in all those cases in which gypsum had acted favorably, it was due to its being a source of sulphur or to its unlocking faculties*[5]. There may exist still other reasons for the favorable action of the gypsum in certain cases as, e.g. might be observed with sandy soils very poor in lime but sufficiently provided with other nutrients in an easily available state. The question is here, whether gypsum would act favorably as a source of lime, since a relative excess of gypsum on light sandy soils is not so much to be feared as an excess of carbonate of lime, its availability only depending upon the amount of water in the soil[6] and not upon the acidity

[3] Landw. Jahrb. 1904. No. III.

[4] Landw. Jahrb. 1891. p. 555.

[5] Heiden, Düngerlehre. p. 779. "Förneren Versuchen bleibt es vorbehalten, das bis jetzt als feststehend zu Betrachtende weiter auszubauen, die Richtigkeit desselben zu bestätigen, resp. an Stelle des einen oder andern Besseres zu setzen und noch Neues hinzuzufügen." Since Heiden wrote this (1887), no other specific actions of gypsum had come to discussion.

[6] Cf. Bul. College of Agr., Tokio. Vol. VI. p. 335. Loew and Aso:—On the different degree of availability of plant nutrients. Barley grown on soil to which 5% of $CaCO_3$ was added contained more lime than on this soil to which 5% of $CaSO_4$ was added.

of the soil and rootlets as in the case of carbonate of lime. Hilgard applied gypsum with great success on the alkaline soils of desert tracts in the Pacific States. *In these cases the alkaline carbonates of the soil, chiefly sodium carbonate, were transformed into sulphate, while gypsum was transformed into calcium carbonate and the soil thus turned neutral.* Now a similar condition exists in a soil manured with sodium nitrate, since sodium nitrate will gradually pass into sodium carbonate which may do harm by its alkaline reaction.[1] In such a case gypsum should prove of value. Should nitrate of soda have been applied repeatedly on a soil *relatively rich in lime* and poor in magnesia, then gypsum would have to be avoided as a corrigens of alkalinity in the soil, because of the further increase of lime. In this case magnesium sulphate would be the proper remedy and serve two purposes at the same time, viz. it would correct the alkalinity of the soil and at the same time diminish or counteract a depressing effect of the relative excess of lime. Of course the soda of sodium nitrate will pass to some extent into the plant body and another portion will be lost by drainage and only a certain part will remain as carbonate in the soil. Hence the amount of gypsum or of magnesium sulphate need not be applied in equivalent quantities to the nitrate but much smaller quantities will suffice.

An experiment with pea and barley was recently carried out by Ishikawa in this college. He applied intentionally a very large amount of gypsum and as a source of phosphoric acid the crystallized disodium phosphate. Four Wagner's pots each holding 8 kilo soil received the following manure, each:

7 g. Disodium phosphate, cryst.
10 „ Sodium nitrate.
5 „ Potassium sulphate.

[1] In cases in which sodium nitrate was applied jointly with superphosphate on a soil free from carbonate of lime, an alkaline reaction would probaly be prevented by the superphosphate.

The alkaline reaction may not only cause a direct damage to the root but also an indirect one in so far as the crumble structure of the soil is transformed into compact structure, thus diminishing the permeability and aëration of the soil.

Two pots received 80 g. gypsum each (=1%). Seeds of pea and barley were sown Nov. 6 and the young plants thinned on Nov. 22 to 6 in the case of pea and to 11 in the case of barley. It was noticed that in the young stage of the plants the leaf surface of pea in the gypsum pot was a little larger than that in the control pot, and the leaves of barley were broader than those in the control pot; further the color of the leaves was of a deeper green in the gypsum pots. Finally also the flowering-and harvest-stage set in earlier 4 days with the gypsum plants than with the control plants. The result observed with the harvest (air dry) was as follows:

Barley.

	Control.	Gypsum.
Number of ears	34	47
Weight of ears...	69.0 g.	82.0 g.
Weight of grains	58.5 „	69.5 „
Total weight	120.0 „	145 0 „
Relative harvest of grains	100	118.8

Pea.

	Control.	Gypsum.
Number of pods	63	75
„ „ seeds	235	240
Weight „ pods	57 5 g.	52.5 g.
„ „ seeds	51.5 „	47.5 „
Aver wt. of one seed	0.219 „	0.192 „
Straw...	30 0 „	27.0 „

Hence, under the conditions of this experiment a favorable action of gypsum on barley but not on pea had taken place. This might have been due to an insufficient manuring with phosphoric acid, since in the case observed by Muramatsu a favorable action of gypsum on pea was

observed; phosphoric acid was in this case supplied nearly in the double dose as here, furthermore the amount of gypsum per pot was much smaller.

I have therefore modified Ishikawa's experiment with pea in the following manner.

The general manure consisted in:

6.25 g. K_2SO_4
15.00 „ $NaNO_3$
8.00 „ $CaHPO_4 + 2\ H_2O$[8],

for 10 kg. soil and 25 g. gypsum was added to one pot but not to the check pot. At the same time a control experiment was started, in which the sodium nitrate was replaced by its equivalent amount of ammonium sulphate (=12 g.). Also here one pot received the same amount of gypsum as above (=25 g.) and another no gypsum. The amount of potassium sulphate was the same. In this control experiment a neutralising action of the gypsum could not come in consideration, as the ammonium sulphate would leave an acid reaction in the soil.

Each pot was prepared in duplicate and the soil was taken from a field that had not been manured for 7 years. Twenty pea seeds were sown in each pot Oct. 23 and the young plants when about 18 cm. high were reduced Dec. 3 to 8 per pot, all of equal size, as far as possible. They were cut May 29 and weighed after drying with the following results:

1st Series.	$(NH_4)_2SO_4 + CaSO_4$	$(NH_4)_2SO_4$	$NaNO_3$	$NaNO_3 + CaSO_4$
Wt. of Straw	37.0 g.	38.7 g.	37.5 g.	43.2 g.
„ „ pods	23.2 „	28.8 „	25.8 „	30.5 „
„ „ seeds	19.0 „	25.5 „	23.2 „	26.9 „
Total weight	60.2 „	67.5 „	63.3 „	73.7 „
Number of pods	47	52	39	45

[8] This precipitated dicalciumphosphate contains nearly double the amount of phosphoric acid as equal quantity of crystallized disodium phosphate.

2nd Series.	$(NH_4)_2SO_4$ + $CaSO_4$	$(NH_4)_2SO_4$	$NaNO_3$	$NaNO_3 + CaSO_4$
Wt. of Straw	38.5 g.	41.5 g.	40.0 g.	45.0 g.
„ „ pods	24.7 „	26.5 „	28.0 „	32.2 „
„ „ seeds	21.5 „	24.8 „	24.5 „	28.5 „
Total weight	63.2 „	68.0 „	68.0 „	77.2 „
Number of pods... ...	44	49	42	46

This result shows that gypsum had a good effect where $NaNO_3$ was used and this beneficial action can in this case only be explained by the neutralising power it exercised, changing the alkaline sodium carbonate produced into the neutral sulphate of sodium while a corresponding amount of gypsum was transformed into calcium carbonate. On the other hand gypsum had depressed somewhat the yield when applied in conjunction with ammonium sulphate. This depression amounted to 8-10%, while the increase gypsum caused when sodium nitrate had been used, was=14—16%.

In a further experiment of my collegue Muramatsu an exhausted loamy soil served for the same test; it was manured partly with gypsum, partly not and while 3 pots (Series A) received acidic, the other 3 (Series B) received alkaline manure. The general manure per pot of 10 kilo was besides 5 g. potassium sulphate:

Series A. acidic	Double superphosphate (41%, P_2O_5)	6 g. = 2.46 g. P_2O_5.
	Ammonium sulphate	10 g. = 2.12 g. N.
Series B. Alkaline	Na_2HPO_4. $12H_2O$	12.4 g. = 2.46 g. P_2O_5.
	$NaNO_3$	12.1 g. = 2.12 g. N.

The pots A_1 and B_1 received gypsum, while two pots A_2 and B_2 each 4.4 g. crystallised $Na_2SO_4 + CaSO_4$ and 2 pots A_3 and B_3 *no gypsum* and no sodium sulphate. 18 pea seeds of equal size were sown Nov. 10 and the young plants reduced to 12 per pot of equal size (15 cm.)

on Jan. 9. The plants were cut May 25 and weighed in the air dry state, with the following results, g.:

Acidic manure	Seeds	Straw	Ratio of total wt.
A_1 with gypsum	30.9	15.8	100.0
A_2 with gypsum + Na_2SO_4 ...	31.3	18.5	106.6
A_3 without gypsum	34.9	18.7	114.8
Alkaline manure	**Seeds**	**Straw**	**Ratio of total wt.**
B_1 with gypsum	44.6	23.7	100.0
B_2 with gypsum + Na_2SO_4 ...	38.5	21.0	87.1
B_3 without gypsum	33.1	16.5	72.6

We observe, therefore, *gypsum has depressed the yield with the acidic manure, while it has increased the yield with the alkaline manure,* which agrees with the result obtained in the first experiment above mentioned; further the weak alkaline manure was in itself more favorable than the acidic manure. Nodules were found only very few in both cases, but this was due probably to the presence of nitrogenous compounds in the manuring mixture.[9]

Some further tests in this line seemed however desirable, and in the next experiment was tested whether gypsum would exert a beneficial effect when applied in conjunction with "lime-nitrogen."

In one case the manure consisted in (10 kilo pot):

A_1 { $CaHPO_4 + 2aq$ ... ...10g. ; $NaNO_3$ ... ...12 „ ; Wood ash ... ... 50 „ } A_2 ... ... A_1 + 20 g. gypsum.

[9] Wohltmann has shown that various leguminous plants do not form nodules or only very scantily, when the soil has received nitrogenous manures.

In another case the following manure was used:

B_1	$CaHPO_4 + 2aq$ ... ... 10 g. CaN_2C[10] ... ... 10.3 „ Wood ash ... ... 50 „	B_2 ... ... B_1 + 20 g. gypsum.

Both these mixtures, A and B, would be weak alkaline manuring mixtures.

20 seeds of oats were sown April 10 and reduced to 8 per pot all of equal size May 2, while 8 beans were sown April 14 and reduced to 4 per pot on the same day with oats. They were cut June 19 and weighed in the fresh state with the following numbers:

Beans.

	Average ht.	Number of stalks.	Total wt.	Wt. of roots (dry matter).
CaN_2C	74	9	163.2 g.	4.2 g.
„ + gypsum ...	77	12	173.0 „	4.5 „
$NaNO_3$	81	7	153.2 „	4.0 „
„ + gypsum ...	83	10	154.5 „	3.8 „

Oats.

	Average ht.	Number of stalks.	Total wt.	Wt. of roots (dry matter).
CaN_2C	98	21	163.0 g.	9.5 g.
„ + $CaSO_4$ ...	103	22	164.8 „	9.8 „
$NaNO_3$	95	19	140.5 „	6.5 „
„ + $CaSO_4$ ...	100	21	156.5 „	5.9 „

The differences in these harvests are not decisive, perhaps because the alkalinity of the manuring mixtures was much less marked than in the above case of Muramatsu where Na_2HPO_4 had been used.

[10] Our sample of commercial calcium cyanamid contains 19.2% nitrogen, hence the amount, equivalent to 12 g. $NaNO_3$. (=1.98 g. N.

A further experiment was made with gypsum in order to test its influence on the availability of tertiary phosphates when sodium nitrate is applied as a nitrogenous manure. This salt depresses the availability of phosphoric acid of bone-dust and of phosphatic rocks, as Prianischnikow and Söderbaum have shown, because of the alkaline reaction produced after decomposition of the nitrate. Gypsum should act here in the opinion of the writer indirectly very beneficially by neutralising the sodium carbonate produced in the soil from sodium nitrate.[11] A very interesting case was observed by S. Suzuki.[12] The pots had been manured with potassium sulphate, sodium nitrate and with disodium phosphate. Some pots had received an addition of gypsum. Upland rice, 11 plants per pot, was grown. We extract from his table the following data, since they show the highly beneficial effect of gypsum in cases of alkalinity of soils:

Addition of phosphate and gypsum.	Relative harvest, taking the yield in the check pot as 100.	
	Total harvest.	Seeds.
No phosphate	100.0	100.0
Disodium phosphate	165.7	192.7
$Na_2HPO_4 + CaSO_4$	201.2	334.1

In the next experiment of the writer were applied steamed bone-dust and woodash (with 12% K_2O), both equivalent to the amounts of P_2O_5 and K_2O used in the first experiment above described; also the amount of sodium nitrate was the same. To the main pot 25 g. gypsum were added, while no gypsum to the check pot. The general manures for 10 kilo soil, which was the same as in the above experiments, was:

[11] Söderbaum obtained 25% more harvest of oats by replacing in a mixture of bonedust with sodium nitrate, the latter by ammonium nitrate (the amounts of nitrogen in both cases being equal); Landw. Versuchs-Stationen. Bd. 63, 247. Cf. Prianischnikow's experiments, ibidem, Bd. 56, 107 and Bd. 65, 23.

[12] These Bul. Vol. VI. No. 4. 1905, p. 347.

28.16 g. Wood ash,
15.00 „ Sodium nitrate.
9.12 „ Bonedust.

For the experiment served pea. 20 seeds were sown on Oct. 30. Later on the young plants were thinned to 8 per pot, at the height of 18 cm. (Dec. 8).

They were cut May 29 and after drying weighed; the results were as follows:

	Wt. of Straw, g.	Wt. of pods, g.	Wt. of seeds, g.	Total weight, g.	Number of pods.	Ratio.
Gypsum 25 g.	36 5	30.5	27.5	66.0	36	100.0
No gypsum	32.5	24.2	21,5	56.7	41	85.9
No gypsum	28.5	25.5	23.0	54.0	42	81.8

This result also shows that gypsum is applied with much increase of yield when the manure has or produces an alkaline reaction in the soil.

Gypsum as an antitode for an excess of magnesia in the soil. A further beneficial action of gypsum may consist in the overcoming of an injurious excess of magnesia in soil or manure. In order to obtain some information in this regard experiments were made with spinach and oats cultivated in pots containing 8 kilo exhausted loamy soil[13], manured per pot with:

5 g. K_2SO_4.
12 „ $NaNO_3$.
6 „ $CaHPO_4 + 2aq$.

While one pot received no further addition, all the other pots received 0.2% artificial magnesium carbonate, a basic carbonate known under the name of magnesia alba.[14] The special additions consisted in:

[13] The loamy soil applied contained in the fine earth < 0.25 m.m. 0.5% MgO and 0.6% CaO soluble in HCl of 10%.

[14] Magnesia alba is much more available than magnesite and some other magnesium compounds; the above dose would be agronomically equivalent to about 8-10 times the amount of magnesite.

0.5 % $CaSO_4$
2.0 % „
0.5 % $CaCO_3$
1.0 % „
2.0 % „

10 seeds of spinach were sown Nov. 9 and the young plants later on reduced to 8 per pot. Gradually a very striking difference became evident. While the gypsum acted very favorably, the carbonate of lime acted injuriously, in fact these plants remained stationary after reaching 3-5 cm. in height; some of the plants even became yellow and died. The plants were cut March 20 and weighed in the fresh state, with the following result, g.:

		Total harvest, g.
A.	Original soil	177.5
B.	Magnesia alba 0.2%	113.3
C.	„ „ „ + 0.5% $CaSO_4$	175.6
D.	„ „ „ + 2.0% „	187.0
E.	„ „ „ + 0.5% $CaCo_3$	15.5
F.	„ „ „ + 1.0% „	1.5
G.	„ „ „ + 2.0% „	1.3

This result shows that the moderate dose of magnesia alba had depressed the yield considerably (36%) and that *lime in the form of gypsum had restored the original productive state while the lime as carbonate had depressed the yield* in a degree as never had been observed before with any other plant in any of our numerous experiments on the lime question.[15] An experiment with spinach in sandculture had been made a year ago by Namikawa to determine the lime factor $\frac{CaO}{MgO}$ for that plant which was found =1. In that case not such an enormous depression was noticed with the increase of carbonate of lime, probably on account of ammonium nitrate having served in this case as source of nitrogen. Our result above mentioned may therefore be connected

[15] Cf. Maki and Tanaka. These Bul. Vol. VII. No. 1, p. 61.

partly with the reaction of the manure. In our case nitrate of soda served as source of nitrogen, a physiologically alkaline compound whose alkalinity was corrected in pots C. and D. by gypsum, but not by carbonate of lime in pots E, F and G. This circumstance would perhaps not have influenced so enormously the result, if the root hairs would secret a certain degree of acidity; but in regard to spinach it seems very probable that the root is of too weak acidity and in this case its absorptive power would be very much depressed by alkalinity of the manure.

The writer has consulted various books on cultivation of special crops but nowhere he was able to find any information on injury to spinach by liming the soil. Hence in order to collect further information on this point a new series of experiments with the same soil were made, containing in the general manure the same amounts of potassium sulphate and secondary calcium phosphate ($CaHPO_4+2aq.$) as in the first case, but in place of the 12 g. sodium nitrate the equivalent amount of ammonium sulphate (=9.3 g.). The further additions were:

A_2 Original soil.
B_2 0.2% Magnesia alba.
C_2 „ „ „ + 0.8% Gypsum.
D_2 „ „ „ + 0.5% Carbonate of lime (equiv.)

In a third series the nitrogen was applied in the form of ammonium nitrate in equivalent amounts (=5.63 g.), otherwise all conditions were the same as in the second series.

15 Spinach seeds were sown per pot April 16, and the young plants reduced to 8 of equal size on May 4. During the month of May considerable differences in development were noticed, calcium carbonate depressing the development also in both these series, altho not so much as in the first case. The plants were cut[16] June 12 and weighed in the fresh state with the following results:

[16] The plants of these two series were cut at a younger stage than those of the first; besides the former developed flowers, the latter had not.

Second Series.

	Average height, cm.	Total weight, g.	Ratio.
A_2	21	47.5	87.1
B_2	19	40.0	73.4
C_2	24	54.5	100.0
D_2	11	12.0	20.1

Third Series.

	Average height, cm.	Total weight, g.	Ratio.
A_3	19	34.5	91.3
B_3	16	32.0	84.7
C_3	18	37.8	100.0
D_3	6	4.5	12.0

The beneficial action of gypsum became here again very marked. It might be supposed that the manure of weak alkaline character depressed the availability of phosphoric acid. But it has been found by various authors that the availability of the phosphoric acid of dicalcium phosphate is not depressed by liming the soil, further Söderbaum[17] has also compared the availability of various phosphates when the nitrogen was offered as sodium nitrate as well as a mixture of sodium nitrate with ammonium sulphate, and found the depression only between 5 and 8%. Hence the alkalinity in itself must have acted injuriously on the activity of the living cells of the root.

The need of spinach for lime seems not very great as may be followed from the following analytical data taken from Wolff's Tables of plant-ashes Vol. I. p. 101. The total ash amounts in average to 16% (of the dry matter).

[17] Landw. Vers.-Stat., Bd. 63, p. 252.

In 100 parts of ash were found:

K_2O...	...23.4— 9.69;	MgO...	...7.4— 5.2;	SO_3...	...4.4--9.3;
Na_2O	...31.4—39.1;	Fe_2O_3	...2.1— 4.6;	SiO_2	...3.1—5.8;
CaO ...	...10.6—13.1;	P_2O_5	...8.5—11.9;	Cl ...	...4.8—7.7;

An experiment with oats was next started Jan. 28 and with the same manure of an alkaline nature as above mentioned with the first spinach experiment. 15 seeds were sown and thinned to 8 per pot of about equal size, Feb. 24. The plants developed at first at about equal rate, but gradually increasing differences were noticed and the condition at the end of May was as follows:

	State of growth.	Average ht. cm.	Number of shoots.
A. Original soil	Developing flowers	93	3
B. Magnesia alba 0.2%	No ears yet visible	81	3
C. „ +0.5% $CaSO_4$ ...	Shows ears	86	3
D. „ +2% $CaSO_4$ ...	Ears just developing	91	3
E. „ +0.5% $CaCO_3$ ...	About like B.	94	2
F. „ +1% $CaCO_3$ ...	Yellowish and no ears	76	2
G. „ +2% $CaCO_3$ ...	Yellowish and no ears	75	2

The plants were cut after the ears had completely developed and weighed in a fresh state, June 10; the results obtained were as follows:

	Average ht., cm.	Total fresh weight, g.	Wt. of root, air dry, g.
A. Original soil...	105	110.0	9.6
B. Magnesia alba 0.2%	101	104.5	9.1
C. „ „ „ +0.5% $CaSO_4$ ...	102	114.0	8.5
D. „ „ „ + 2 „ „ ...	109	132.0	10.2
E. „ „ „ +0.5 „ $CaCO_3$ ...	107	101.0	7.8
F. „ „ „ + 1 „ „ ...	93	80.5	4.5
G. „ „ „ + 2 „ „ ...	92	77.0	4.3

This result shows again that gypsum may be applied with success in soils with a relative excess of magnesia, especially when the total manure led to an alkaline reaction in the soil.

Summary.

Gypsum is a very valuable addition to manure when sodium nitrate had been applied, or more generally when there is any alkaline reaction produced in the soil. If, however, an acidic manure, as superphosphate and ammonium phosphate, had been applied, gypsum has rather a depressing effect.

Gypsum has also a favorable effect in overcoming the injurious action that a certain relative excess of magnesia can exert on the plants.

Spinach is injured considerably by carbonate of lime, but not by sulphate; provided the reaction of soil and manure is not acid.

On the Depression of Growth by Large Doses of Lime.

BY

C. Kanomata.

Depressions in harvest by liming soils have been observed to take place occasionally on poor sandy soils, further when bone dust or phosphatic rock had been used as phosphatic manure. On the other hand it has been observed by various authors, that the availability of superphosphate and of dicalcium phosphate was not depressed by liming the soil. This would again confirm that carbonate of lime does not transform dicalcium phosphate into tricalcium phosphate.

For a series of years, however, it has been shown by experiments at this college that a depression of the harvest by liming can be caused when there is relatively but very little magnesia in the soil. Thus an unfavorable ratio of lime to magnesia may be caussed by excessive liming. In such a case the remedy consists in the application of magnesium salts in order to change the unfavorable ratio again to the favorable one[1]

A case was recently reported by Kraus, in which all the plants, growing on the so called "Wellenkalk" soil in the Würzburg district of Bavaria are characterised by attaining only a very low growth, viz. $^1/_5 - ^1/_{10}$ of the normal size, which phenomenon was attributed by that author to the deficiency of water caused by the looseness of the coarse soil. But this view was not supported by experiment and the real cause might be a deficiency of magnesia. In order to observe the degree of depression caused by a great excess of lime over magnesia,[2] the writer observed

[1] Compare these communication of Maki and Tanaka in these Bulletins Vol. VII. No. 1.

[2] These experiments at this college have thus for only considered a moderate excess of lime over magnesia.

the development of several plant species in sand culture, at a ratio $\frac{CaO}{MgO} = {}^{100}/_{1}$ (Series A) which was compared with a check culture containing the ratio $\frac{CaO}{MgO} = {}^{1}/_{1}$ (Series B)[3].

Each pot held 2.5 kilo of quartz sand and received the following manures, g.:

K_2SO_4	1.0
NH_4NO_3	0.8
$Na_2HPO_4 + 12$ aq.	0.5
$FeSO_4 + 5$ aq.	0.1
Magnesite	0.6

The main pots A further contained 56 grm. of $CaCO_3$ (=28 grm. CaO), while the control pots B only 0.56 grm of $CaCO_3$ (=0.28 grm CaO)[4].

For this experiment served barley, oats, rice, buckwheat, mustard and onion (small variety). 10 seeds of barely were sown Oct. 30th, 1906, and the young plants reduced to three of equal size, when they had reached 10—12 cm.

Since there existed danger from attack by fungi, the plants were cut, Jan. 29th, 1907, and weighed in the fresh state with the following result:

[3] Lime and magnesia were supplied as natural carbonates in the form of the finest powder (< 0.25 mm.)

[4] These data would therefore correspond for 100 parts of sand:

K_2O		0,0202
P_2O_5		0,0039
N		0,0128
FeO		0.0012
MgO		0.0112
CaO	Series A	1,1200
	Series B	0,0112

$\frac{CaO}{MgO}$		Average height in cm.	Number of stalks.	Weight of shoots in grm.
$\frac{100}{1}$	A_1	31.3	30	27.5
	A_2	31.1	28	26.4
$\frac{1}{1}$	B_1	34.2	43	38.9
	B_2	33.5	35	37.2

This result shows that altho the plants were still very young, a considerable depression had already been caused by the excess of calcium carbonate. This depression would have increased later on, to judge from former similar experiments.

The other plants above mentioned were sown in March and April; after five weeks a great difference in height was noticed with buckwheat and mustard. The results are seen from the following table:

Lime factor.	$\frac{CaO}{MgO} = \frac{100}{1}$ (A)		$\frac{CaO}{MgO} = \frac{1}{1}$ (B)	
	Average height, cm.	Fresh weight, grm.	Average height, cm.	Fresh weight, grm.
Buck wheat, 3 plants ...	10.1	3	23.2	17
Mustard, 2 plants	4	2	13	8

In regard to the rice plants there was a yellow coloration of the leaves noticed in the series A, perhaps a case of so called lime-chlorosis but this phenomenon was not observed with oats under the same condition; all oat plants showed a normal deep green. The oat plants were harvested shortly before showing the ears; the result observed were as follows:

Number of plants per pot = 3
Number of shoots developed = 6 in A
= 9 in B
Fresh weight of plants per pot = 20.1 g in A
= 33.0 g in B

The shoots were measured and gave the following figures, cm.

	A	B
	—	136
	—	93
	—	90
	91	70
	89	57
	62	54
	60	53
	51	46
	48	23
Total sum.	401	622

By the excessive dose of carbonate of lime, the weight was therefore reduced by 39% and the total length of shoots reduced by 35%.

It might be here pointed out that this depression would be simply due to a *decrease* of the *availability* of the *phosphatic manure*. But such a view can not be correct, because in our sand culture a *soluble* phosphate (viz. Na_2HPO_4) has been applied, and this salt even after transformation into $CaHPO_4$ would not suffer any loss in the degree of availability. Furthermore it must be considered that high diluted secondary sodium phosphate acts at common temperature only gradually on calcium carbonate with the production of dicalcium phosphate, altho at boiling heat this decomposition is rapid.

The rice plants were harvested June 3 with the following result:

	Series A.	Series B.
Height of plants, cm.	35	62
	36	63
	29.5	61
Color of plants.	yellowish	dark green.
Number of shoots	4	5
	2	6
	3	6
Fresh weight, g.	4.2	20.0[5]

On the onion plants. the following osbervations were made, June 17.

	Series A.	Series B.
Number of living shoots	6	12
Average height of shoots, cm.	7.3	12.3

Various authors might here object that what causes the depression in all these cases there is merely the undue excess of a carbonate and not an unfavourable ratio of lime to magnesia. But that such an opinion is erroneous follows from many experiments made thus far at this college.

A further proof that the depression by the large dose of $CaCO_3$ is not due to this large amount in itself, is furnished by the fact that an addition of so much pulverised magnesite that the ratio $\frac{CaO}{MgO} = {}^{100}/_{100}$ is produced, *has again a favorable effect,* as an experiment with buckwheat had demonstrated. The manure for each pot of 2.5 kilo sand was the same as above mentioned, and both the pots received 56 g. $CaCO_3$; but one of the pots only 0,6 g. magnesite while the other 60 g. The result was that in the latter case the buckwheat plants showed after 3 weeks with the ratio ${}^{100}/_{100}$ the same height (20 cm.) as the buckwheat plants had shown at ${}^{1}/_{1}$ while at ${}^{100}/_{1}$ the plants had reached only 8.5 cm. Hence the ratio ${}^{100}/_{100}$ was far superior to the ratio ${}^{100}/_{1}$. This experiment further shows that buckwheat is not injured by an excess of carbonate of lime in itself, which agrees very well with a former observation

[5] In a former experiment with rice only one shoot was produced when the lime amount was increased to thirty times of that of magnesia and the ripening process was much retarded, phenomena also observed by Mr. Kumakiri (these Bul. VII., No. 1.) at an excess of magnesia.

of T. Furuta,[6] who obtained, by adding to 8 kilo soil 42.6 g. of quick lime corresponding to 76.08 g calcium carbonate, a great increase of harvest, namely an increase from 190 g. in the check pot to 382 g. In a second series, the former pots yielded 240 g. while the check pots only 281 g. harvest of buckwheat. In a recent experiment described in the 'Journal für Landwirthschaft (55, p. 82),' a similar dose of calcium carbonate caused a depression of the yield. But in this case no *determination of the magnesia* content of the soil was made; perhaps this amount was relatively very small and in this case the liming would naturally produce a still more unfavorable ratio of lime to magnesia and hence the depression. The most favorable result with buckwheat in Furuta's case was observed with the ratio $\frac{CaO}{MgO} = {}^{3}/_{1}$, while the check pots showed the ratio ${}^{1}/_{1}$.

Experiment with soil culture.

In another experiment with oats an exhausted loamy soil, containing 0.6% CaO and 0.5% MgO, soluble in 10% HCl, received the following manure per pot, g.

$(NH_4)_2SO_4$	6
NO_3Na	4
Double superphosphate	5
K_2SO_4	4

4 Pots, each holding 10 kilo soil served for the test.

2 pots A received each 786 g. $CaCO_3$, corresponding to 440 g. CaO, which in addition the available CaO already present in 10 kilo soil would make the total amount of CaO=500 g.=10 times that of magnesia, while 2 pots B received only 10 g. $CaCO_3$ more than sufficient for correcting any acidity of the humus present.

15 Seeds were sown in each pot, and later on the young plants were reduced to 10 per pot, all of nearly equal size. After nearly 4 months a very great difference in development was noticeable, see Plate XIV on

[6] These Bulletins, Vol. IV. No. 5.

which the photograph was reproduced. Shortly after developing the ears, May 30, the plants were cut and weighed with the following result (average of 2 pots), g.

Lime ratio.	Greatest height, cm.	Number of shoots.	Fresh weight of stalks.	Dry weight of root.
$\frac{CaO}{MgO} = \frac{1}{1}$	126.5	39	290.5	17.7
$\frac{CaO}{MgO} = \frac{10}{1}$	101.0	28	140.7	8.2

Hence it will be noticed that the increase of the lime content in the soil[7] from 0.6% to 5.0% caused a depression of harvest by nearly 48%. Also here the objection that the availability of phosphoric acid was depressed, would not be permissible.

It was further very interesting to observe the great difference of the development of the roots.[8] The roots were very carefully collected from each pot and well washed in order to remove every visible particles of soil, and well dried.

Another experiment in a similar line and with the same soil had been made at the same time by Mr. H. Yokoyama of this college and from his results the following data are taken:

Manure for 10 kilo soil, g:

Double superphosphate	8
K_2SO_4	3
$NaNO_3$	10
$(NH_4)_2SO_4$	5

15 Seeds of oat sown, reduced later on to 10. Harvested just at the beginning of showing ears.

[7] Also in this case there was no trace of lime chlorosis noticed with oats.

[8] It deserves to be mentioned that the roots from the pot with the excess of lime showed a more intense order of fresh malt than those from the other pot.

	Lime ratio.	Fresh weight from two pots, g.
Original soil	$\frac{CaO}{MgO}=\frac{1.2}{1}$	153.0
Original soil+6g. $CaCO_3$ for 10 kilo...		153.2
Original soil+152g. $CaCO_3$ for 10 kilo.	$\frac{CaO}{MgO}=\frac{4}{1}$	73.0

Finally the result recently obtained by Mr. H. Hamasaki at this College with oats under the influence of different ratios of lime to magnesia may be mentioned[9].

Each pot of 2.5 kilo quartz sand received as general manuring, g

K_2SO_4 1
NH_4NO_3 .. .. 0.8
$Na_2HPO_4 + 12aq.$ 0.5
$FeSO_4 + 5aq.$ 0.05

A... ... { 2 pots received: 4.3 g. limestone ... ... and 5.0 g. magnesite... ... ... } $\frac{CaO}{MgO}=\frac{1}{1}$

B... ... { 2 pots received: 43 g. limestone ... ... and 50 g. magnesite .. .. } $\frac{CaO}{MaO}=\frac{10}{10}$

C... ... { 2 pots received: 4.3 g. limestone ... ... and 50 g. magnesite... ... ... } $\frac{CaO}{MaO}=\frac{1}{10}$

Four oat plants were grown in each pot and the plants harvested at the flowering stage[10] with the following result:

[9] The doses of lime and magnesia were such as occur frequently in soils and by no means abnormously high.

[10] There is very little difference naticeable in the first few weeks of development but later on the differences became more and more pronounced; There can exist little doubt, that at ripening time of seed they would be still greater.

	Lime ratio.	Number of stalks.	Total fresh weight, g.
A	$\frac{CaO}{MgO} = \frac{1}{1}$	14	93.0
		15	93.5
B	$\frac{CaO}{MgO} = \frac{10}{10}$	11	79.5
		12	82.5
C	$\frac{CaO}{MgO} = \frac{1}{10}$	9	56.2
		8	52.5

This result shows that the great depression caused by the increase of magnesite (C.) was considerably diminished again by adding a corresponding dose of $CaCO_3$ (B.) However the original yield at the ratio $^1/_1$ in (A) was not reached again might be due to the smallness of the dose of phosphate applied.

Summary.

When the amount of lime is increased in undue proportion to the amount of magnesia present, the yield of oats is considerably depressed. In sand culture, there was a decrease by 39% of the weight of shoots before flowering time, when the amounts of limestone and magnesite differed so much that the ratio $\frac{CaO}{MgO}$ was changed from $^1/_1$ to $^{100}/_1$.

In soil culture the decrease was 48% some time after the flowering, when that ratio was changed from $^1/_1$ to $^{10}/_1$.

Corresponding observations were made with upland rice, barley, buckwheat, mustard and onion. If by proper increase of magnesia in the over limed sand again the ratio $^1/_1$ is produced, there is again a considerable increase of yield.

These experiments form an analogy to those of Maki and Tanaka who regenerated the over limed soil by application of magnesium sulphate[11].

It is certainly not the absolute amount of magnesite or of limestone which comes in consideration but the ratio of lime to magnesia which determines—ceteris parvibus—the height of the harvest.

[11] These Bulletins, Vol. VII. No. 1.

On the Depression of Growth by large Doses of Lime.

On the Agronomical Equivalent of Artificial Magnesium Carbonate.

BY

S. Kanamori.

It has been shown by various experiments carried out at this college that barley and rice show under otherwise favorable conditions the best development when magnesia and lime are offered to the roots in equal quantities. This observation was made, however, under the condition that both these bases are present in about equal degree of availability.

But it is natural that when this degree of availability is not equal, for instance when the lime compound is difficultly soluble, while the magnesia compound is easily soluble, then the best ratio of lime to magnesia will be very different from the above, since of the more easily available compound also much more will enter the plant than of the difficultly soluble compound. It was therefore necessary to compare the action of magnesite with that of the artificial magnesium carbonate by a practical test.

Even the finest powder of magnesite that can be prepared, consists of relatively compact particles when we compare with it the artificial magnesium carbonate, which latter is an exceedingly loose voluminous product and is much more easily dissolved by dilute organic acids than the powder of magnesite. Therefore the artificial magnesium carbonate added to soil will be much more easily dissolved not only by the carbonic acid of the soil water but also by the acid of the root fibres, and consequently much less of this product will be required than of magnesite when a soil too poor in magnesia has to be enriched with this base.

Various authors have found, however, that equal quantities of artificial and natural magnesium carbonate produce a very different result upon

the vegetation. The harvest was in some cases several times higher with the artificial carbonate than with the natural. On the other hand when relatively large doses were given, the artificial carbonate was found to be much more poisonous than the natural carbonate or magnesite, because a much greater excess of magnesia was absorbed by the plant in the former case than in the latter. The artificial magnesium carbonate is further not identic with the natural. While this latter is the neutral salt=$MgCO_3$, the artificial carbonate is a hydrated basic salt of the formula=$4MgCO_3 + Mg(OH)_2 + 4H_2O$[1].

The experiment of the writer was carried on with a sand culture of barley, each pot containing 2½ Kilo sand, purified with dilute hydrochloric acid. The general manure per pot consisted in, g:

K_2SO_4	1.0
NH_4NO_3	0.8
$Na_2HPO_4 + 12H_2O$	0.5
$FeSO_4 + 5H_2O$	0.1
Finest Powdered limestone	4.3

Two pots A. received so much magnesite (5 g.), as a finest powder, that the amounts of lime and magnesia were equal.

Two pots B received	0.1 g.	Artificial magnesium carbonate.
„ „ C „	0.3 „	
„ „ D „	0.6 „	
„ „ E „	0.9 „	
„ „ F „	1.5 „	
„ „ G „	2.0 „	

[1] The composition, however, is subjected to little differences according to the conditions of the precipitation. While there exists a great difference as to the availability of natural and artificial magnesium carbonate, this is not the case in regard to the availability of natural and artificial calcium carbonate (provided the limestone is applied in an exceedingly fine powder), as experiments with oats in sand culture at this college have shown. There exists also *not such a chemical difference* here, as is the case with the natural and artificial magnesium carbonate.

Six seeds of barley, previously soaked in water, were sown Nov. 15 in each pot. The young plants showed gradually a very great difference in development. The average height was on Dec. 20 as follows, cm:

A = 13.9	D = 8.6
B = 9.5	E = 8.2
C = 12.5	F = 6.0
	G = 4.6

Since there existed danger from attack by fungi the plants were harvest long before flowering on Jan. 29th and weighed in the fresh state.

The observative were as follows.

	Average height, cm.	Number of stalks per pot.	Average fresh weight of a plant.
A. magnesite 5g	29 28	34 35	average = 7.01
Artificial magnesum carbonate. B. 0.1g	25.8 29	24 23	„ 8.95
C. 0.3g	29.9 28.1	33 36	„ 8.73
D. 0.6g	28.5 26.3	31 24	„ 6.03
E. 0.9g	25.6 24.6	23 18	„ 4.05
F. 1.5g	14 11.7	7 9	„ 0.60
G. 2.0g	10.5 11.4	6 8	„ 0.33

A second experiment under the same conditions was made with oats. Six seeds were sown March 4 and the young plants reduced to 3 per pot later on.

The differences in growth and the gradually increasing damage caused by the moderate increase of the doses of *magnesia alba* became also here soon very marked, and will be recognised from the photagraph of some of the pots reproduced on Plate XV.

The plants were cut at the flowering time of the Check pot, and weighed in the fresh state with the following result, g.:

Checkpot : magnesite 5 g.		30.4 35.7
Magnesia alba.	0.1 g.	30.8 30.0
	0.3 „	32.3 29.7
	0.6 „	33.5 34.0
	0.9 „	28.3 22.8
	1.2 „	13.3 11.7
	1.5 „	6.6 1.8
	2.0 „	4.3 2.5

It will be seen from this result, that doses of 0.1—0.6 g. *magnesia alba* were agronomically equivalent to 5g magnesite; a further increase of magnesia alba led to a depression of harvest. The question will be further studied also in relation to seed-production.

On the Agronomical Equivalent of Magnesia Alba.
I. Magnesite 5 g.; II. Magnesia alba 0.1; III. Magnesia alba 0.3; IV. Magnesia alba 0.9; V. Magnesia alba 1.5.

Topdressing with Magnesium Sulphate.

BY

J. N. Sirker. (Calcutta).

Since a high lime content of the soil may cause some depression of the yield in barley and in similar crops it was desirable to be tested by a field-experiment whether magnesium sulphate in small doses would act favourably in overcoming the effects of a relative excess of lime.

The experiment of the writer was carried out on the loamy soil of the college-farm which contains fine earth 70%, CaO 0.6%; and MgO 0.5% easily soluble in HCl of 10%: A plot of land, 50 sq. metres in area, was limed with slaked lime at the rate of 10,000 kilo per hectar and manured later on at the rate of:—

100 Kilo Superphosphate
200 Kilo $NaNO_3$
600 Kilo Kainit
} per hectar

The plot was divided into equal parts, and an equal quantity of barley grains sown on both divisions Dec. 9.

Owing to severeness of winter, the growth of the plants was very slow for nearly three months. With the spring the growth was however quite rapid.

When the plants had reached 25—30 c.m., one half of the plot received a topdressing of 250g crystallised magnesium sulphate i.e. at the rate of 10 kilo per hectar on March 4, 1907.

A perceptible difference in the development of the plants on the two divisions was noticed as early as one week after the topdressing.

The crops were harvested and weighed in the fresh state June 15, with the following result:

(a) Limed plot 10370g
(b) Limed plot, topdressed with mgnesium sulphate ... 13660g } as 100 : 131.7

This result shows that a topdressing with a small quantity of magnesium sulphate at the rate of 10 kilo per hectar had the favourable effect of increasing the harvest by 31.7% on a plot too rich in lime relatively to magnesia.

Why are Poor Sandy Soils Often Easily Injured by Liming?

BY

H. Yokoyama.

It is a fact frequently observed that poor sandy soils containing but little lime are much more easily damaged by liming than similar soils richer in lime. Some authors believed that useful soil-bacteria might have been killed in such a case while others ascribed injury to the depression of the availability of phosphoric acid. The latter explanation however can only be admitted when bone dust or phosphorite had been used as manure on that soil, while the former assumption has not yet been proved. If, however, soils naturally poor in lime are poor in magnesia also, what frequently happens, then a simple explanation may be given.

Let us compare such a soil with one moderately rich in lime and magnesia and apply to both the same amount of lime. Then the poor soil will show now a much more unfavourable ratio of lime to magnesia for Gramineæ and other plants than the latter soil will. An experiment on oats was made to illustrate this case. Well purified quartz sand was mixed with so much finely pulverised limestone and magnesia that it contained 0.04% CaO and 0.04% MgO. Four pots each holding 2.5 Kilo sand were filled with this mixture. Another series of four pots were prepared, containing 0.2% CaO and 0.2% MgO, i.e., five times as much of those bases as the pots of the first series. Two pots of each series received now equal doses of precipitated calcium carbonate namely 0.3%=0.16% CaO, therefore with the sand poor in lime and magnesia the ratio CaO: MgO=5:1 is reached, while with the sand moderately rich in lime and magnesia the ratio CaO: MgO=1.8:1. Two pots A

received 1g CaO=1.7g limestone and 1g MgO=2.1g magnesite respectively, representing the poor sandy soil, while two pots B, representing the richer soil in regard to lime and magnesia, received 5g CaO=8.2g $CaCO_3$ and 5.5g MgO=10.5g $MgCO_3$.

Two pots A, prepared like A, received further 4g CaO=7.1g $CaCO_3$, and two B, prepared like B, the same dose.

All eight pots received the same general manure, namely;

K_2SO_4 .. .. 1.0g
NH_4NO_3 .. .. .8g
$Na_2NPO_4 + 12H_2O$ 5g
$FeSO_4 + 5H_2O$ 2g

Seeds of oats were sown, ten in every pot, Febr. 28, and when the young plants had reached about 14 cm in height, they were thinned, leaving five plants of nearly equal size per pot. The development appeared for a certain time to be about equal in all the eight pots, later on, however, some difference was noticeable, in regard to height and intensity of the green color.

The plants were cut in the flowering stage, June 4, and weighed in the fresh state; the result is seen from the following table:

	Ratio of CaO : MgO	Color.	Number of stalk.	Average hight, cm.	Total fresh weight, g.	Ratio of green harvest.	Weight of root dried at 100°	Ratio of dried root.
A	$\frac{0.04}{0.04} = \frac{1}{1}$	Dark green	8	98.2	77	100	8.5	85
A			7	103.0	80		10.0	100
B	$\frac{0.20}{0.20} = \frac{1}{1}$	Dark green	9	102.1	72	89.8	7.2	72
B			8	92.1	69		7.0	70
A_1	$\frac{0.04}{0.04} + 0.16_{CaO} = \frac{5}{1}$	light green	6	83.6	53	71.9	4.5	45
A_1			6	86.9	60		5.2	52
B_1	$\frac{0.20}{0.20} + 0.16_{CaO} = \frac{1.8}{1}$	Dark green	7	89.3	63	82.8	6.5	65
B_1			7	96.3	67		7.0	70

It will be seen that the liming of the sand poor in lime has led to a greater depression than the liming of the sand rich in lime. Since the lime was applied as carbonate and further since the phosphatic manure was a soluble salt and the formation of tricalcium phosphate was impossible, there remains no other possibility as to the cause of the depression, than to ascribe the result to the unfavorable ratio of lime to magnesia created by the liming on the poor sand, as the same dose of calcium carbonate produced here the ratio CaO: MgO: 5.1 which in the rich sand produced the ratio 1.8:1. The former ratio is much less favorable than the latter.

It must therefore be infered: Soils poor in lime and magnesia should only be limed with *dolomitic limestone*. Thus the production of an unfavorable ratio of lime to magnesia would be avoided.

On the Composition of Rice-Straw.

BY

T. Takeuchi.

There exist thus far no detailed analyses of rice straw and not even an ash analysis is found in the great work of Wolff on "Aschen Analysen." But there exist some partial analyses. E. Wolff[1] found in air dry rice straw,

Dry matter	85.6%
Proteïn	5.6%
Crude fat	2.0%
Nitrogen free extract	28.8%
Crude fibre	36.4%

Further analytical data were published by Kellner in conjunction with Kozai, Mori and Nagaoka[2] and recently also by Galli who found in rice-straw 0.77% N, while other analyses gave 0.65% N. Galli found further in rice straw 0.25% P_2O_5, 1.88% K_2O, and 0.81% CaO. There may exist some considerable differences in regard to the composition of other kinds of straw, inasmuch as the rice plant requires much longer time for maturity than wheat or barley (from germination in April until harvesting in middle of November, i.e. 30 weeks), further since the paddy rice grows in swampy soil it will no doubt also contain a very considerable amount of silica like the Cyperaceæ and Juncaceæ do. A point, however, of special interest would be to compare the rice-straw from years of very poor harvest with straw from a very rich harvest. It is

[1] Mentioned in Wender, Landw. Chemie, p. 243.

[2] These Bulletins, Vol. I., and Landw. Vers.-Stat. 41, (1892).

very well known that when no seed is produced, for instance when the flowers of maize are cut off, that in the stalk can accumulate a considerable amount of cane sugar which otherwise under normal condition would have been deposited in form of starch in the seeds. It has been observed further that farmers in this country in times of a very poor harvest of rice-grains, cut the lower portion of the stalks into small chips and mix it wtih flour bake it and consume this product as a food. It is easily to observe sometimes in the lower portions of such rice-stalks some substance which gives the red iodine reaction for erythrodextrin. From these points of view the writer compared the rice-straw from stalks that bore a rich amount of seeds with rice-straw of very poor harvest. Attention was paid to the determination of cane sugar, reducing sugar, starch and pentosans; further crude protein, crude fat, crude fibre and ash were compared in both cases.

The result of the analysis was:—

In % of air dry matter.	Straw from favorable harvest.	Straw from poor harvest.
Hygroscopic water	12.31	9.85
Dry matter...	87.69	90.15
Total nitrogen	0.97	1.48
Crude protein	6.05	8.82
Crude fat	1.36	1.65
Crude fibre...	31.16	28.72
Crude ash	11,42	12.35
Silica	5.39	6.13
Dextrose	2.25	3.28
Cane sugar...	0.79	0.96
Starch+Hemicelluloses	14.86	18.75
Pentosans	14.28	16.55

A comparison of the silica contents with that of other Gramineæ-straw is of some interest.

Percentage of SiO_2 in the dry matter of Gramineæ-straw.

Rice.	From Wolff's tables:			
	Summer wheat.	Barley.	Oat.	Maize.
6.15	2.12	2.73	3.36	1.54

Conclusion.

In comparing the results obtained, it is found that the straw from rice plants of a poor seed production is somewhat richer in protein, fat and carbohydrate than the straw at a favorable harvest of grains.

On the Behavior of Algae to Salts at Certain Concentration.

BY

T. Takeuchi.

Between the concentration of salts which leads to a rapid plasmolysis of the cytoplasm and that which is favorable for nutrient purposes there exists a concentration which is gradually injurious on account of a certain degree of osmotic action, which however is too weak to cause soon a normal plasmolysis of the cytoplasm.

Similar studies were recently carried on with marine algæ by Duggar,[1] who argued as follows:

"There seems to be no easy explanation of the relative toxic values of NH_4, K, Na, Ca and Mg, as indicated by the results given. By considering the factor of electrolytic dissociation there seems to be nothing of special interest; for if we take the concentrations at which the ammonium salts are toxic as the points of comparison, we find that the dissociation of practically all the salts used is very nearly the same. At greater concentrations there would be, of course, marked differences in the amount of dissociation in the various salts. However, the fact remains that mere differences in the degree of dissociation may not be invoked to explain these relations."

The writer has compared the action of K, Na, and Ca-salts[2] in equimolecular and in equivalent concentrations. As standard solution served a 1% solution of potassium nitrate$=^1/_{10}$ gramm-molecule. For comparison served the following salts:

[1] Transactions of the Academy of Science of St. Louis. 1906, Vol. XVI. p. 487.

[2] Mg-salts were here excluded on account of the strongly specific toxic action exerted in absence of Ca-salts.

Salt.	$\frac{1}{10}$ gramm-molecule.	Percentage.[3]
KNO_3	1	1.01
,, ...	2	2.02
$NaNO_3$	1	0.85
,, ...	2	1.70
KCl	1	0.75
,, ...	2	1.49
NaCl	1	0.58
,, ...	2	1.17
K_2SO_4	1	1.74
Na_2SO_4	1	1.42
K_2HPO_4	0.5	0.87
,, ...	1	1.74
Na_2HPO_4	1	1.42
$K_1H_2PO_4$	1	1.36
,, ...	2	2.72
$Na_1H_2PO_4$	1	1.20
,, ...	2	2.40
$CaCl_2$	1	1.11
$Ca(NO_3)_2$	1.0	1.64
,, ...	1.2	2.07
,, ...	1.5	2.46

For the observation served *Spirogyra nitida,* rich in starch and containing active albumin in the cell sap and also traces of tannin.

A small amount of threads was placed in 100 c.c. of the salt solution. The temperature of the room ranged from 8 to 20° C. Bright sunlight was admitted to the flasks.

The threads were from time to time examined, whereby in some cases normal plasmolysis, in other anomalous plasmolysis and again in other a peculiar phenomenon was noticed which the writer would propose to

[3] All figures refer to the anhydrous state.

call: *Subdivided normal plasmolysis* since in place of one large globe 5—8 and sometimes more globes were formed, each containing a piece of the chloroplast. (Fig. I.)

The observation resulted as follows:

	After 24 hours.	After 72 hours.
KNO_3 $\frac{1}{10}$ gramm-molecule $=1.01\%$	Still healthy.	In about 30% of the cells the subdivided normal plasmolysis was seen, others showed anomalous plasmolysis.
KNO_3 $\frac{2}{10}$ gramm-molecule $=2.02\%$	Very few cells still alive; many cells show subdivided normal plasmolysis.	Anomalous plasmolysis noticed in a few cells, most are dead.
$NaNO_3$ $\frac{1}{10}$ gramm-molecule $=0.85\%$	Moderate number of cells attacked. Normal plasmolysis was seen in a number of cells.	In some cells the subdivided normal and anomalous plasmolysis were noticed. About $\frac{1}{3}$ of the cells still alive, but showing commencing injuries of chlorophyll.
$NaNO_3$ $\frac{2}{10}$ gramm-molecule $=1.7\%$	Almost all cells killed, some show anomalous, others subdivided normal plasmolysis.	All cells killed but not yet bleached.
KCl $\frac{1}{10}$ gramm-molecule $=0.75\%$	Most cells still alive; subdivided normal plasmolysis was seen in about one third of the cells.	Some show anomalous and subdivided normal plasmolysis. Many cells still alive.
KCl $\frac{2}{10}$ gramm-molecule $=1.49\%$	Many cells killed, some died after undergoing subdivided normal plasmolysis.	All cells killed.
NaCl $\frac{1}{10}$ gramm-molecule $=0.58\%$	Almost all cells still normal.	About 15% of the cells still alive, but showing injured chloroplast.
NaCl $\frac{2}{10}$ gramm-molecule $=1.17\%$	In about one half of the cells normal and subdivided normal plasmolysis was seen, other cells being killed before these phenomena appeared.	All cells killed and bleached.
K_2SO_4 $\frac{1}{10}$ gramm-molecule $=1.74\%$	In some cells anomalous plasmolysis and in others the normal plasmolysis was seen.	Only about 5% of the cells still alive. Some died after normal plasmolysis. In some cells the tonoplast alone was still alive.

	After 24 hours.	After 72 hours.
Na_2SO_4 $\frac{1}{10}$ gramm-molecule $=1.42\%$	Some cells show the subdivided normal plasmolysis.	About 25% of the cells still alive. In many dying cells a separation of active albumin in a granular state took place forming a dense precipitate and changing very soon to the passive state.
$K_2H_1PO_4$ $\frac{1}{20}$ gramm-molecule $=0.87\%$.	A number of cells still alive: a number of celles showing subdivided normal plasmolysis, others anomalous plasmolysis and again in others the chloroplast alone had separated in 6–10 globular masses.	Some cells still alive, some showed subdivided normal plasmolysis, others anomalous plasmolysis.
$K_2H_1PO_4$ $\frac{1}{10}$ gromm-molecule $=1.74\%$.	Many cells attacked some cells show anomalous plasmolysis with small granulations in the vacuole probably due to separated active albumin.	Almost all cells dead.
$Na_2H_1PO_4$ $\frac{1}{10}$ gramm-molecule $=1.42\%$.	Most cells still alive, some showed anomalous plasmolysis.	Almost all cells killed. Some showed anomalous plasmolysis and in a few cases a small precipitate of active albumin was seen.
$K_1H_2PO_4$ $\frac{1}{10}$ gramm-molecule $=1.36\%$.	Almost all cells still healthy.	Few cells still normal, many showed normal plasmolysis. A great number dead and bleached previously passing through the normal plasmolysis. In no other solution so many cases of normal plasmolysis were noticed.
$K_1H_2PO_4$ $\frac{2}{10}$ gramm-molecule $=2.72\%$.	Almost all cells showed contracted cytoplasm and were dead. Some cells, however, showed normal plasmolysis and were still alive.	All cells killed and bleached.
$Na_1H_2PO_4$ $\frac{1}{10}$ gramm-molecule $=1.2\%$.	Almost all cells healthy; some show normal plasmolysis.	Only few cells still alive; no normal plasmolysis could be seen.
$Na_1H_2PO_4$ $\frac{2}{10}$ gramm-molecule $=2.4\%$.	Almost all cells killed.	All cells killed and bleached.
$CaCl_2$ $\frac{1}{10}$ gramm-molecule $=1.11\%$.	Healthy.	Healthy.
$Ca(NO_3)_2$ $\frac{1}{10}$ gramm-molecule $=1.64\%$.	Healthy.	Healthy.

	After 24 hours.	After 72 hours.
$Ca(NO_3)_2$ $\frac{1.2}{10}$ gramm-molecule $=2.07\%$.	Healthy.	Most cells alive; in some cells the nucleus was transformed to a globe and had by means of the plasmastrings drawn the chloroplast somewhat into the interior.
$Ca(NO_3)_2$ $\frac{1.5}{10}$ gramm-molecule $=2.46\%$.	Healthy.	Some cells still alive, few showed normal and others anomalous plasmolysis.

While all cells in KNO_3 solution of 1.01% were killed after 8 days standing, the cells remained alive and healthy much longer in the equimolecular amounts of $Ca(NO_3)_2$, but even when the amount of $Ca(NO_3)_2$ was increased to $\frac{1.2}{10}$ gramm-molecule (=2.07%), most cells were still alive after 18 days; further even in the solution of $\frac{1.5}{10}$ gramm-molecule (=2.46%) of $Ca(NO_3)_2$, most cells seemed nomal after 2 weeks, while a few showed normal plasmolysis. But little injury was also noticed after 2 weeks with some cells in the solution of $^1/_{10}$ gramm-molecule (=1.11%) $CaCl_2$, this injury consisting only in the local contraction of the cytoplasm where the plasmastrings were attached. (Fig. II.) The further observations are seen in the following table.

	After 20 days.	After 25 days.	After 30 days.	After 35 days.	After 70 days.
$CaCl_2$ $\frac{1}{10}$ gramm-molecule $=1.11\%$	Healthy.	Healthy.	Healthy.	Healthy.	Almost all cells perfectly healthy and normal. Many small elliptic bodies (p)[4] with a central perforation, and also some crystals of calcium oxalate were noticed. Many cells still healthy after 4 months.

[4] These bodies are not stained by iodine and not attacked by acetic acid, but dissolved by hydrochloric and sulphuric acid. They are further very strongly stained after 1 hour in a highly diluted methyl-violet solution. Perhaps they have some connection with the pyrenoids.—It may be mentioned that those cells showing these formations never contained any starch in the chloroplast, and further the chloroplast in such cells looks generally somewhat emaciated.

	After 20 days.	After 25 days.	After 30 days.	After 35 days.	After 70 days.
$Ca(NO_3)_2$ $\frac{1}{10}$ gramm-molecule $=1.64\%$	Healthy.	Healthy.	Healthy; some of the cells show contraction of plasmastrings, (see d and d_1). producing constriction of the cytoplasm.	Healthy: some crystals of calcium oxalate were seen with the elliptic bodies mentioned above.	Cells normal and healthy, but these cells are in average only about $\frac{2}{3}$ as long as those in $CaCl_2$ 1.11% solution. In numerous cells starch was recognized in both cases.[5]
$Ca(NO_3)_2$ $\frac{1.5}{10}$ gramm-molecule $=2.07\%$	Contraction of plasmastrings, see d and d_1.	Crystals of calcium oxalate and elliptic bodies formed.	Almost all cells killed.	All cells killed	——
$Ca(NO_3)_2$ $\frac{1.5}{10}$ gramm-molecule $=2.46\%$	Normal and anomalous plasmolysis.	Almost all cells killed.	All cells killed	——	——

It will be seen, therefore, that lime salts are at moderate concentrations less injurious than equivalent and equimolecular quantities of Na- and K-salts.[6] In regard some of the phenomena observed compare the attached drawings.

[5] 8 days later a fungus spread rapidly and killed many cells.

[6] It may be mentioned here that the injurious action of Mg-salts can only completely be overcome by Ca-salts, and not by Na-or K-salts, what has been observed not only with algæ, but also with young plants of barley and maize which were deprived of their endosperm. Three young barley plants 12-15 cm. high were deprived of their endosperm and placed in 0.4% solution of $Mg(NO_3)_2$ to which 0.2% K_2SO_4 was added, while to a second flask 0.2% of $CaSO_4$ was added instead of K_2SO_4. In the former case the plants were dead in 4 weeks, while in the latter case some leaves were still alive after 6 months. During that long time altogether have been developed 27 small leaves of which however only the 5 youngest were still alive and these showed a much lighter green than normal. Evidently every new leaf that developed had received some food from the neighbouring dying leaves.

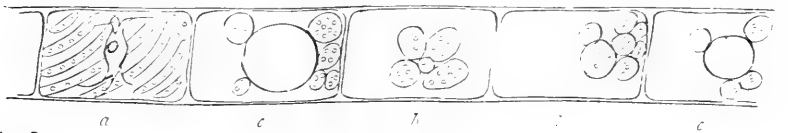


Fig. I.
a... ... ...Normal cell.
b... ... ...Subdivided normal plasmolysis.
c... ... ...Anomalous plasmolysis with plasmolysed chloroplast.

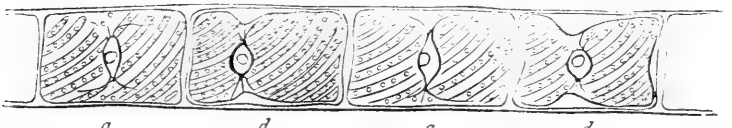


Fig II.
a... ... ...Normal cell.
d and d_1... ...Cells with beginning contraction of the plasmastrings leading to the constriction of the cytoplasm at their place of attachment.

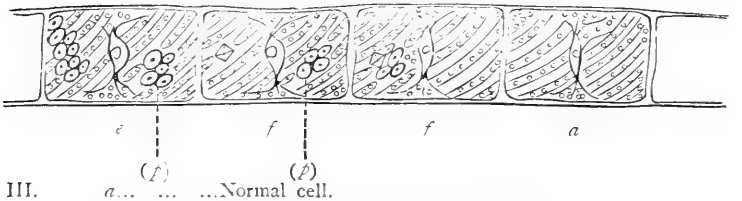


Fig III.
a... ... ...Normal cell.
e... ... ...Cells with the elliptic bodies (*f*).
f... ... ...Cells with calcium oxalate.

On the Efficacy of Calcium-cyanamid under Different Manuring Conditions.

BY

I. Namba and C. Kanomata.

In a former paper[1] Inamura had communicated that calciumcyanamid (lime-nitrogen) applied in conjunction with superphosphate yielded a better harvest of *Brassica chinensis*, then when applied in conjunction with disodiumphosphate. It was desirable however, to observe also the behavior of other plants under these conditions. For the trial served oats and onion.

The lime-nitrogen was applied to the soil two weeks before the other manure, and the mixture kept sufficiently moist in order to have the decomposition of that lime compound thoroly realized.

Eight pots each holding 8 kg. soil served for the experiment.

The general manure was per pot:

Calcium cyanamid .. .. 4 g.

Potassium sulphate .. .. 6 g.

Four pots A received 10 g. crystallized disodium phosphate, while four pots B 5 g. double superhosphate, the equivalent in P_2O_5.

Two pots A and two pots B received 20 seeds of oat each, further two pots A and two pots B received each 20 seeds of onion, Nov. 22nd.

Later on the young oat plants were thinned to ten per pot, all of nearly equal size, while the young onion plants were reduced to five.

After 4 months a considerable difference was observed with the oat plants, the superphosphate plants showing a greater height and a darker green color. The photograph taken on May 5th, reproduced on plate Pl. XVI. shows this difference.

[1] These Bul. Vol. VII. No. 1.

Between May 10 and 14 the oat plants were flowering. In this stage they were cut and weighed in the fresh state with the following result:

	yield.
Sodium phosphate	A_1 = 275g.
	A_2 = 277g.
Double superphosphate	B_1 = 280g.
	B_2 = 305g.

With the onion plants a difference in height was hardly noticeable, altho also here a small difference in favor of the superphosphate was revealed, when the plants were cut (May 13th) and weighed in the fresh state.

	yield.
Sodium phosphate	A_1 = 25 g.
	A_2 = 22 g.
Double superphosphate	B_1 = 27 g.
	B_2 = 28 g.

If now the average is taken and the yield with sodium phosphate taken =100, we obtain the following result, to which we add for comparison the result obtained a year ago by Inamura with Brassica.

	Sodium phosphate + Lime-Nitrogen.	Double superphosphate + Lime-Nitogen.
Avena sativa	100	106
Allium, fistulosum...	100	117
Brassica chinensis	100	109

Altho these differences are not very great, they show that *lime-nitrogen* (an alkaline manure) yields a better result in conjunction with superphosphate, than in conjunction with a neutral phosphate, doubtless due to the former mixture coming nearer neutrality than the latter.

In another experiment with *lime-nitrogen*, carried out by Mr. C. Kanomata, bonedust was applied in conjunction with it, and not superphosphate as in the former case.

4 pots each holding 8 kilo soil, received the following manure, g:

2 Pots, A. each:

K_2SO_4	6
Bone dust	11.2
Calcium cyanamid	4

2 Pots, B. each:

K_2SO_4	6
Bone dust	11.2
$(NH_4)_2SO_4$	3.4

(Equivalent to N in 4 g. calcium cyanamid).

The special maure, the lime-nitrogen or calcium cyanamid[2] was applied to the soil 2 weeks before the other general manures, and this mixture kept sufficiently moist in order to support the decomposition by microbs into calcium carbonate and ammonia.

10 Seeds of *Brassica chinensis* were sown Nov. 13, 1906, and when the young plants were about 15 cm. high, they were reduced to 4 per pot, all of equal size.

The weight in the fresh state, Jan. 25 was:

$$CaCN_2 \begin{cases} A = 118\text{ g.} & B = 113.6\text{ g.} \\ A = 122.6\text{ g.} & B = 117.6\text{ g.} \end{cases} (NH_4)_2SO_4$$

These differences with Brassica show that lime nitrogen compared with ammonium sulphate does not depress the availability of bonedust.

A further experiment was made with oats. 20 seeds per pot were sown and later on when 15-18 cm. high the number of plants was reduced to 10 per pot, all of equal size, April 18.

The plants were cut soon after the flowering period with the following result:

	A	B
Greatest height, cm.	125	115
	120	118
Fresh weight, g. ...	155.5	156.0
	152.0	153.0

[2] The sample of calcium cyanamid at my disposition contained 18 per cent. nitrogen.

This result shows again that lime-nitrogen—unlike sodiumnitrate—does not depress the availability of bonedust; it resembles ammoniumsulphate in this regard.

On the Efficacy of Lime-nitrogen in Presence of Different Phosphates.

On the Behavior of Onion to Stimulants.

BY

I. Namba.

Former experiments at this College have shown that the growth of various plants was stimulated by a dose of 0.5 g. of manganous sulphate added to 8 kg of our soil. But the onion plant showed a marked exception, as its development was depressed by this dose. Hence further experiments seemed desirable to decide whether stimulation would take place with still smaller quantities of manganese.

At the same time with this test the writer has observed the behavior of onion to small quantities of sodium fluorid.

As general manure for each pot filled with 8 kg loamy soil served, g:

Potassium sulphate	5
Steamed bone dust	10
Ammonium sulphate	8

Twenty seeds of onion were sown into each pot November 4.

A. received 0.1 g $MnSO_4 + 4$ aq.
B. „ 0.2 g „
C. „ 0.01 g NaF
D. „ 0.05 g „
E. „ 0.2 g „
F. served as check pot.

These salts were applied in high dilution in the form of topdressing, after the young plants had reached about 5 cm. in height.

The plants were harvested June 14 and weighed in the fresh state with the following result:

	Wt. of leaves.	Wt. of bulbs and roots.	Total weight.		Bulbs and roots leaves.
			Absolute.	Relative.	
A	38.0	22.5	60.5	159.2	.59
B	35.5	16.5	51.0	134.2	.46
C	44.0	24.5	68.5	180.2	.55
D	33.5	16.5	50.0	131.5	.49
E	30.0	11.0	41.0	107.8	.36
F	29.5	8.5	38.0	100.0	.28

These figures show that onion can be considerably stimulated in bulbs and leaves by small doses of manganese, and also by fluorine compounds.

The stimulating doses in A and C would correspond to 22 Kilo $MnSO_4$ + 4aq. and 2.2 Kilo sodium fluorid per ha.

An increase of this dose will lessen the stimulating effect.

On the Influence of Didymium on Plants.

BY

C. Kanomata. (With Plate).

The observation that various mineral salts in high dilution exert a stimulating action on plant growth, led me to an experiment with a didymium salt[1].

To 4 pots each holding 10 kilo loamy soil, were applied the following manure:

KNO_3	6 g.
$NaNO_3$	6 „
$CaHPO_4$	8 „
$CaSO_4$	10 „

Twenty seeds of barley were sown per pot on Nov. 28. When the young plants were about 10 cm. high, they were reduced to 10 per pot, all of equal height:

Pot A served as control pot.

Pot B received 10 milligrams didymium nitrate dissolved to 200 ccm. of water.

Pot C 100 milligrams.

Pot D 500 milligrams.

This last pot received one half of the salt at the same time as the others and one half 4 weeks later.

[1] Altho. didymium was shown to be not element by Auer, but a mixture of proseodym and neodym. the salts of didymium still occur in commerce. Such a salt was the sample of nitrate used, imported from Germany, through Dr. H. König Leipzig. It was a crystalline powder of a weak violet color. According to Matignon the compounds of neodym stand as to certain properties those of magnesium and calcium.

Since didymium nitrate is of strong acid reaction, it was necessary to neutralize the solution with very dilute NaOH, and to apply then the liquid thru some small holes made in the soil[2]. The precipitate of didymium hydroxid produced by sodium hydroxid dissolved on dilution to 1 p. mille gradually, probably forming a colloidal solution.

Some months later a difference in height was noticeable, the plants that had received 10 milligrams didymium nitrate being the highest.

The photograph taken on April 12, reproduced on the adjoining plate, shows the differences very clearly.

The plants were cut shortly after the flowering stage and weighed in the fresh state with the following result:

	Contral	10 mg.	100 mg.	500 mg.
Total weight, g....	244.5	287.2	214.5	190.0
Weight of ears, g.	42.2	60.1	41.0	22.3
Number of ears ...	29	32	24	17

It will be seen that 10 milligram didymium nitrate (after neutralization with NaOH) produced not an inconsiderable stimulation, while the increase to 100 and 500 milligrams produced an injurious action.

Another experiments made with mustard, Raphanus sativus radicula and tobacco under the same conditions and with application of 1 milligramm neutralized didymium nitrate per kilo soil. The plants cut after 6 weeks yield the following weight, g. in the fresh state:

[2] This crystallized didymium nitrate has following formula,

$$Di_2(NO_3)_3 + 12 \text{ aq.}$$

Our product gave, however, not a weak red precipitate with NaOH as it should produce, but a white precipitate.

	Check.	With Didymium.
Mustard, (5 plants) total weight	145	165
Raphanus sativus radicula, 4 plants total weight ..	37	47
Ditto weight of roots	22	30
Tabacco, 8 plants. total weight	35.5	47

These further tests confirm the above observations with barley, that *didymium nitrate (neutralized) at the rate of one milligramm per kilo soil can cause a stimulation of plant growth.*

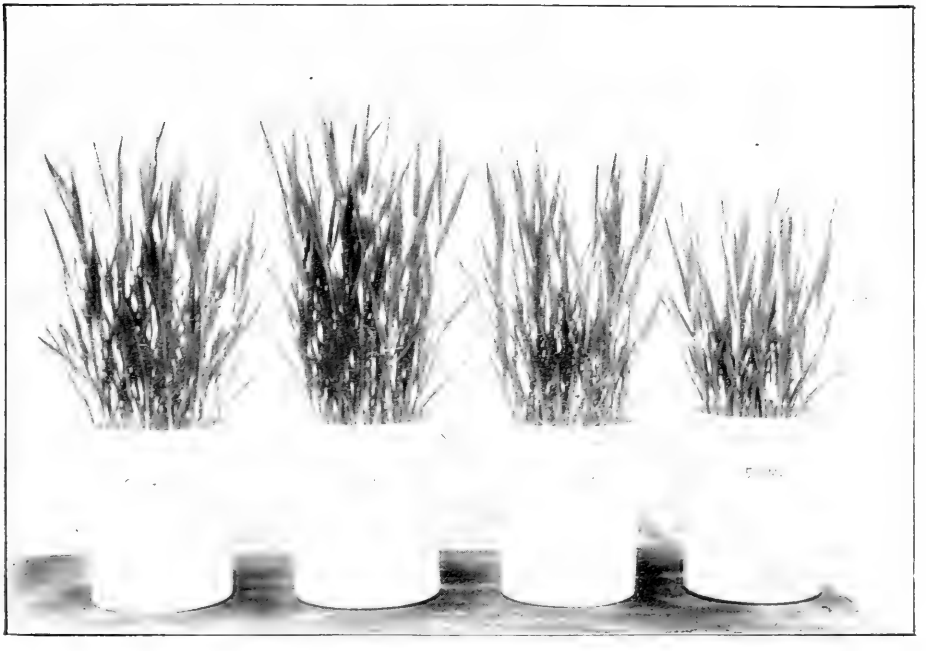
If now the yield in the check pots taken= 100, it shows the following comparative result:

Name of plants		Check.	With 10 mg. Didymium.
Barley	Total weight.	100	117.5
	Weight of ears.	100	142.4
Mustard	Total weight.	100	113.7
Raphanus sativus radicula ...	Total weight.	100	127.0
	Weight of roots.	100	136.0
Tobacco	Total weight.	100	132.1

An experiment under the same conditions was carried out nearly at the same time by H. Hamasaki on oats with Beryllium nitrate, which on account of its acid properties was neutralized by sodium carbonate in high dilution. The plants were cut shortly before showing the ears and weighed in the fresh state, g:

10 mg.	178.8 g.
100 „	151.0 „
500 „	156.0 „
Control	171.0 „

This result shows that a decisive stimulation by beryllium nitrate did not take place at the rate of 1 milligramm per kilo soil, while an increase to 10 times the amount caused a depression. Some further experiments with beryllium are intended.



Action of Didymium on Plants.

Young Bees as a Delicacy.

BY

M. Takaishi.

In the province of Shinano in Japan a kind of wild bees (Japanese name jibachi or anabachi), which live in earth holes, serve as food, the young bees as well as their larvæ. Considered in the proper light, this dish is valued rather than as a delicacy than as a real food in that province, otherwise the high price would not be justifiable. The dish is prepared with sugar and shoyu-sauce; the larvæ and bees thus prepared are now sold also in tin boxes, like canned meat, and about one thousand boxes are now exported annually to other provinces of Japan. One kilo of this preparation costs yen 2.50. The insects are caught in the autumn by firing some gun powder at the entrance to the nest; the smoke spreading throu the underground cavities stupifies the insects. The place is rapidly digged up and the insects caught in a basket which is then covered with cotton cloth and placed for a moment in hot water.

For my analysis served the preserved content from a tin box 13 g of this content were ground in a mortar; 1 g. served for determination of total nitrogen, 1 g. for ash, and 1 g. for water content. The remaining 10 g. were washed well with water, to remove shoyu and sugar, and after drying served for determination of fat, which amount was calculated for the original dish. The wash water served for the determination of sodium-chlorid and sugar.

The analytical results were as follows:—

Water	28.15%
Crude protein.	13.69%
Crude fat ..	11.15%
Glucose ..	5.71%
Cane sugar..	5.81%
NaCl	6.23, corresponds to 41.53 parts shoyu[1]
Ash	10.92%

[1] The Shoyu sauce contains generally about 15% NaCl.

東京帝國大學

農科大學

學術報告

第八卷

THE

BULLETIN

OF THE

COLLEGE OF AGRICULTURE,

TŌKYŌ IMPERIAL UNIVERSITY

JAPAN.

VOL. VIII.

1908–1909.

NOTICE.

The Bulletin of the College of Agriculture, Tōkyō Imperial University, will be continued under the name of the "Journal of the College of Agriculture, Imperial University of Tokyo," Vol. VIII, No. 2 being the last number of the Bulletin.

CONTENTS.

No. 1, September, 1908.

No. 2, April, 1909.

A List of Panorpidae of Japan, with Descriptions of Ten New Species.[1]

BY

T. Miyake, *Rigakushi.*

With Plate I.

The Panorpidae hitherto known to occur in Japan are as follows:—

1. **Panorpa japonica** Thunberg, West., Trans. Ent. Soc. Lond., Vol. IV. p. 188 (1845); M'Lach., Trans. Ent. Soc. Lond., 1878, p. 183.
2. **Panorpa macrogaster** M'Lach., Trans. Ent. Soc. Lond., 1878, p. 184.
3. **Panorpa Klugi** M'Lach., Trans. Ent. Soc. Lond., 1878, p. 185.
4. **Panorpa Pryeri** M'Lach., Trans. Ent. Soc. Lond., 1878, p. 185.
5. **Panorpa leucoptera** Uhler, Trans. Ent. Soc. Lond., 1878, p. 186.
6. **Panorpa Wormaldi** M'Lach., Trans. Ent. Soc. Lond., 1878, p. 186.
7. **Panorpa Lewisi** M'Lach., Bull. Soc. Ent. Suiss., 1887, p. 402.
8. **Panorpa cornigera** M'Lach., Bull. Soc. Ent. Suiss., 1887, p. 404.
9. **Panorpa bicornuata** M'Lach., Bull. Soc. Ent. Suiss., 1887, p. 403.

[1] Contribution from the Zoological Laboratory.

10. **Panorpa communis** L., Matsumura, Senchūzukai (Thousand Insects of Japan), Vol. I. p. 164, pl. XI, fig. 6 (1904).
11. **Leptopanorpa Ritzemae** M'Lach., Trans. Ent. Soc. Lond., 1878, p. 187.
12. **Leptopanorpa Sieboldi** M'Lach., Trans. Ent. Soc. Lond., 1878, p. 188.
13. **Panorpodes paradoxa** M'Lach., Trans. Ent. Soc. Lond., 1878, p. 189.
14. **Panorpodes decorata** M'Lach., Bull. Soc. Ent. Suiss., 1887, p. 405.
15. **Bittacus sinensis** Walk., M'Lach., Bull. Soc. Ent. Suiss., 1887, p. 406; Matsumura, Senchūzukai (Thousand Insects of Japan), Vol. I, p. 165, pl. XI. fig. 5 (1904).

Now, while engaged in examining the collections of the Agricultural College and of the Imperial Central Agricultural Experiment Station of Nishigahara, I have discovered a number of specimens belonging to the family, but which are apparently not referable to any of the above mentioned species. On studying them, I have come to the conclusion that they include at least ten species, all which I consider to be new to science. They shall therefore be described in the present paper.

It is a well known fact that in the family Panorpidae the most important characters for the distinction of species are offered by the last four abdominal segments, especially by the form of appendages of the 9th segment (cheliferous segment) of male individuals. The characters just referred to are however of no use for systematic purpose in cases in which only females are known but not males. Under such circumstances, the wing-markings might be utilized to a certain extent for the purpose of specific distinction. This holds good for at least the better differentiated forms, in which the wing-markings, subject as they are to much individual variations, present to a greater or less extent features peculiar to the species, irrespective of the sexes. In fact, the

course indicated had been taken by several entomologists when they had only female specimens to deal with. So that, it seems to me justifiable if I describe in the following lines five new species on the basis of female specimens alone, laying weight principally on their wing-markings.

The Panorpidae of Japan is rich in species, beautiful in colouration and remarkable in structure. All the following ten new species, like the rest of the family (excepting *P. communis*), show in common the well known peculiality mentioned by M'Lachlan, that "the subcosta in all the wings scarcely extends beyond the middle of costal margin." So that this interesting and remarkable feature may still be said to characterize the Japanese members of the Panorpidae.

1. **Panorpa ochracea** n. sp. (*Kihada-shiriagemushi*).

(Pl. I. figs. 9, 9a 9b ♂.)

Body brownish ochraceous; head black, ocelli and compound eyes brown; rostrum purplish brown; prothorax black except the hind margin; anterior half of the mesothorax blackish; a black line on the anterior margin of the metathorax. Antennae black; legs brownish ochraceous.

Wings moderate; apex elliptical tinged with ochraceous, the basal half more deeply coloured; a narrow brownish black fascia rather beyond the middle, and a brownish black apical space with slight internal sinuation; two small spots (in the specimen these spots are indistinct in the right wing) before the fascia in the fore-wing and a small spot near the posterior margin in the hind-wing. Longitudinal veins blackish; transwerse veins whitish.

Abdomen moderately long, brownish ochraceous; a transverse black line on the 1st dorsal segment; two irregular black patches on the 2nd dorsal segment; the posterior margin of the 3rd dorsal segment produced into a short broad median lobe as in *P. japonica, P. Klugi* and its allies; 6th and 7th segment thick, cylindrical, truncate and equal in length; a slight prominence on either side of the two segments beyond the middle;

8th slightly longer than the 7th, cylindrical; 9th (cheliferous segment) short; lateral pieces less stout than in *P. japonica* and *P. Klugi*, the chelae brownish ochraceous with brown tips, longer in proportion than in the preceding species, the appendages ochraceous brown, linear, rather short as in *P. japonica* but much more curved than in that species.

Expanse 37 mm.

A single male specimen captured by Mr. TSUCHIDA at Yoshino, Aug., 1902.

This species is closely allied to *P. Klugi* in the colouration of body and in the markings of wing, but is readily distinguished from the latter by the difference in size (wing-expanse in *P. Klugi* 27-30 mm.) and by the more elongated cheliferous appendages. In structural respect, it resembles *P. japonica;* but the colouration of body, wing-markings and the structure of the cheliferous segment well separates the two species.

2. **Panorpa sinanoensis** n. sp. (*Usumon-shiriagemushi*).

(Pl. I. figs. 7, 7a, 7b ♂.)

Head and thorax black; rostrum and antennae also black; legs ochraceous yellow.

Wings whitish; a very broad greyish fuscescent (not deep) fascia beyond the middle, the apex also very broadly greyish fuscescent, so as to leave a very narrow untinged space between the fascia and the apical dark portion; an angulate spot on the posterior margin in this space; two small greyish spots before the fascia in the fore-wing, the smaller one of which is on the anterior, and the other larger and more irregular one on the posterior margin; the former spot absent in the hind-wing; another small spot near posterior margin in the fore-wing; longitudinal veins black; transverse veins whitish.

Abdomen rather slender, ochraceous fuscescent, all segments much as in *P. japonica*, the posterior margin of the 3rd dorsal segment produced

in the middle into a broad short lobe; 8th longer than the 7th; 9th (cheliferous segment) short as in *P. japonica;* lateral pieces less stout as in *P. ochracea,* the chelae long, the appendages as in *P. ochracea* but more linear, ochraceous brown.

Expanse 36 mm.

A single male specimen captured at Sakaki in Shinano by the author on May 22, 1906.

Allied to *P. japonica* in wing-markings and in structure, but differs from it in the appendages of the cheliferous segment, in lighter wing-markings and also in the colouration of abdomen.

3. **Panorpa rectifasciata** n. sp. (*Obi-shiriagemushi*).

(Pl. I. figs. 10, 10a, 10b, ♂.)

Varying from black to brown, the cheliferous segment reddish brown; rostrum black to reddish brown; antennae black to yellowish brown; legs ochraceous yellow.

Wings rather narrow, whitish, the apex rounded; a rather broad black fascia (in some specimen not deeply coloured) beyond the middle; a broad black apical space, this space is very slightly incurved on its inner margin; both with very sharply defined edges; neither line nor spot anywhere present; longitudinal veins of basal half and the portion where they cross the markings black; the rest and transverse veins whitish.

Abdomen black to brown; a broad median lobe on the 3rd dorsal segment as in the preceding species; 9th segment relatively smaller than in *P. Klugi* although somewhat larger in some specimens; lateral pieces rather stout, the chelae not longer than *P. Klugi,* the appendages very short, yellowish brown, the divided portion much broader and shorter in proportion than in *P. Klugi* and the preceding species and more curved.

Expanse 28-30 mm.

Collected by Mr. Tsuchida at Chūzenji, Nikkō on July 31,

1885, a male; at Kominato, Aomori, on Aug. 20, 1902, two males; at Sanbogi, Aomori, on Aug. 15 and 29, three females.

Allied to *P. Klugi*, but certainly distinct in the structure of the appendages of the cheliferous segment.

A large female specimen (Exp. 32 mm.) obtained by me in Kii has apparently the same wing-markings as above mentioned form; only the inner margin of the apical black portion is straight and not incurved, the ground colour of wings very ochraceous and the whole body black in colour. Whether this constitutes a variety of the present species or not cannot be exactly determined without an examination of the male.

4. **Panorpa striata** n. sp. (*Suji-shiriagemushi*).

(Pl. I. fig. 1, 1a, 1b, ♂.)

Body black, the cheliferous segment ochraceous brown; rostrum black; antennae black; legs fuscescent yellow.

Wings with elliptical apex, the hind-wing somewhat shorter than the fore-wing; whitish, with black markings as follows:—the subcostal vein with a streak from base to end; a small elongated spot connected transversally on the end of the vein; three conjoined spots along the posterior margin, which are in the hind-wing less emphasized; an irregular fascia, broader than the others, beyond the middle of the wing; three elongated spots on the posterior margin between the two fasciae just mentioned; a curved line just before the apex; apex with a narrow dark portion; longitudinal veins brownish black; transverse veins mostly whitish.

Abdomen black, the posterior margin of the 3rd segment produced into a short median lobe; 6th segment larger than the others; 7th and 8th segment not so long as the others (except the 1st segment), 8th segment scarcely longer than the 7th; 9th segment stout; lateral pieces larger, fuscescent yellow, the chelae very short, the appendages of the segment black, rounded, short and very broad in proportion to same

of the species hitherto examined, the divided portions extremely short, the distal part of the appendages bent downwards between the two lateral pieces of the cheliferous segment (no such case in any other species) so that they represent a transverse ridge above.

Expanse 27 mm.

A single male specimen in the collection of the Imperial Central Agr. Exp. Station at Nishigahara without date of capture.

This species I have supposed at first to be the male of *P. Wormaldi* M'L., of which only the female has been described by M'Lachlan in Trans. Ent. Soc. Lond., p. 186 (1875). But after a careful examination I have decided to consider the specimen as representing another and distinct species. There is a female specimen of *P. Wormaldi* in my collection, which very closely agrees with the original description of M'Lachlan in all parts. Comparing this specimen with the present and taking into consultation the original description, the differences are as follows:—wing-markings of the present species are deeply blackish, while in *Wormaldi* they are light coloured; beyond the middle of wings there are three fasciae in both species, of which the first fascia is nearly the same, but the second fascia of the present species is topographically the same as the third of *Wormaldi*, so that the second fascia of the last species is wanting in *striata*; the third fascia of *striata* is to be recognized as a part of the apical black portion of *Wormaldi*; longitudinal veins of *Wormaldi*, where they do not cross the fascia, are mostly whitish, while the same of *striata* are mostly black.

5. **Panorpa nihonensis** n. sp. (*Ko-shiriagemushi*).

(Pl. I. figs. 3, 3a, 3b. ♂.)

Body totally black; legs fuscescent.

Wings rather broad, the apex rounded, whitish; a very broad blackish fascia beyond the middle; apex also broadly blackish, sinuated internally; two blackish spots before the fascia; longitudinal veins

blackish; transverse veins whitish; markings mostly similar to those of *P. japonica;* the posterior margin of the 3rd abdominal segment produced into a short broad median lobe; 6th, 7th and 8th segment much as in *P. japonica;* 9th (cheliferous segment) stout, the appendages blackish, very long, slender, straight, the branched portions widely divaricate.

Expanse 28 mm.

A single male specimen captured at Nojiri in Shinano, by Mr. TSUCHIDA on July 24, 1887.

Allied in colouration of body and wing-markings to *P. japonica*, and in size to *P. Klugi;* but readily distinguishable from both by the structure of the cheliferous segment (see figs. 3a, 3b, 3c, 3d), from the former by size, and from the latter by wing-makings and colouration of body.

6. **Panorpa pulchra** n. sp. (*Aya-shiriagemushi*).

(Pl. I. fig. 4, ♀.)

Body totally black; legs yellowish.

Wings rather broad, whitish; apex somewhat elliptical; a very broad fascia (broader than in any other species) beyond the middle; the fascia furcate externally at a point a little lower than the middle, forming a narrow branch which terminates on the posterior margin, running obliquely in a direction contrary to that of the fascia; apex also very broadly black, its inner margin sinuated; between this black apical space and the fascia a narrow untinged portion is left, diverging towards the posterior margin; another narrow fascia before the middle of wing, oblique in directon to that of the above stated broad fascia (this narrow fascia is reduced to two irregular spots in hind-wings); an irregular spot near the anterior margin between the two above mentioned fasciae; a short basal streak along the posterior margin; longitudinal veins black especially in basal portion, those in the outer untinged portion whitish; transverse veins white, even in the apical black portion, so that they constitute two very fine white striae in it.

Expanse 33 mm.

A single female specimen captured in Tosa by Mr. TAKENOUCHI (without date of capture).

This species resembles *P. japonica* to a certain extent, but differs in the more pronounced wing-markings and in the presence of two fine white striae in the apical black space.

7. **Panorpa trizonata** n. sp. (*Misuji-shiriagemushi*).

(Pl. I. fig. 11. ♀.)

Body black; eyes yellowish; legs ochraceous yellow.

Wings very narrow, the apex rounded, yellow; a broad black fascia before the middle of wings; a likewise broad fascia beyond the middle, in the fore-wing the fascia is furcate externally in the middle forming a narrow obliquely running branch; apex also broadly black, its inner edge slightly incurved; transverse veins ochraceous; longitudinal veins colourless.

Expanse 32 mm.[1]; 38 mm.[2]

1) two females captured on Nachi in Kii by the author on July 24, 1906; 2) a female captured on Takaoyama in Musashi by Prof. SASAKI, on Sept. 21, 1902.

The Takaoyama specimen has a black spot in fore-wing between the two black fasciae.

8. **Panorpa brachypennis** n. sp. (*Maruhan-shiriagemushi*).

(Pl. I. fig. 6, ♀.)

Body blackish or testaceous; rostrum ochraceous in testaceous specimen and black in the blackish specimen; legs testaceous.

Wings broad towards apex, broader in proportion than any of the other species, narrower in basal portion; apex rounded, ground colour

[1] The Nachi specimen.

[2] The Takaoyama specimen.

somewhat ochraceous; a narrow brownish black fascia rather beyond the middle, sinuated externally; apex also broadly brownish black, internally incurved in the middle; (in a specimen a small spot near the anterior margin before the fascia present); longitudinal veins ochraceous; transverse veins whitish.

Expanse 28-33 mm.

Three female specimens obtained at Nikkō by Mr. TSUCHIDA on Aug. 28, 1885; a female by Mr. MURATA on Sept. 6, 1902.

9. **Panorpa Takenouchii** n. sp. (*Hoshi-shiriagemushi*).

(Pl. I. fig. 5, ♀.)

Body black; basal joints of antennae and rostrum ochraceous; prothorax with yellowish posterior margin (in one specimen obsolete); some yellowish patch on the dorsal sides of meso- and metathorax (in one specimen less emphasized); antennae black; legs ochraceous.

Wings rather broad; apex rounded; white; four somewhat quadrate black spots along the anterior margin, of which the external one is largest; five likewise quadrate spots along the posterior margin, of which the second spot from the base of wing is smallest; external second spot connected with the last external spot of the anterior margin; a very small linear spot on the posterior margin near the base in the fore-wing; apex black (originally consisted in two conjoined spots), with acute internal edge; main longitudinal veins blackish; the rest and transverse veins whitish.

Expanse 35 mm.; 30 mm.

Two female specimens captured in Tosa by Mr. TAKENOUCHI (without date).

10. **Panorpa nikkoensis** n. sp. (*Nikkō-shiriagemushi*).

(Pl. I. fig. 2, ♀.)

Head black; rostrum yellowish; thorax and abdomen ochraceous brown; a yellowish patch on the mesothorax; (antennae lost); legs ochraceous.

Wings rather broad; apex elliptical; white with slight brown tinge; three small brownish black spots along the anterior margin; a small spot on the posterior margin beyond the middle in the fore-wing, and just at middle in the hind-wing; a small spot just at the apex.

Expanse 32 mm.

A single female specimen obtained at Chūzenji in Nikkō by Mr. MURATA, on Aug. 28, 1887.

P.S.—There are two female specimens in the collection of the Agr. Exp. Station at Nishigahara, obtained at Chūzenji in Nikkō (June 13,1902 Coll. MURATA), which bear a close resemblance to *P. Pryeri*. They are very large in size (exp. of wings in the two specimens 40 mm.) and the wing-markings are strongly pronounced; moreover the apex of wings is suffused with black (See Pl. I. fig. 8). Whether they repsesented a new species or not, cannot be determined unless the male be obtained and examined. However, I consider the specimens to deserve being made into at least a variety of *P. Pryeri* if not into a new and distinct species. I shall call them *P. Pryeri* var. *major*.

October, 1907.

Explanation of Plate I.

(*Panorpa*; *a* denotes apex of abdomen; *b* appendices.)

Fig. 1. *Panorpa striata* n. sp., ♂. (1*a*, 1*b*).

Fig. 2. *P. nikkoensis* n. sp., ♀.

Fig. 3. *P. niphonensis* n. sp., ♂. (3*a*, 3*b*). (3*c* do. of *P. japonica*; 3*d* do of *P. Klugi*).

Fig. 4. *P. pulchra* n. sp., ♀.

Fig. 5. *P. Takenouchii* n. sp., ♀.

Fig. 6. *P. brachypennis* n. sp., ♀.

Fig. 7. *P. sinuatensis* n. sp., ♂. (7*a*, 7*b*).

Fig. 8. *P. Pryeri* var. *major*. ♀.

Fig. 9. *P. ochracea* n. sp., ♂. (9*a*, 9*b*).

Fig. 10. *P. rectifasciata* n. sp., ♂. (10*a*, 10*b*).

Fig. 11. *P. trizonata* n. sp. ♀.

Miyake et Yokoyama del.

Contributions to the Study of Japanese Aphididae.[1]

I. On the Structure of the Antennae of Aphididae.

BY

G. Okajima.

With Plates II and III.

The antennae of Aphides (Plant-lice) are, as is well known, long and filiform, and inserted in front of the eyes, directly on the head or on raised protuberences, the so called frontal tubercles. The length is very variable, some being longer than the width of head while others are more than twice as long as the body. In the Subfamily Aphidinae, and especially in the Genus *Siphonophora*, the antennae are almost always very long, while in the Subfamilies Callipterinae, Lachninae, Eriosomatinae and Phylloxerinae, they are usually short. The antennal joints vary from three to six in number. The chief object of this paper is to discuss their number. Some give it as three to seven, others as five to seven, while still others give it as invariably six. Up to the present, the first of the above statements appears to have been regarded as true by many entomologists. J. F. Judeich and H. Nitsche[2] state in their work as follows: "Die ältere Angabe, die Gattung *Aphis* L. habe siebengliederige Fühler, beruht darauf, dass bei ihr das Glied sechs sich gegen das Ende zu plötzlich verdünnt. Sie tragen an den letzten Gliedern kleine Riechgrüben." And they add: "bei den jungen Larven 5-gliedrig, bei den erwachsenen Formen dagegen meist 6-gliedrig und oft länger als der Körper, nur selten 5-gliedrig." Mr. E. Witlaczil[3] after criticizing briefly the observations of Kaltenbach, Koch and Buckton,

[1] Contribution from the Zoological Laboratory.

[2] Lehrb. der Mitteleurop. Forstinsekt. 1895. p. 1197.

[3] Zur Anatomie der Aphiden. 1882. p. 10.

says, "Wie ich schon bemerkt habe, ist dies unrichtig. Ich fand bei den Aphiden allgemein sechs Fühlerglieder. Bei der ebenfalls als mit fünf Fühlergliedern unterschiedenen Gattung *Pemphigus* zeigen alle geflügelten agamen Weibchen und die Männchen sechs Fühlerglieder. Allerdings nur im vollkommen ausgebildeten Zustande, indem die Larven derselben nur 4-5 Fühlerglieder besitzen, von denen aber oft eines einen Einschnitt in der Mitte zeigt, so andeutend, dass wir nach der nächsten Häutung ein Glied mehr zählen werden." Buckton,[1] the eminent author, in the first volume of his celebrated Monograph states that: "in *Aphis* proper there are seven joints; *Pemphigus* and *Schizoneura* have six antennal joints, while in *Phylloxera* the number of joints is reduced simply to three." Even Mr. D. Sharp[2] expresses the doubt "whether the antennae have ever really more than six joints, the apparent seventh joint being actually a sort of appendage of the sixth."

Thus it appears that the exact number of the antennal joints remains still unsettled, but many authorities agree in regarding the number three, found in the Gen. *Phylloxera,* as the lowest limit. Unfortunately I have not been able to obtain any species with three jointed antennae, so I shall here confine my discussion to forms having more than three jointed antennae. Many entomologists are of the opinion that most Aphides possess seven joints, while Judeich, Nitsche, Witlaczil and others affirm that the antennae are composed of only six joints. On this point Buckton[3] says as follows: "the terminal joint of the antennae affords good discriminating characters. In *Siphonophora* and like genera, the seventh or the last joint is very long and imbricated. In *Lachninae,* the seventh joint, although obvious, is much curtailed in length, and here it shows the first tendency to abort."

It is not needless to make a further study of the number of primary antennal segments of Aphides. In the Subfamily Aphidinae (Fig. 23) the first and second joints of the antenna are usually short globous, and

[1] Monogr. of the Brit. Aphides. Vol. I. 1876. p. 12.

[2] Insects. Part II . 1901. p. 581.

[3] Monogr. of the Brit. Aphides. Vol. I. 1876. p. 12.

freely movable. The third joint, usually the longest, is equal to or longer than the length of the two next combined. The fourth and the fifth, mostly short, are equal to each other and longer than half the length of the third joint. The sixth, which is usually longer than or equal to the third in length, is divided into two portions, a short, stout, proximal and a long, slender, filiform or sometimes whip-like distal portion (Figs 1, and 8-15). In the Subfamily Aphidinae, the distal is, with certain exceptions, usually much longer than the proximal portion (Figs. 21-23 and 27). This sixth joint is the very point of discussion, for some regard the joint as one, while others look upon it as two combined. The two portions are not only different in form, but on the so-called node between them there lies a small tubercle. If this node be a true joint, then the two portions must be capable of bending towards each other; but as a matter of fact they are inflexible. Further there is no articular membrane which is characteristic of a true node (Figs. 1, 3 and 13-15).

It is convenient to remark here on the tubercles or sensoria[1] of the antennae. They are usually small circular spots, but sometimes large and oval or irregular in shape, they are moreover, ring-shaped, so as to impart a rough surface to the antennae (Figs. 5 and 16-19). They are covered with a membrane like drums and look much like ocelli (Figs. 1, 18 and 20). These spots are present commonly on the third joint, and often on the fourth, fifth and sixth, but never on the first and second joints. Near the tip of the last joint and the one before the last, one or more of these sensoria are invariably to be found. It is one of these sensoria that divides the sixth joint into two portions, and simulates a node between them (Fig. 15). Their number, size and arrangement are quite different in different species. They are sometimes arranged in a single row (Figs. 3, 7, 19, 21, 24 and 25), and sometimes irregularly aggregated (Figs. 22, 23 and 27). There are two forms of annulated sensoria, one is merely linear and raised around the antennae, or semi-circular (Fig. 7), the other ridged along the raised portion so that the

[1] Oestlund, O. W., Synopsis of the Aphididae of Minnesota, 1887, p. 2.

antennae appear dentate on a side view (Figs. 16, 17 and 19). Mr. O. W. OESTLUND, who adopted this character in describing species for the first time, state that, "they are considered by entomologists to be organs of smell or hearing, or both. I have always found them present and they often give good specific characters, though very few writers have yet made use of them in describing species." In fact, these sensoria afford a discriminating character for species as well as for genera. Moreover, the five subfamilies proposed by Mr. G. W. KIRKALDY[1] can also be distinguished by this antennal character.

1. In Aphidinae, generally long, frequently reaching to twice the body length. Six jointed. Distal part of the sixth joint always very long. Small circular sensoria mostly at irregular distances, present on the third joint only or on third to fifth.

2. In Callipterinae, long, and more slender than in the preceding subfamily. Six jointed. Distal part of the sixth joint usually short or sometimes equal to the proximal. Sensoria more or less oval in shape, present on the third joint alone. They are close together on the basal half of the joint and very few in number.

3. In Lachninae, short, mosty less than the body length. Six jointed. Distal part of the sixth joint shorter than the proximal, and looks like a nail-like process of the latter. Sensoria, round or oval in shape, arranged in a single row and very few in number. The presence of long hairs is peculiar to this subfamily.

4. In Eriosomatinae, very short, not more than half the length of the body. Six or five jointed. The last joint with a short spur (distal). The third and following joints are annulated or furnished with transverse sensoria, at regular or sometimes irregular distances.

5. In Phylloxerinae, exceedingly short. Five or three jointed. In the genus *Chermes*, the antennae are hardly equal to the width of the head. They are furnished with comparatively large sensoria one on each of the three distal joints.

[1] Catalogue of the genera of the Hemipt. Fam. Aphidae. Can. Ent. 1906.

Conclusion.

I. The antennae of Aphides are composed of not more than six joints.

II. Numerous sensory pits are present on the third and following joints, in particular they are never absent from the third.

III. Near the tips of both the last joint and the one before last, there are always one or more circular sensoria.

IV. These sensoria divide the last joint apparently into two parts, of which the distal is usually slender than the proximal.

V. This distal part may be called "flagellum" as in Crustacea and others.

March, 1908.

Explanation of Figures.

Plate II.

(Joints of the Antenna.)

Fig. 1. Sixth and a part of fifth joint of *Aphis sp.* *a*, sensoria on the sixth joint; *b*, the same on the fifth. (4×D. Zeiss.)

Fig. 2. *Schizoneura ulmi* L. *a*, sensoria on the sixth. A. 3-6 joints. B. side view of the sixth. (4×D.)

Fig. 3. *Lachnus sp.* (4×A.)

Fig. 4. *Lachnus sp.* Third to sixth joints.

Fig. 5. *Schlechtendalia chinensis* Bell. (1×D.)

Fig. 6. *Schlechtendalia sp.* (1×D.)

Fig. 7. *Pemphigus sp.* (1×D.)

Fig. 8. Sixth joint with tip of fifth of *Siphonophora sp.* (1×A.)

Fig. 9. Fifth and sixth of *Phorodon sp.* (1×A.)

Fig. 10. *Pterocallis tiliae* L. Fifth and sixth. (1×A.)

Fig. 11. *Callipterus castaneae* Buck. Fifth and sixth. (1×A.)

Fig. 12. *Melanoxanthus sp.* Third to sixth. (1×A.)

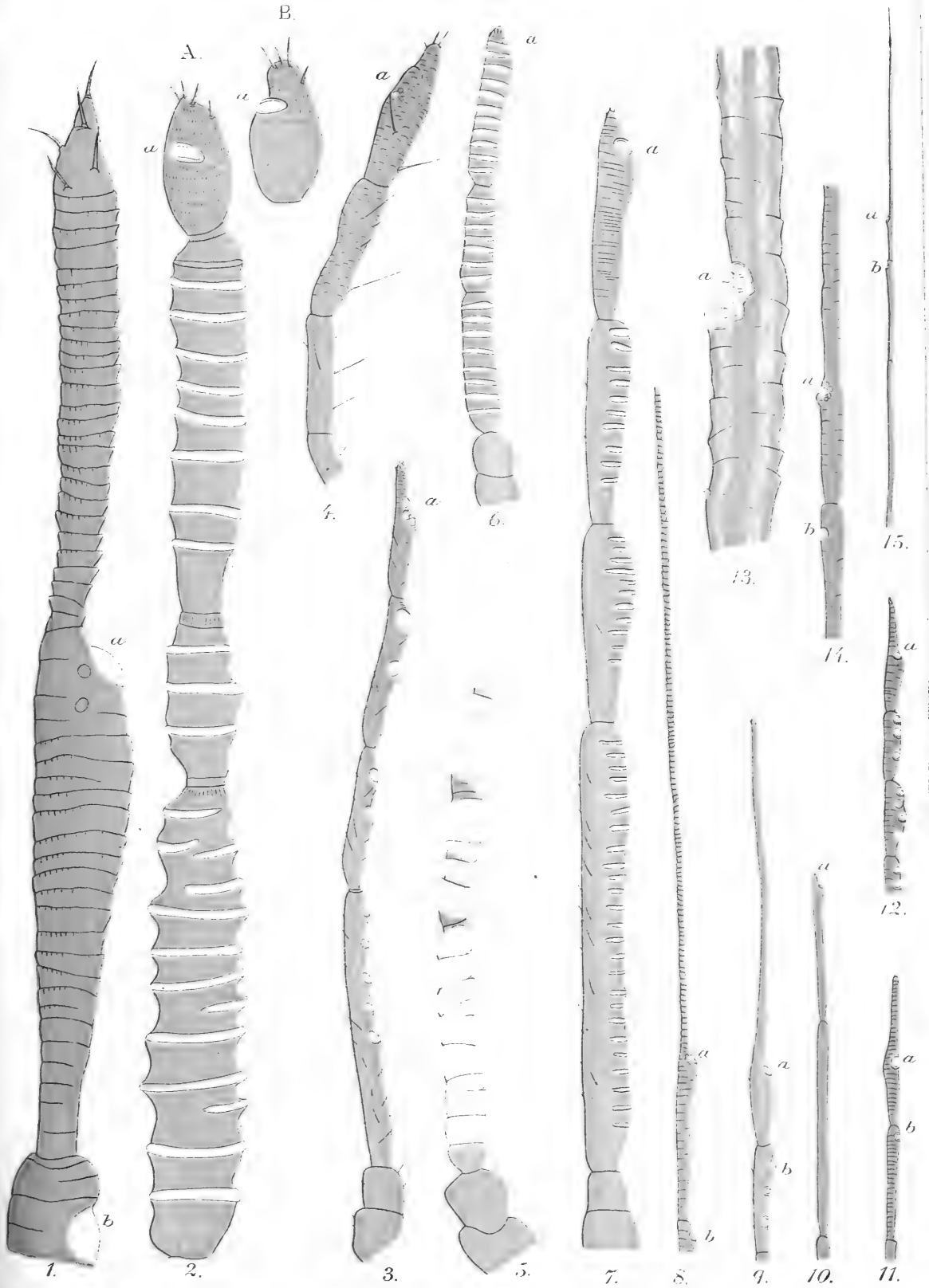
Fig. 13. A part of sixth joint of *Phorodon galeopsidis* Kalt., showing the sensoria upon it. (4×D.)

Fig. 14. Parts of fifth and sixth joints of the above species. (I×D.)
Fig. 15. Fourth to sixth of the same. (I×B.)

Plate III.

(Antenna.)

Fig. 16 *Hamamelistes sp.* (I×D.)
Fig. 17. *Astegopteryx nekoashii* Sasaki.
Fig. 18. *Schizoneura corni* Fab.
Fig. 19. *Schizoneura lanigara* Hans.
Fig. 20. *Chermes laricis* Hartig.
Fig. 21. *Aphis sp.*
Fig. 22. *Phorodon galeopsidis* Kalt.
Fig. 23. *Aphis brassicae* L.
Fig. 24. *Callipterus sp.*
Fig. 25. *Callipterus sp.*
Fig. 26. *Cryptosiphum artemisae* Buck.
Fig. 27. *Megoura viciae* Buck.



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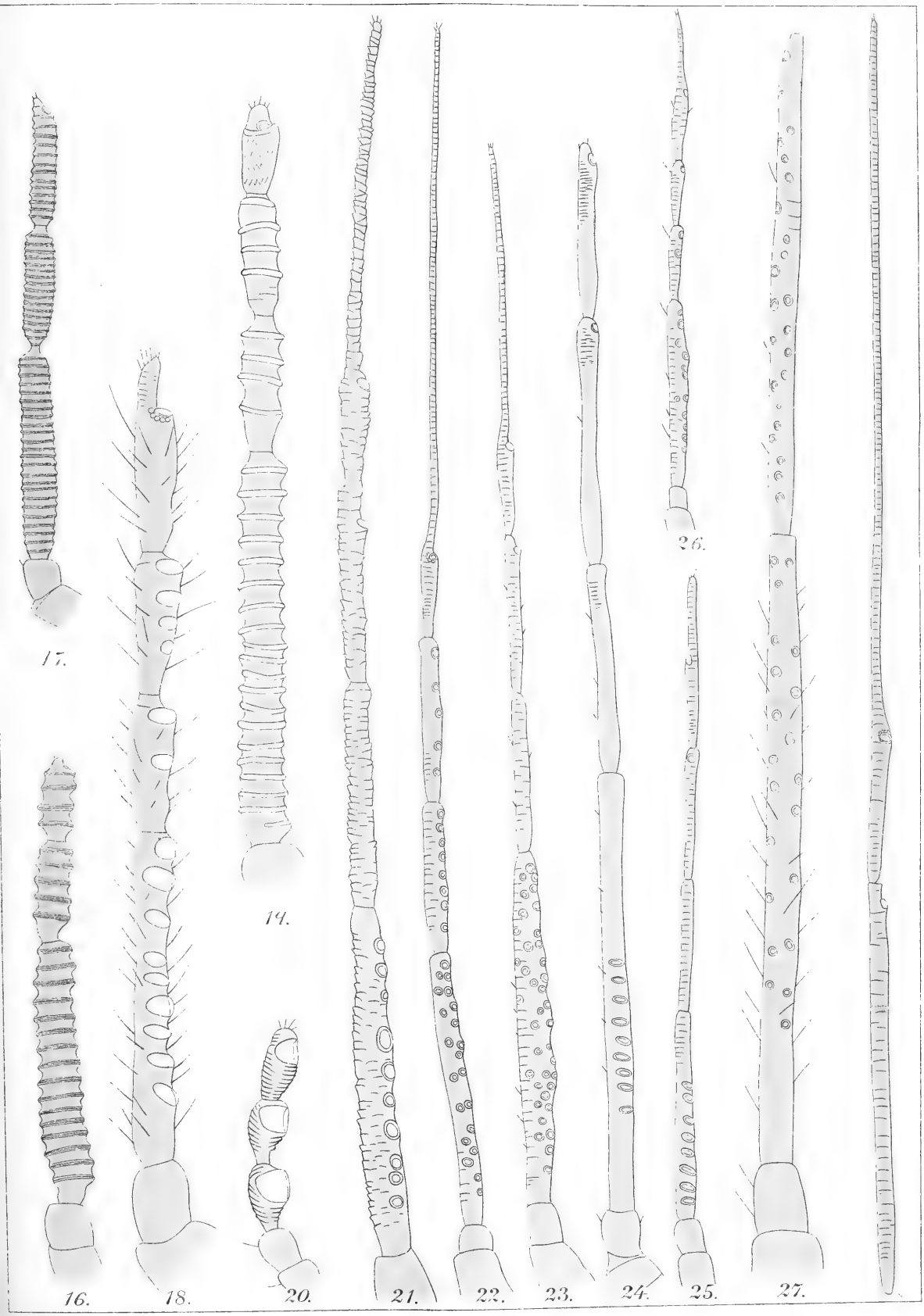

G. Okajima del.

Contributions to the Study of Japanese Aphididae.[1]

II. Three New Species of Trichosiphum in Japan.

BY

G. Okajima.

With Plates IV and V.

Species of *Trichosiphum*, a new genus created by Mr. T. PERGANDE for one of our plant-lice have lately been found in the entomological collection of the Imperial Agricultural Experiment Station at Tokyo. The plant-lice belonging to this genus are very peculiar in shape as well as in habit and deserve special attention. A plant-louse from Ceylon, which Prof. J. O. WESTWOOD[2] described and figured in 1890 under the name of *Siphonophora Artocarpi*, is in my opinion a species of *Trichosiphum*. In our *Trichosiphum*, the cornicles on the abdomen are hairy and exceedingly long (Figs. 16, 18, 20 and 21). The second oblique vein of the fore wing is curved in its outer half. The stigma is short and broad, but Prof. WESTWOOD appears to have erroneously figured the antennae as being eleven jointed. My studies of the insects are still incomplete and require further examination, but a few results obtained thus far may be briefly given.

Genus **Trichosiphum** Pergande, 1906.[3]

Rostrum long, reaching to the coxa of hind legs. Antennae nearly

[1] Contribution from the Zoological Laboratory.

[2] Trans. Ent. Soc. Lond. 1890. p. 649.

[3] Duplicate specimens of some Japanese Aphides were sent to Mr. T. PERGANDE, U.S. Department of Agriculture, by Mr. I. KUWANA, Imperial Agricultural Experiment Station at Tokyo. To one of them, the American entomologist gave this generic name with reference to the remarkable character of the cornicles. As no original description is at hand, I drawn up the generic as well as a specific characters (*Trichosiphum kuwanea*) according to my own observations. This genus has a very remarkable character, so that it may perhaps represent a new subfamily.

equal to the length of the body; first and second joints short, third joint longest, equal to the length of the three following taken together. The tubercles on the third joint are arranged in a single row along the entire length. Fourth and fifth equal in length. Sixth, the last, somewhat longer than the preceding two joints, and divided into two halves, of which the distal is slender and usually longer than the proximal. Compound eyes large. Supplementary eyes prominent. Wings laid vertically when at rest. Venation similar to that of the Gen. *Aphis* excepting the following points. Second oblique vein arising near the first and strongly curved for the last third of its length. Cubital vein lying parallel to the second oblique vein, obsolete at the base. Inframarginal cell very large. Cornicles exceedingly long, mostly straight, but sometimes slightly curved. Cauda blunt. Legs short and stout. The entire body and especially the long cornicles are covered thickly with hairs, which are never found in other genera, hence the generic name *Trichosiphum*.

Prof. J. O. Westwood[1] states that, "a striking character of the species (*Siphonophora Artocarpi*) consists in the enlarged size of the cornicles. These tubes are stated by Mr. Green to be carried diverging and elevated at an angle of 45°; they are sometimes as long as the whole remainder of the insect, and are strongly setose, the fine bristles set on nearly at right angles.....When alarmed the insects suddenly dropped from the leaves to the ground. They are very active, and walk rapidly." This description also applies to the genus before us.

1. **Trichosiphum kuwanea** Perg. (*Ō-kebakaarimaki*).

(Figs. 1-5, 15 and 16).

a. Winged viviparous female.

Expanse of wings	7.0 mm.
Length of body	2.7 „
„ „ antenna	2.0 „
„ „ cornicle	1.5 „

[1] Trans. Ent. Soc. Lond. 1890. p. 649.

Head large. First antennal joint large, third joint longest, provided with about thirty tubercles of various sizes. Fourth and fifth equal in length, the latter with a single tubercle near the apical end. Sixth equal to the length of the preceding two taken together. The distal part of the sixth twice as long as the proximal. Ocelli distinct. Compound eyes large, bright red. Supplementary eyes remarkably prominent. Rostrum long, reaching to the third coxa. Prothorax with a transverse black band. Mesothorax well developed, thoracic lobes large and black. Scutellem dark brown. Abdomen oval, with four blackish broad bands on the dorsal and ventral surface, and large black spots lying on the sides of each segment Cornicles very long, cylindrical, almost equal to the length of the thorax and abdomen. Cauda conical blunt. Wings ample. Cubitus brown. Stigma short, thickend, and grayish in colour. Hooklets on hind wings three or four. Legs short and stout, dark brown. Body looks dark brown and thickly covered with hairs.

This may, perhaps, be the female of the second generation or migrant form of the species. It is found forming small colonies on the young shoots of *Quercus serrata* Thumb. and *Q. acuta* Thumb. Appear in June. Habitat, Tokyo.

b. Apterous viviparous female (stem mother).

Length of body	.. ..	2.7 mm.
„ „ antenna	.. ..	1.6 „
„ „ cornicle	.. ..	0.6 „

Dark brown. Body much swollen, round in shape, ventral surface flat. Head comparatively small. Antennae similar to those of the winged form. Compound eyes bright red. Supplementary eyes prominent. Rostrum long, reaching to the third coxa, thorax small, abdomen hemispherical, cornicles short, somewhat sickle-like in shape. Cauda conical and blunt.

c. Pupa.[1]

Oval and somewhat corpulent. Antennae and compound eyes same

[1] Nymph in strict sense.

as in stem mother. Legs same as in the winged females. Cornicles short stick-like. Head, prothorax, wing case, legs and cornicles dark brown. Dorsal surface of the abdomen with numerous dark spots of variable size, symmetrically arranged on each side. Body hairy, pale brown with a slightly yellow shade.

2. **Trichosiphum tenuicorpus** nov. sp. (*Hosonaga-kebukaarimaki*).

(Figs. 6-10, 17 and 18).

a. Winged viviparous female.

Expanse of wings	5.6 mm.
Length of body	3.0 „
„ „ antenna	2.2 „
„ „ cornicle	3.0 „

Body dark brown or black, slender and almost linear. Head of moderate size. Antennae inserted upon a gibbous tubercle, nearly two-thirds as long as the body. First and second joint comparatively small. Third longest, as long as the three following taken together. Fourth and fifth equal. Sixth, the last, divided by a tubercle into two nearly equal halves. Tubercles on the third joint arranged in a single raw, about twenty in number. Compound eyes small, bright red. Supplementary eyes prominent. Rostrum long, reaching to the third coxa. Rectangular band on the prothorax black. Thoracic lobes black. Abdomen somewhat fusiform, and blackish. On the ventral surfce of the abdomen run two longitudinal lines, on the outside of which there are four dark spots on either side. Cornicles exceedingly long, nearly equal to the body, lying vertically, or sometimes horizontally. Legs short and black. Wings thin and delicate, venation as in the preceding species. Stigma black, very long, equal to two-third of the fore wing. First oblique vein comparatively broad and short. Infra-marginal cell large. Hind wings small and narrow, hooklets two. Found on the young shoots of *Pasania cuspidata* Oerst.

This form, I think, may be a migrant, though it has been captured

in September or later. In the colony I have found pupae and larvae of various stages, as well as a few apterous viviparous female; but no males or oviparous females have been met with.

b. Apterous viviparous female.

Length of body	2.1 mm.
„ „ antenna	2.0 „
„ „ cornicle	1.7 „

Body brown, pear-shaped, broader behind. Frontal tubercles gibbous, first antennal joint big, third longest, equal to the length of the three following combined, fourth and fifth equal, sixth divided into two halves, with a knee-shaped angle between, giving rise to the appearance of two different joints. Compound eyes bright red. Supplementary eyes prominent. Rostrum long reaching to the third coxa. Head and thorax equal in width. Abdomen broadens toward the caudal end. Cauda blunt. Cornicles equal to the length of abdomen. Legs short and stout. Body much covered with hairs, dirty brown.

c. Pupa.

Body slender, hairy. Pale brown to brownish yellow. Antennae, legs, cornicles and anal plate dark coloured. Antennae and cornicles equal separately to the length of abdomen. Compound eyes bright red. Legs short and stout.

3. **Trichosiphum pasaniae** nov. sp. (*Ko-kebukaarimaki*).

(Figs. 11-14, 19 and 20).

a. Winged viviparous female.

Expanse of wings	4.9 mm.
Length of body	1.6 „
„ „ antenna	1.1 „
„ „ cornicle	0.8 „

Body small, dirty brown. Head of moderate size. Antennae two-third as long as the body; first and second joints small, third longest,

equal to the two following joints combined, fourth equal in length to the fifth of the six-jointed antennae of the preceding species, short, with a tubercle near the apical end, fifth, the last, almost twice as long as the preceding, and divided as usual into two halves by an intervening tubercle. Distal half twice as long as the proximal. Tubercles on the third joint arranged along its entire length, about twenty in number. Compound eyes prominent and bright red. Supplementary eyes very prominent. Rostrum long, reaching to the third coxa. Transverse band on the prothorax black. Thoracix lobes black. Adbomen oval with a large irregular black patch on the dorsal surface, the three lateral patches black. Cauda blunt. Ventral surface of thorax and fifth abdominal segment black. Cornicles long, as long as the abdomen, slightly thickened towards the base in the distal two-thirds of their length. Wings ample, venation same as in the previous species. Stigma very long, almost equal to one-third of the costal margin of the fore wing. Hooklets of the hind wings two or three. Infra-marginal cell exceedingly broad. Legs short.

b. Pupa.

Oval, light brown or dirty yellow, tips of antennae, prothorax, cornicles, cauda, tibial end and tarsi darker coloured. Dorsal patches of the abdomen and the terminal antennal joint similar to those of *Trichosiphum kuwanea*. Cornicles somewhat sickle-shaped. Legs short. Found on the young shoots and underside of leaves of *Pasania cuspidata* Oerst. *Quercus serrata* Thunb. and *Q. acuta* Thunb. The colony is not so large and frequently found together with that of *Trichosiphum kuwanea*.

The antennae (Fig. 19) of this species are only five jointed. At first I was inclined to suppose that the species had more than five-jointed antennae, but careful examination of ten specimens (all in my possession) has made it clear that the antennae are normally 5-jointed in this species.

Synopsis of the three Species.

A. { Body slender, cornicles equal to or longer than the body length .....*tenuicorpus.*
Body oval, cornicles shorter than the body length.....B.

B. { Antennæ with five joints, stigma long.....*pasaniae.*
Antennæ with six joints, stigma short .....*kuwanea.*

The author expresses his cordial thanks to Prof. C. Sasaki to whose instructions and permission of the free use of the valuable literature in his possession this study is chiefly due. Thanks are also due for the kindly encouragements given him by Profs. C. Ishikawa, S. Goto and K. Toyama. Moreover I have great pleasure in acknowledging the friendly advices of Mr. I. Kuwana.

March, 1908.

Explanation of Figures.

Plate IV.

Fig. 1. *Trichosiphum kuwanea* Perg. Vivip. ♀.
Fig. 2. Ditto. Ventral side.
Fig. 3. Stem mother.
Fig. 4. Hook angle of the hind wing of the species, with four hooklets.
Fig. 5. Pupa or the last larval stage.
Fig. 6. *Trichosiphum tenuicorpus* nov. sp. Vivip. ♀.
Fig. 7. Ditto. Ventral side.
Fig. 8. Viviparous wingless female.
Fig. 9. Hook angle of the hind wing of the species.
Fig. 10. Pupa.
Fig. 11. *Trichosiphum pasaniae* nov. sp. Vivip. ♀.
Fig. 12. Hook angle with two hooklets.
Fig. 13. Ventral side of the winged viviparous female
Fig. 14. Pupa.

Plate V.

Fig. 15. Antenna of *Trichosiphum kuranoi* Perg.

Fig. 16. Cornicle of the same.

Fig. 17. Antenna of *Trichosiphum tenuicorpus*.

Fig. 18. Cornicle of the same.

Fig. 19. Antenna of *Trichosiphum pasaniae*.

Fig. 20. Cornicle of the same.

Fig. 21. Cornicle of *Siphonophora sp.* probably the longest among the subfamily.

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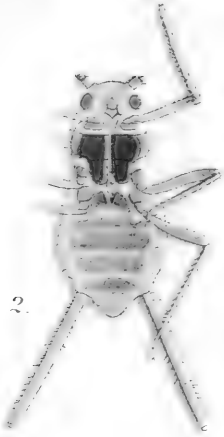

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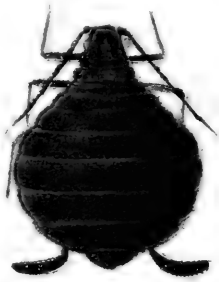

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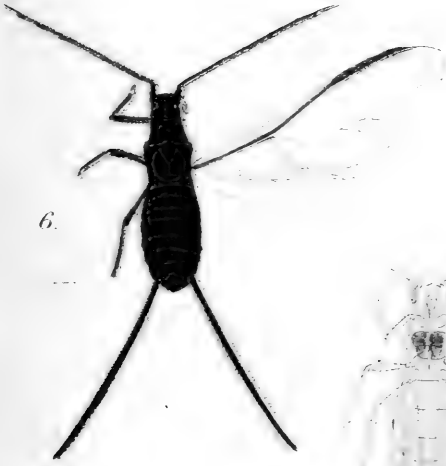

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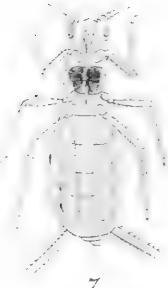

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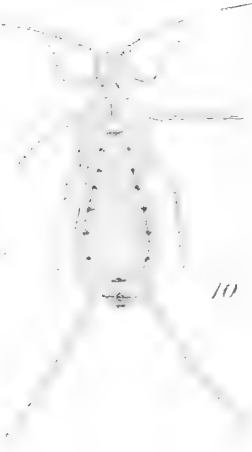

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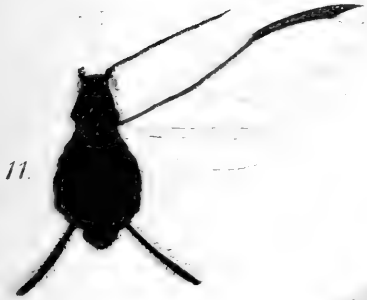

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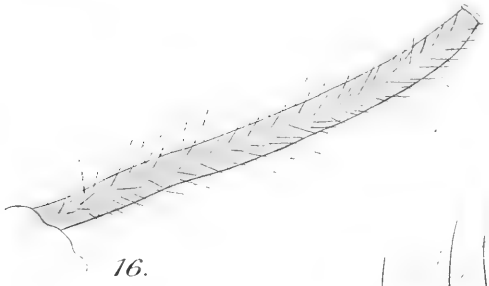

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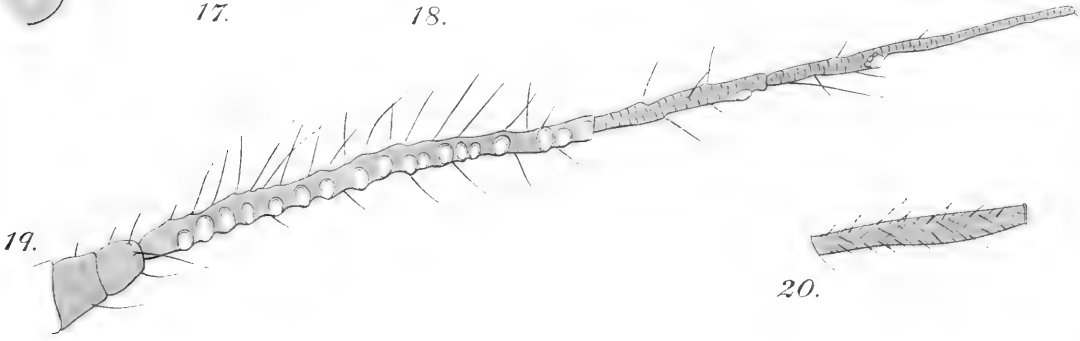

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Biology of the Chrysanthemum-Rust.[1]

BY

S. Kusano.

With one Figure in the Text.

Black Rust (*Puccinia Chrysanthemi* Roz.).

Some years ago a most dreadful rust appeared suddenly in several countries of Europe and America, and furiously spread in a short interval of time, inflicting no inconsiderable damage upon chrysanthemum-cultivation.[2]

Although it was thought at that time that the rust was imported from Japan, where the same rust had long been known to attack the same plant, yet the correct specific name of the fungus has remained undetermined. Some held the view that it was identical with *Puccinia Hieracii* Mart[3] while others took it for *P. Tanaceti* De. or *P. Balsamitae* (Str.) Rbh. Roze after a more careful study has, however, arrived at the conclusion that it was different from any known species, and consequently called it *P. Chrysanthemi* n. sp.[4]

Subsequently Hennings described the Japanese species as new to science under the name of *P. Chrysanthemi chinensis*[5] which, however, P. Sydow has identified with the known species, *P. Pyrethri* Rab.[6] This

[1] Contribution from the Botanical Laboratory. A short account has already appeared in Japanese in Bot. Mag., Tokyo, Vol. XVIII, 1904, p. 99.

[2] See Jacky, E., Der Chrysanthemum-Rost. Zeitschr. f. Pflanzenkrankh., Bd. X, 1900, p. 132.

[3] Massee, G., Chrysanthemum-Rust. Gardener Chron., Vol. II, 1898, p. 269.

[4] Roze, E., Le Puccinia Chrysanthemi, cause de la Rouille du Chrysanthemum indicum L. Bull. de la Soc. myc. de la France, T. XVI, 1900, p. 88.

[5] Hennings, P., Einige neue Japanische Uredineen. Hedwigia, Bd. XL, 1901, p. (26).

[6] Sydow, P., Monographia Uredinarum, I, p. 45.

confusion in the identification of the chrysanthemum-rust seems to have arisen from the small morphological difference exhibited by the allied species of the genus. JACKY[1] then undertook a comparative study of Japanese and European rusts. His infection-experiments proved that the rust of *Chrysanthemum indicum* L. did not infect other *Chrysanthemum* or other allied plants of Compositae, except *Chrysanthemum sinense* Sab., the natural host of the rust in Japan, and that the Japanese rust of *Chrysanthemum sinense* could easily infect *Chrysanthemum indicum,* the host of the European rust. There being no morphological difference both in uredo and teleutopores taken from both hosts, he came to the conclusion that the Japanese rust was identical with the European, and hence he referred it to *Puccinia Chrysanthemi* Roz.

But curiously enough, there exists a great difference between the Japanese and European rusts in their mode of development. The European species repeats the uredogenerations through the year, and the uredospores can winter on the young shoots of the host kept in the house. The formation of the teleutospores is exceedingly rare. When they are formed, mesospores accompany them invariably. Further, we have in almost all cases variously formed and two-celled uredospores in the European species. As JACKY noted, the formation of mesospores and two-celled uredospores, abnormal as it may be, is almost constant[2]. In the Japanese species such cases seem to occur very seldom. In Tokyo and its vicinity the uredogeneration is regularly followed by the teleutostage, and the hibernating teleutospores germinate at once in the early spring. The following is the development of the rust observed by myself in Tokyo.

The first appearance of the uredosori takes place at the end of May or at the beginning of June, when the host has attained the height of 30cm. or thereabout. Among several garden-varieties of the host-plant placed side by side a certain variety was first attacked by the rust. At the Botanic Garden of the Agricultural College, Komaba, it was observed

[1] JACKY, *loc. cit.*: ——. Der Chrysanthemum-Rost. II. Centralbl f. Bakteriol., II. Abt., Bd. X, 1903, p. 369.

[2] JACKY, *loc. cit.* Centralbl. f. Bak.

that a variety called "Ōmihakkei" first produced the uredosori on its lower leaves. The rapid spread of the rust later on to other varieties seemed to be due to infection from this source. At the beginning of July almost all the varieties were attacked by the rust.

A most careful examination has failed to reveal any spermogonium in the first generation of uredosori, so that it is most probable that the fungus belongs to Hemipuccinia.

In the autumn, when the host approaches its flowering season, the uredostage is followed by the teleutostage. I have found it at the beginning of October. Afterwards the teleutosori spread over most rapidly from the lower portions of the stem up to the bracts of the heads.

It may be noticed that in the first generation of the teleutostage the sori form a ring of 1.5-3.0 mm. in diameter around each uredosorus of the last generation, so that there is no doubt that the teleutosori may originate from the mycelium around the uredosorus. Numerous isolated teleutosori developed afterwards seemed to originate from the urdeospores of the year.

In early winter, when the stems begin to wither from injury by the frost, young shoots appear from the mother-stock. The fungus then invades these vigorous offsprings perhaps by means of the uredospores, and forming there the teleutosori it can winter on the living host. Though rare, a few new teleutosori made their appearance even in the midst of the winter (end of January), but later the formation of the sori seemed to have ceased entirely.

The teleutospores on the dead host of the last year germinated at once in April. The uredosori which, as stated above, suddenly appear at the end of May probably owe their origin to the sporidia thus produced from the teleutospores.

Such being the course of development of the rust in Tokyo, I will now give a few observations made in a warmer region in Japan. At my request Mr. Yoshinaga has made some observations since a few years on the development of the rust in Prov. Tosa, and he has kindly put

the specimens he has collected at different localities from time to time at my disposal. An examination of these specimens shows beyond doubt that the rust produces in these warm coastal localities, as for instance Akimachi and Kōchi[1], only uredosori generation after generation through the year without the formation of teleutospores even in the midst of the winter. It suggests to us the possibility of the uredospores retaining in these localities their germinating power through the winter and becoming a new starting point for the rust in the next year. This must be admitted to be perfectly possible, as the uredospores exposed to -25° C. may retain, according to JACKY[2], their germinating power.

As in this way the rust develops in the coastal region of Tosa just as in Europe without the formation of the teleutospores, I have given special attention to the abnormal formation of spores at the localities mentioned above. In the specimens collected at Kōchi[3], December 13, 1907[4], which consisted entirely of vigorously developed uredosori, I could without difficulty find numerous abnormal and two-celled uredospores, as in the European specimens. Further, the formation of mesospores was ascertained at somewhat colder localities in Tosa. Mr. YOSHINAGA has already found in November, 1901 abundant teleutospores at Sakawamachi[5], 4 *ri* (*ca.* 10 miles) distant from the sea-shore, and quite recently he has collected them again in a mountainous region Ujimura, 1.5 *ri* west from Kōchi on December 15, 1907[6]. In both specimens the teleutospores were developed as much as in those of Tokyo, but their form was much irregular, and the sori contained mesospores. These facts show clearly that the abnormal formation of spores is not a characteristic of the rust introduced to Europe, but takes place in its native country, though not constantly.

As regards the constant formation of abnormal spores in Europe

[1] The frost is rare in these localities.

[2] JACKY, *loc. cit.* Zeitschr. f. Pflkrkh., Bd. X, p. 141.

[3] A town near the sea-shore.

[4] In February the host withered away without forming any teleutosorus.

[5] HENNINGS, Fungi japonici. V. Engler Bot. Jahrb., Bd. XXXIV, 1905, p. 595.

[6] At a place a little nearer to Kochi he found on the same day only uredosi.

several views may be proposed. JACKY remarked about this point, "es dürfte die Bildung derartig anormaler Sporen vom Standort und der Beschaffenheit der Nährpflanze abhängig sein."[1] That the rust develops differently in Tokyo and Tosa makes it probable that the formation of abnormal spores is due to the influence of the locality where the fungus occurs, but the exact nature of this influence remains still unknown. In Japan the cessation of the development of the teleutospores in the coastal region of Tosa is probably due to the much warmer climate there, but we can not as yet conclude on that account that the climate is the cause of the abnormal formation of spores.

Next we may ask whether the rust on *Chrysanthemum indicum* in Japan develops differently from that on *C. sinense*, as it does in Europe. In Japan I endeavoured to get the specimen of the rust on *C. indicum*, but as most of the cultivated chrysanthemums belong to *C. sinense*, it was not easy for me to find abundant material for study of the rust on the desired plant. However, I was fortunate enough to find a few diseased leaves of this plant[2] in the herbarium in the Botanical Institute, Science College, Tokyo Imperial University. The plant was collected by T. MAKINO at Mt. Hie, Prov. Yamashiro on Nov. 7, 1904. The teleutospores were found abundantly as on *C. sinense*, but neither mesospores nor two-celled uredospores were found, showing that the rust may develop in the same way on *C. indicum* and *C. sinense*.

In this connection I may mention the rust on a wild chrysanthemum, *Chrysanthemum Decaisneanum* Max. This is a coastal plant limited to the warmer region. At the beginning of January, 1907, I collected the rust on it at Hanemura, a coastal region in Prov. Tosa. At that time the host had nearly withered from frost, but the rust was present all over. On bringing it back to Tokyo I found that the sori were almost entirely occupied by uredospores, of which the form, structure of the wall, and

[1] JACKY, *loc. cit.* Centralbl. f. Bakt., Bd. X, p. 375.

[2] *Chrysanthemum indicum* L. var. *genuinum* Max.

the number of the germ-pores did not differ from those of the uredospores of the typical *Puccinia Chrysanthemi*. Exceedingly rarely, I found a few teleutospores whose morphology coincided also with that of the teleutospores of *P. Chrysanthemi*. Moreover, remarkably enough I could find the irregular and two-celled uredospores, and numerous mesospores.[1] So that the rust on *Chrysanthemum Decarisneanum* is quite the same in every respect as that on *C. indicum* in Europe. This gives a strong evidence for the assertion given above that the abnormal development is by no means characteristic of the European rust, but may take place in its native country.

We see thus a great variation in the development of the rust in different localities in Japan. In the coastal region of Tosa irregular and two-celled uredospores are very common, while the teleutospores develop sparingly. In inland localities the rust has a tendency to develop the teleutospores regularly, and inhibit the formation of abnormal uredospores. In Tokyo and its vicinity the formation of these abnormal spores ceases apparently, while the teleutospores follow the uredospores. It also seems probable that the chrysanthemum-rust now so widely found originated from *Chrysanthemum Decaisneanum*, on which the warmer climate brought about the inhibition of the formation of the teleutospores; it then spread to the cultivated chrysanthemum, retaining still the character it has assumed on *C. Decaisneanum*. As it spread wider and wider on the cultivated chrysanthemum in much colder regions it acquired the character of forming the teleutospores on the one hand, and inhibited the formation of abnormal spores on the other. This character has becomes nearly fixed in Tokyo and its vicinity, but in Tosa, where the occasional infection of *C. sinense* by the rust of *C. Decaisneanum* is possible, the character of the rust can still be observed unchanged on the former. That the direct influence of the environment is not concerned on this point may be partly demonstrated by JACKY's infection-experiments.[2] He succeeded in getting

[1] The difference between the sea-shore and inland forms was also described by ARTHUR in *Cromyces acuminatus* Arth. (ARTHUR, J. C., The Uredineae occurring upon Phragmites, Spartina, &c. in Bot. Gaz., Vol. XXXIV, 1902, p. 1).

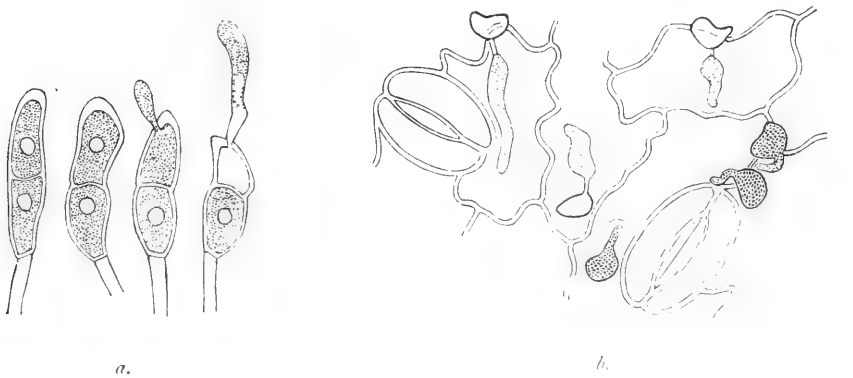
[2] Centlbl. f. Bakt., Bd. X, p. 370.

abundant teleutosori by infecting *C. indicum* with the spores of the Japanese rust,[1] but not with the European rust, which shows that there is no immediate change in the developmental habit of the Japanese rust when transferred to Europe.

The specific name of the host in Europe still remains a question for Japanese botanists. It is there assumed to be *Chrysanthemum indicum*, but if it has all been imported from Japan, we have a strong ground for believing it to be *C. sinense*. The Japanese chrysanthemum with large flowers belongs exclusively to *C. sinense*, though some small-flowered forms belong to *C. indicum*, which is, however, cultivated very little. Hence it is very probable that the host of the rust is the same species in Japan and abroad, being either *C. sinense* or *C. indicum*, so that there is but little doubt about the identity of the Japanese chrysanthemum-rust with the European

White Rust (*Puccinia Horiana* P. Henn.).[2]

This is another chrysanthemum-rust recently made known botanically, though gardeners seem to have been acquainted with it long since. It



a. *b.*

Fig. 1. *a*, teleutospores; *b*, germination of sporidia on the leaf of the host (3 days after sowing). ×400.

[1] The materials were collected by N. Nambu at Urawa near Tokyo on December 4, 1900.

[2] Hennings, P., Einige neue japanische Uredineen. Hedwigia, Bd. XL, 1901, p. (25).

is characterised by having large, white, waxy sori, 2-3 mm. in diameter on the undersurface of the leaf. The sori consist entirely of comparatively small thin-walled teleutospores which germinate as soon as they become mature (Fig. 1*a*). The rapid propagation of the rust is due to infection by the sporidia thus produced. Sown on a leaf of the host kept in a moist chamber, they soon produce germinating tubes which penetrate through the outer wall of the epidermis, and send out swollen haustoria, or intercellular mycelia (Fig. 1*b*). As no spermogonium has ever been found, the fungus should be referred to Leptopuccinia.

The development of this rust proceeds almost without interruption through the year, though its activity varies more or less according to the condition of the host and the condition of the environment. In Tokyo the host-plant suffers most generally in May or June, while it is still young, and the propagation of the rust seems to be exceedingly rapid in a moist place, or where the host-plants are densely planted. The rainy seasons are especially favourable for the development of the fungus: in Tokyo they occur in the spring, early summer, and September. The intimate connection of the development of the rust with moisture is due to the circumstance that the teleutospores and sporidia germinate immediately after maturation.

When the old stems of the host die in the autumn, the rust directly attacks the new shoots, and young and old sori, though not vigorous in development, can pass the winter in the field. There is no doubt that low temperature checks the development of the fungus, but the ripe spores can resist it, and germinate soon when favourable conditions return the next spring.

The white rust is more injurious than the black rust because of its attacking young hosts and of its rapid propagation. However, many forms of *Chrysanthemum sinense* are quite free from its attack. The edible variety of the host-plant called "Ryorigiku" is the one which is most commonly attacked. In the Botanic Garden, Koishikawa, Tokyo, a form called "Kiazami," perhaps closely allied to "Ryorigiku," is also attacked every year. Further, we have observed at several localities that

the rust is found on *Chrysanthemum sinense* Sab. var. *japonicum* Max. ("Ryūnōgiku"), the common wild chrysanthemum. In Tosa it is also found on *C. Decaisneanum* together with other rusts.

Bordeaux mixture is very effective, when used repeatedly, in preventing the propagation of the rust.

Brown Rust (*Uredo autumnalis* Diet.)[1]

This rust is characterised by its light brownish colour. The sori are very small, and appear densely scattered over the surface of the host. In contradistinction to the rusts previously described the sori are produced much more on the upper than on the under surface of the leaves. So far only the uredosori have been observed, but it is probable that they represent a stage of certain *Puccinia*. In external form the present rust is hardly distinguishable from *Uredo Artemisiae japonicae* Diet[2] widely distributed on *Artemisia*. The character of the uredospores is also like that of the latter rust, having a faintly coloured thin wall. However, in the materials examined by me the sori are not provided with paraphyses, which occurs invariably in *Uredo Artemisiae japonicae*, as DIETEL has already observed.[3]

The mode of wintering has not yet been ascertained, but it is most probable that the uredospores can winter without losing their germinating power, as they do in *Puccinia Chrysanthemi* in Europe. This must be quite possible in warmer regions like Tosa where it occurs commonly. In the vicinity of Tokyo the rust is only known on *Chrysanthemum sinense* var. *japonicum*, but in Tosa it occurs on several chrysanthemums, viz. *C. Decaisneanum*, *C. sinense* (cultivated form), and *C. indicum*. From the communication of Mr. YOSHINAGA we know that the cultivated chrysanthemums there suffer as much from this as from the black rust.

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[1] DIETEL, P., Uredineae japonicae. VI. Engl. bot. Jahrb., Bd. XXXVII, 1905, p. 108.

[2] DIETEL, P., Uredineae japonicae. V. Engl. bot. Jahrb., Bd. XXXIV, 1905, p. 591.

[3] DIETEL, P., Uredineae japonicae. VI, p. 108.

We see from the observations described above that all the rusts known to occur on *Chrysanthemum* attack *Chrysanthemum Decaisneanum* in Tosa, where they also have a tendency to occur on *C. sinense*, but in Tokyo and its vicinity they show more or less specialisation among themselves with regard to their hosts: thus *Puccinia Chrysanthemi* occurs on *C. sinense* and *C. indicum*, *P. Horiana* on certain garden-varieties of *C. sinense* and on *C. sinense* var. *japonicum*, and *Uredo autumnalis* on *C. sinense* var. *japonicum* only. We also see that the rust on the cultivated chrysanthemum in Europe and that on *Chrysanthemum Decaisneanum* in Japan show no difference so far as their mode of development is concerned. These facts justify our assumption that the chrysanthemum-rusts now occurring on several hosts have originated from *C. Decaisneanum*.

Notes on Japanese Fungi.

V. *Puccinia* on the Leaves of Bambuseae.[1]

BY

S. Kusano.

With Plate VI and one Figure in the Text.

Five species of *Puccinia* have been known to occur upon the leaves of bamboo-plants, of which two, viz. *P. longicornis* Pat. et Hariot and *P. Kusanoi* Diet., are indigenous to Japan, while the rest are exotic.[2] My attention has been directed to this subject for some years and an exhaustive examination of all the species of bamboo to which I have been able to get access has enabled me to add two more species and one variety still undescribed. To a description of these indigenous rusts I propose to append a complete list of their hosts, which now amounts to eighteen in number, belonging to three genera (*Phyllostachys*, *Arundinaria*, and *Sasa*), and thus place the relation of the rust to the several species of its host in a clear light. As is well known, the vegetative organs of the different species of bamboo exhibit hardly any distinctive features characteristic of each, while the floral organs, the most important for the study of affinity and specific characters, are developed exceedingly rarely. On this account, although the study of our bamboo-plants made recently by Makino and Shibata[3] from the systematic and anatomical standpoints

[1] Contribution from the Botanical Laboratory.

[2] *Puccinia Arundinariae* Schw. in America (Syn. Fung. Car., 1822, p. 72; Sacc., Syll. Fung., Bd. XIV, p. 355; Bot. Gaz., Vol. XXXIV, 1902, p. 1; Syd., Monogr. Ured I, p. 731), *P. xanthosperma* Syd. in East India (Ann. Myc., Bd. IV, 1906, p. 437), and *P. melanocephala* Syd. in East India (Ann. Myc., Bd. V, 1907, p. 500).

[3] Makino, T., Description des Produits forestiers envoyés à l'Expostion universelle de 1900 à Paris par le Ministère de l'Agriculture et du Commerce;—, Bambusaceae Japonicae. Bot. Mag., Tokyo, Vol. XIV, 1900; Shibata, K., Beiträge zur Wachstumgeschichte der Bambusgewächse. Journ. of Coll. Sci. Tokyo Imp. Univ. Vol. XIII, 1900; Makino, T. and Shibata, K., On Sasa, a new genus of Bambuseae, and its Affinities. Bot. Mag., Tokyo, Vol. XV, 1901, p. 18.

has rendered the systematic position of each species very definite, it seems to me that an account of the specialisation of the rust on certain species of bamboo is important from a practical point of view for the determination of the genera and species, in case only their branchlets or leaves are accessible.

In order to avoid any possible error regarding the specific name of the hosts, due to an examination of sterile specimens in a dried state, I have consulted as far as possible living specimens which had been identified by Mr. MAKINO, the authority on Japanese bamboo. They are all cultivated in the Botanic Gardens both of the Science College and the Agricultural College in Tokyo, with the single exception of *Sasa ramosa* which is found flourishing in Sōma in Prov. Iwaki.

I desire in this place to acknowledge my indebtedness to Prof. MIYABE who has generously put at my disposal the rich herbarium specimens of the Museum of the Agricultural College of Sapporo, which have been particularly useful for a study of the distribution of the fungi concerned.

Puccinia Phyllostachydis Kusano n. sp.

(Figs. 1, 2, 10, 11.)

Uredosori hypophyllous, isolated, early becoming naked, pulverulent, ferruginous; paraphyses hyaline, swollen and thick-walled at the top, with a septum near the top[1]; spores obovate, 30-33×23-25 μ; epispore brown, coarsely echinulate; pores 4 (rarely 5), equatorial.

Teleutosori hypophyllous, isolated, scattered, prominent, round, compact, 0.5 mm. in diameter, dark brown or black; spores oblong, rounded at the upper end, slightly tapering towards the lower end, constricted at the septum, wall scarcely or not thickened at the apex, finely dotted, brown, 45-70×15-20 μ; pedicel persistent, slender, hyaline, more than 150 μ long.

Common in various places in Tokyo.

[1] I can not yet exactly decide whether they are young sporophores or not.

On *Phyllostachys bambusoides* Sieb. et Zucc. (= *Ph. megastachya* Steud. *Ph. macrantha* Sieb. et Succ., *Ph. Quirioi* Riv., *Ph. Mazeli* Hort.) B.G.[1]; Komaba; Kyoto*[2] (Y. Takahashi, July 4, 1895; N. Hiratsuka, June 12, 1895).

Phyllostachys bambusoides Sieb. et Zucc. var. *aurea* Makino (=*Ph. aurea* Riv.). B.G.

Phyllostachys bambusoides Sieb. et Succ. var. *Marliacea* Makino (=*Ph. Marliacea* Mitf.). B.G.

Phyllostachys bambusoides Sieb. et Zucc. forma *Kasirodake* Makino. B.G.

In general feature the teleutospores appear to resemble those of *Puccinia Arundinariae* Schw. with thin-walled apex, judging from the figures of J. C. Arthur,[3] but the present species is distinguished by the presence of paraphyses in the uredosori.

It is a very interesting fact that this species is confined to the four forms above mentioned, which according to Makino must all be referred to *Ph. bambusoides*.[4] As may be seen from the synonyms, these had been regarded as so many different species, but the specialisation of *Puccinia Phyllostachydis* on these bamboos points to a more close relationship among themselves in contrast to the others, and so Makino's revision appears justified from the mycological point of view.

When the branchlets and leaves alone are taken into consideration, *Phyllostachys bambusoides* var. *aurea* resembles very closely *Ph. puberula* Munro, but the occurrence of the rust, if any, will enable one to distinguish the two.

[1] B. G.=Botanic Gardens, Koishikawa, Tokyo.

[2] *=Dried specimen.

[3] J. C. Arthur. The Uredineae occurring upon *Phragmites*, *Spartina*, and *Arundinaria* in America. Bot. Gaz., Vol. XXXIV, 1902, p. 1.

[4] The genus *Phyllostachys* includes 4 species and a number of varieties. Bot. Mag., Tokyo, Vol. XIV, 1900, p. (61).

Puccinia longicornis Pat. et Hariot.

(Figs. 3, 4.)

Bull. Soc. Myc. France, 1891, p. 143; Sacc., Syll. Fung., Bd. XI, p. 200; Syd., Monogr. Ured., I, p. 734; —, Exic. Ured., n. 1314.

This is a species of common occurrence. As far as my observations go, it is found every year in the Botanic Garden at Koishikawa and other localities in Tokyo. The formation of the teleutospores at a certain season of the year is so vigorous that they form solid, ferruginous to dark brownish, orbicular pustules of 1 mm. or sometimes more in diameter, and cover densely the under surface of the leaves, doing much damage to the host.

In general features the uredosori resemble those of the preceding species. They are however characterised by having clavate paraphyses (Fig. 4). The spores are globose or oval, and coarsely echinulate. Four or rarely five germ-pores are present in the equatorial plane.

The typical teleutospores are elongated, fusiform, well constricted at the septum, and tapering at both ends. Some deviating forms resemble those of the typical spores of *P. Kusanoi*, or its variety to be described below (Fig. 3*a*, *b*). The distinctive character of the spore is the extraordinary thickening of the wall of the apex so as to form a long papilla of 32 μ in length. The typical ones measure 80-100 × 15-20 μ. Pedicel is hyaline, slender, and more than 200 μ long.

Only two species of bamboo have so far been ascertained as host:—

Sasa paniculata Makino et Shibata (=*Bambusa tessellata* Munro,[1] *B. palmata* Marl., *B. paniculata* Makino, *B. senanis* Franch. et Sav., *Arundinaria palmata* Bean, *A. kurilensis* var. *paniculata* Fr. Schw., etc). B.G.; Sapporo* (T. Miyake, May 4, 1902).

Arundinaria japonica S. et Z. B.G.; Nagoya in Prov. Owari* (Y. Takahashi, July, 1895).

1 Dietel, Uredineae Japonicae. I. Engl. bot. Jahrb., Bd. XXVIII, 1899, p. 568.

The fact that the present rust is confined to the two hosts mentioned above seems to show the close relationship of the latter, though they are now included in different genera. The bamboos now included in *Sasa* were formerly considered to belong to *Bambusa* in the section Eubambuseae, but their anatomical as well as morphological characters necessitated a revision, and thus a new genus *Sasa* was erected conjointly by MAKINO and SHIBATA.[1] To this genus are now referred eight species which were all formerly included in *Bambusa*. Taking the rust as our guide it appears that *Sasa* and *Arundinaria* are allied genera. The above authors have already remarked that *Arundinaria japonica* is a somewhat aberrant form in the genus, and stands rather close to *Sasa* in the character of the culm and leaves, and in having at times more than 3 stamens[2]: it is in fact a transitional form between the two genera. Hence it is not strange that *Arundinaria japonica* should harbour the rust apparently peculiar to *Sasa*, instead of the one which occurs commonly on several species of *Arundinaria*.

Puccinia Kusanoi Diet.

(Figs. 5, 13, 14.)

Engl. bot. Jahrb., Bd. XXVIII, 1899, p. 568; SACC., Syll. Fung., Bd. XVI, p. 309; SYD., Monogr. Ured., I, p. 732; —, Exic. Ured. n. 1313, 1373.

(=*Uredo Arundinariae* Syd.). Hedwigia, 1898, p. (208); SACC., Syll. Fung., Bd. XIV, p. 406; Syd., Exic. Ured. n. 1239.

(=*Puccinia Bambusae* Schroet. in herb.)

This rust is most common owing to the wider distribution of its host. It is found mostly on species of *Arundinaria*, thus:—

Arundinaria Simoni A. et C. Riv. B.G.: Tokyo* (S. HORI, March 19, 1891; K. MIYABE, Sept., 1889; K. ŌNUMA, April 30, 1883; K. SENGOKU, Nov. 25, 1895, Jan. 14, 1896; T. NISHIDA, Oct. 24, 1899);

[1] MAKINO, T. and SHIBATA, K., *loc. cit.*

[2] *Phyllostachys* and *Arundinaria* have 3 stamens, while in *Bambusa* and *Sasa* they are 6 in number.

Ōmiya near Tokyo* (N. Ichikawa, Oct., 1886); Chiba in Prov. Kazusa* (K. Sengoku, Jan. 1, 1896); Hakone* (S. Hori, April, 1891; K. Miyabe, April 12, 1901); Gifu* (N. Tokubuchi, Oct., 1889, Dec. 3, 1898).

A. Simoni A. et C. Riv. var. *Chino* Makino. B.G.; Komaba; Sendai* (K. Sengoku, Oct. 5, 1895).

A. Simoni A. et C. Riv. var. *variegata* Hook fil. B.G.

A. variabilis Makino var. *viridi-striata* Makino (=*A. Fortunei* Riv.[1]). B.G.

A. variabilis Makino var. *Tanakae* Makino. Komaba.

A. variabilis Makino forma *foliis pubescens* Makino. Hirosaki in Prov. Mutsu* (N. Hiratsuka, Oct. 26, 1896); Shiroishi in Prov. Iwaki* (Y. Takahashi, Aug. 1, 1895); Nagoya in Prov. Owari* (Y. Takahashi, July 19, 1895).

A. variabilis Makino forma *foliis glabris* Makino. Tokyo* (Y. Takahashi, July 21, 1895).

A. Narihira Makino. B.G.; Komaba.

A. Narihira Makino forma *Yashadake* Makino. Komaba.

Sasa sp. (*S. nipponica* Mak. et Shib.?). B.G.; Prov. Rikuchū* (Y. Takahashi, Nov. 7, 1897, May 13, 1893).

Thus, out of eight species of our native *Arundinaria*[2] the fungus occurs on three species and their varieties. Of these hosts, the first two are most widely distributed, from south to north, especially however in the central part of Japan, and are everywhere subject to attack by the rust.

The uredosori do not differ essentially in appearance from those of the other species, except that they lack paraphyses which characterise two species described before. The spores are obovate to subglobose, with coarsely echinulate wall. The size varies, more or less, according to the host. For instance, it is smaller on *Arundinaria Simoni*, measuring 20 × 25 to 21 × 30 μ, but on the other hosts it may attain

[1] Engl. bot. Jahrb., Bd. XXVIII, 1899, p. 569.

[2] Bot. Mag., Tokyo, Vol. XIV, 1900, p. (61).

to 27×28 or even 27×35 μ. There are apparently three or four germ-pores in the equatorial plane.

The teleutospores form solid, firm, orbicular sorus just as in the preceding species. The typical ones are oblong or fusiform, rounded at both ends, the wall conically thickened at the apex. They measure 61×17 μ or thereabout, but there may occur even in the same sorus very short and broad spores measuring from 42×22 to 40×20 μ. Moreover, slight variation in form and size may occur according to different hosts. For instance, on *A. Narihira* forma *Yashadake* large spores predominate which may measure as much as 88×16 or 70×15 μ. Sometimes they are linear oblong, not constricted at the septum and tapering gradually at both ends. On *A. Simoni* and its varieties *Chino* and *variegata* the constriction at the septum is somewhat conspicuous; on *A. Narihira* the apex of the spore is rounded, the constriction is more obvious, and the shorter ones are more predominant; and on *A. variabilis* var. *viridi-striata* and *A. Narihira* forma *Yashadake* larger ones with a slight constriction at the septum are more usual.

With respect to the occurrence of the rust upon a species of *Sasa* a question arises again concerning the affinity of *Arundinaria* and *Sasa*, and also concerning the specific name of *Sasa* in question. The plant has been cultivated for a long time in the Botanic Garden of Tokyo but has never produced floral organs. Mr. Makino inclines to the view that it is *Sasa ramosa* Makino et Shibata, though the sterile specimens do not show distinctly any character to distinguish it from *Sasa nipponica* Makino et Shibata. As the rust on the *Sasa* in question is somewhat different from that on the typical *Sasa ramosa*, which will be given below, I believe it better to assume at present the questionable *Sasa* as *S. nipponica*.

The rust on *S. nipponica* bears a more close resemblance to that on *Arundinaria Narihira* forma *Yashadake* (Fig. 5 *c-f*). The uredo and teleutosori are smaller than on other hosts; the oval uredospores measure from 40×20 to 42×25 μ; the teleutospores are long and regular in form, well constricted at the septum; and the typical ones measure

from 72 : 19 to 65 × 20 μ, but deviating ones may be 80 × 17 — 55 × 20 μ. The general characters of the rust on *S. nipponica* are therefore quite the same as those of the rust on most *Arundinaria*.

This singular fact indicates that some species of *Sasa* may be closely allied to the genus *Arundinaria*. As has been ascertained from anatomical as well as floral characters[1] certain species of *Sasa* resemble *Arundinaria Simoni*, just in like manner as *A. japonica* approaches to *Sasa*. In the present case it seems probable that *S. nipponica* stands next to *S. ramosa* which in turn is closely related to *A. Simoni*: *S. nipponica* is phylogenitically nearer to *A. Simoni* than to *A. japonica*[2].

Puccinia Kusanoi Diet. var. **azuma** Kusano n. var.

(Figs. 6, 12, 15.)

Uredosori hypophyllous, isolated, small, naked, pulverulent, ferruginous, without paraphyses; spores oval to obovate, 25-28×20-25 μ, epispore brown, coarsely echinulate, germ-pores 4-3, equatorial.

Teleutosori hypophyllous, isolated, scattered, prominent, round, compact, 0.5-1.0 mm. in diameter, black to brown; spores linear oblong, tapering towards both ends, not or rarely constricted at the septum, wall conically thickened at the apex, brown, finely verruccse, 55-87 × 12-20 μ; pedicel persistent, slender, hyaline, more than 200 μ long.

It has been found at Koganei near Tokyo (T. MAKINO, May, 1894)*, and Sōma in Prov. Iwaki.

On *Sasa ramosa* Makino et Shibata (= *Bambusa ramosa* Makino, *Arundinaria ramosa* Makino).

In the general form of the teleutospores the present species resembles *Puccinia longicornis*, but differs from it in having the wall at the apex less thickened. Generally the constriction at the septum is exceedingly slight, and the spores are longer than those of *P. Kusanoi*. At times we find some short and broad spores which resemble the typical

[1] MAKINO and SHIBATA, *loc. cit.*

[2] See MAKINO and SHIBATA, *loc. cit.*, p. 30.

spores of *P. Kusanoi*, but since the above mentioned characters of the spores as well as others remain constant on *S. ramosa*, I think it better to distinguish the rust as a new variety. This will seems natural when we take into consideration the relationship of the host to *Arundinaria*. The authors of the genus *Sasa* remark, "*Sasa ramosa*, which we include provisionally in our new genus, resembles, however, *Arundinaria Simoni* in its floral characters, and has often less stamens than 6. Further study will decide whether it represents the type of a distinct genus or not"[1] Thus, the host seems to be different from other *Sasa*, intermediate between *Arundinaria* and *Sasa*, and for this reason it is not strange that its parasite is closely related to *P. Kusanoi*. Infection-experiments will perhaps decide whether the rust on *S. ramosa* is or is not specifically identical with that on *Arundinaria Simoni*.

Puccinia Sasae Kusano n. sp.

(Figs. 7-9.)

Uredosori hypophyllous, isolated pulverulent, ferruginous; paraphyses hyaline, swollen at the top; spores globose or subglobose, 32-35 μ; epispore very thick, brown, coarsely echinulate; germ-spores 5, equatorial.

Teleutosori hypophyllous, isolated, prominent, round, compact; spores oblong, rounded at the upper end, tapering towards the lower, constricted at the septum, lower cell generally longer, wall not or slightly thickened at the apex, finely verrucose, brown, 37-80 × 14-20 μ.

On *Sasa borealis* Makino et Shibata. Nikko* (T. Makino, Aug., 1905).

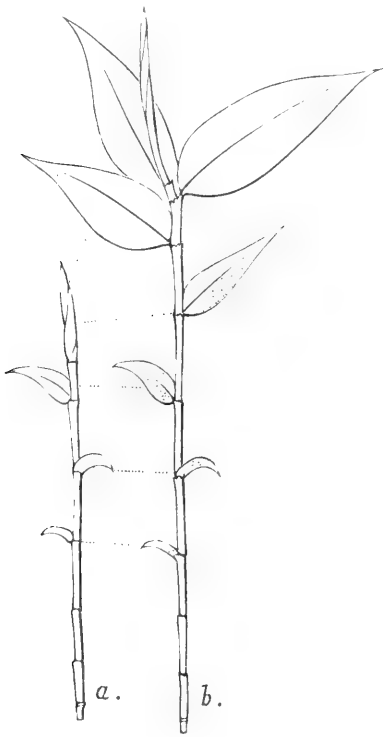
In a single dried specimen the rust was imperfectly preserved and almost all the teleutospores had germinated. In general appearance the teleutospores resemble those of *Puccinia Phyllostachydis*, but the upper cell is mostly wider and shorter than the lower. Among a large number of longer, thin-walled, light brownish teleutospores we find a few shorter

[1] Makino and Shibata, *loc. cit.*, p. 30.

and broader, thick-walled, dark brownish ones. The paraphyses resemble those of *P. longicornis*. The uredospores are the largest and have the thickest wall of the rusts described above, and they are also characterised by having 5 germ-pores. Although the specimen is somewhat imperfect, still these characters show beyond doubt that it can not be referred to any of the other species that are found on the bamboo-leaves.

General Characters and Development of the Fungi.

All the known species of *Puccinia* on the leaves of our bamboo-plants present externally nearly the same characters and can scarcely be distinguished from each other. The only differences which can be regarded as of diagnostic value are the structure of the uredosori and



Text-Fig. 1.

Young shoot of *Sasa paniculata*. *a*, at the time the teleutospores germinate; *b*, at the end of May.

the form of the teleutospores. In *Puccinia Phyllostachydis*, *P. longicornis*, and *P. Sasae* characteristic paraphyses occur which are wanting

in *P. Kusanoi* and its variety; and the thickening at the apex of the teleutospore is exceedingly slight in *P. Phyllostachidis* and *P. Sasae*, prominent in *P. Kusanoi* and its variety, and most remarkable in *P. longicornis*.

The most vigorous formation of the teleutospores takes place, so far as observed in Tokyo, in February and March, and they come to germinate at the beginning of April, whenever moisture is supplied in sufficient quantity. The uredosori appear all at once on the same leaves on which the teleutospores are developed, or on the young leaves of new shoots, attaining their full development at the end of May. The portions of the young leaves on which the uredosori first appear, are strictly those that were exposed exactly at the time the teleutospores were germinating (Text-fig. 1). It is therefore highly probable that the uredosori originate from the sporidia. The rust then proceeds to attack the young leaves as they come out one after another, developing successively new uredosori upon them. The development, however, is exceedingly retarded in the summer and autumn, and so the leaves developed later are for the most part apparently healthy, though a few discoloured spots may be observed, which are points infected by the uredospores. As winter approaches we recognize here and there a few teleutosori appearing from these diseased spots, but the number of the sori is not so numerous in midwinter as in the early spring. This is an ecologically interesting fact. Usually the teleutospores are the hibernating spores in many Uredineae, and they are formed at the beginning of winter, or sometimes when the host has completed its vegetation period, which occurs in some species in the summer. In bamboo-plants, the leaves are all perennial, and survive mostly till the end of the second year. Hence, if the cold season is concerned in the formation of the teleutospores in the bamboo-rust, it seems that the teleutospores must all be formed in or before the winter, instead of in the next spring. The fact being, however, otherwise, we must ascribe this unusual mode of development to another unknown cause, for which a further study is required.

The unfavourable conditions of the autumn and winter check the

development of the fungus, and it is practically in a resting state during these seasons. The numerous fine discoloured spots which we find during the winter on the leaves are produced by the uredospores disseminated during summer, and the intercellular mycelium developed in these spots is mostly inhibited from further development till the spring of the next year: the fungus can therefore winter in the form of sterile mycelium in the tissue of the host, as many perennial fungi do. The formation of the teleutospores in the spring appears to be a means of propagation for the fungus upon the young leaves by the formation of sporidia. As the function of the teleutospores is thus somewhat different from that of many other rusts, it does not seem strange that the wintering mycelium in the infected spots does not always develop teleutospores, but uredospores as is often observed especially during warmer winter[1].

The mode of germination of the teleutospores is quite similar in all the species. Kept in a moist chamber they germinate in from 24 hours to a few days, the first noticeable change being the production from the upper cell of a short curved promycelium consisting of four cells each bearing a pyriform sporidium. In water they send out a long germ-tube which may sometimes grow to 700 μ, usually with no septum (Fig. 11), or produce unusually long and thick sterigmata (175 μ) with or without sporidia (Figs. 12, 13).

The formation of abnormal promycelium is common in the rust on *Sasa nipponica*, and appears to take place under the same conditions as in other species. While the sterigma is usually produced near the upper septum, the second sterigma appears in this species constantly at the lower septum (Fig. 13). This is the character by which we can distinguish the rust on *Sasa nipponica* from *Puccinia Kusanoi* on *Arundinaria*, but it is of too little value to be taken as a specific character of the rust on this problematic *Sasa*.

March, 1908.

[1] It was most remarkable in the spring of 1907 on *Arundinaria Simoni*, *A. Varihira* f. *Yashadake*, and *Phyllostachys bambusoides*.

Key to the Species.

I. The wall at the apex of the teleutospore thickened very slightly.
   a. Uredosori with septate paraphyses ..... *P. Phyllostachydis* Kusano.
   b. Uredosori with non-septate paraphyses ..... *P. Sasae* Kusano.

II. The wall at the apex of the teleutospore thickened conically.
   a. Teleutospore broad and constricted at the septum ... *P. Kusanoi* Diet.
   b. Teleutospore long and not constricted at the septum .....
   P. Kusanoi Diet. var. *azuma* Kusano.

III. The wall at the apex of the teleutospore produced into a long papilla; uredosori with paraphyses ..... *P. longicornis* Pat. et Hariot.

Host-Index.

Arundinaria japonica Sieb. et Zucc.	*Puccinia longicornis* Pat. et Hariot.
A. Narihira Makino	*P. Kusanoi* Diet.
A. Narihira Makino, forma *Yashadake* Makino	*P. Kusanoi* Diet.
A. Simoni A. et C. Riv.	*P. Kusanoi* Diet.
A. Simoni A. et C. Riv. var. *Chino* Makino	*P. Kusanoi* Diet.
A. variabilis Makino var. *Tanakae* Makino	*P. Kusanoi* Diet.
A. Simoni A. et C. Riv. var. *variegata* Hook fil	*P. Kusanoi* Diet.
A. variabilis Makino var. *viridi-striata* Makino	*P. Kusanoi* Diet.
A. variabilis Makino forma *foliis glabris* Makino	*P. Kusanoi* Diet.
A. variabilis Makino forma *foliis pubescens* Makino ...	*P. Kusanoi* Diet.
Phyllostachys bambusoides Sieb. et Zucc.	*P. Phyllostachydis* Kusano.
Ph. bambusoides Sieb. et Zucc. var. *aurea* Makino ..	*P. Phyllostachydis* Kusano.
Ph. bambusoides Sieb. et Zucc. var. *Marliacea* Makino...	*P. Phyllostachydis* Kusano.
Ph. bambusoides Sieb. et Zucc. forma *Kashirodake* Makino.	*P. Phyllostachydis* Kusano.
Sasa borealis Makino et Shibata	*P. Sasae* Kusano.
S. nipponica Makino et Shibata (?)	*P. Kusanoi* Diet.
S. paniculata Makino et Shibata	*P. longicornis* Pat. et Hariot.
S. ramosa Makino et Shibata	*P. Kusanoi* Diet. var. *azuma* Kusano.

Explanation of Figures.

All figures except Figs 7—9 are drawn from fresh specimens and magnified 400 times.

Fig. 1. Teleutospores of *Puccinia Phllostachydis*. *a*, longer spore; *b-e*, typical spores.

Fig. 2. Paraphyses in the uredosori of *Puccinia Phyllostachydis*.

Fig. 3 Teleutospores of *Puccinia longicornis*. *a*, *b*, shorter spores; *c-f*, typical spores.

Fig. 4. Paraphyses in the uredosori of *Puccinia longicornis*.

Fig. 5. Teleutospores of *Puccinia Kusanoi*. *a*, *b*, on *Arundinaria Simoni* var. *Chino*; *c-f*, on *Sasa nipponica* (?).

Fig. 6. Teleutospores of *Puccinia Kusanoi* var. *azuma*. *a*, short and broad spore; *b-e*, typical spores.

Fig. 7. Teleutospores of *Puccinia Sasae*. *a-c*, typical spores; *d-e* round form.

Fig. 8. Uredospores of the same. *a*, ripe spore; *b*, young spore.

Fig. 9. Paraphyses in the uredosori of *Puccinia Sasae*.

Fig. 10. First stage of germination of *Puccinia Phyllostachydis* on *Phyllostachys bambusoides* var. *aurea* (after a week).

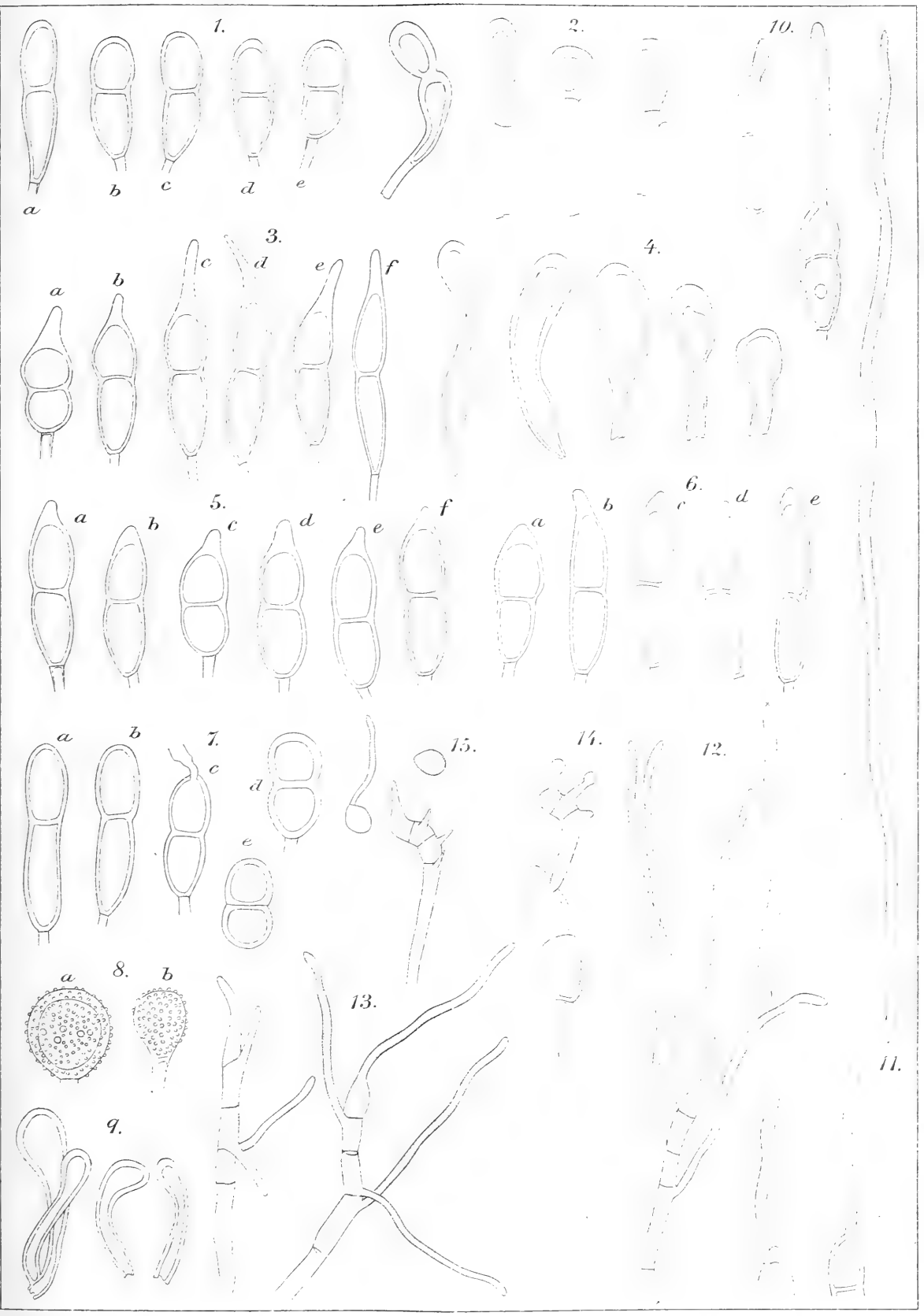
Fig. 11. Germination of the same in water (after a week).

Fig. 12. Germination of *Puccinia Kusanoi* var. *azuma* in water (after 24 hours).

Fig. 13. Germination of *Puccinia Kusanoi* on *Sasa nipponica* (?) in water (after 24 hours).

Fig. 14. Germination of the same in moist air.

Fig. 15. Basidium and sporidia of *Puccinia Kusanoi* var. *azuma*.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S. Kusano del.

On the Parasitism of *Siphonostegia* (Rhinantheae).[1]

BY

S. Kusano.

With five Figures in the Text.

Since the discovery of the parasitism of Rhinantheae by DECAISNE[2] numerous genera belonging to this subfamily of Scrophulariaceae have been ascertained as hemiparasites[3]. Although there is but little doubt that the remaining genera may perhaps also contain such plants, yet no one appears to have examined closely their root-system; so that many of them have still to be studied on this point. Of our Rhinantheae we have two widely distributed but little studied indigenous genera: *Monochasma* and *Siphonostegia*. Without detailed descriptions the former plant (*M. Sheareri* Max.) was already enumerated among parasites in SHIRAI's "Diseases of Plant" written in Japanese (1894), and I can now confirm his statement by my own examination of its root-system, but nothing has yet been recorded about *Siphonostegia*, whether it is an autophyte or a hemiparasite.

During my study of Japanese phanerogamic parasites, I became convinced already in 1898 that *Siphonostegia chinensis* Benth. was a hemiparasite, but at that time I thought it better to report this fact after studying its biology and physiology. Owing to various circumstances I have however been unable to carry my intention into effect, so that I shall give in this paper only an account of the structure of the haustorium.

[1] Contribution from the Botanical Laboratory. A short account has already appeared in Japanese in Bot. Mag., Tokyo, Vol. XVIII, 1904, p. (144).

[2] DECAISNE, J., Ann. d. sci. natur., III Série, t. VII, 1847, p. 5.

[3] *Melampyrum*, *Tozzia*, *Euphrasia*, *Orthantha*, *Odontites*, *Bartschia*, *Alectrolophus* (*Rhinanthus*), *Pedicularis*, etc.

Siphonostegia chinensis is a herbaceous plant common on grassy fields in the central part of Japan. Over its whole root-system, which is as well developed as that of an autophyte, there occur numerous haustoria of various size. Many of them appear as lateral swellings of the root, but old haustoria occupy frequently a terminal position of the older roots, similarly as in *Lathraea*[1], *Buckleya*[2], and *Santalum*[3]. The form is mostly globular or oval, being nearly sessile, and the size varies according to that of the mother-root, the largest one that has come under my observation being 2 mm. in diameter (Fig. 1).

As to the anatomical structure the haustorium of *Siphonostegia* is very different from the same organ of other Rhinantheae, so far as the arrangement and relative size of the three main parts are concerned, namely cortex ("Rinde"), nucleus ("Kern") and sucker ("Saugfortsatz"). According to PITRA[4], SOLMS-LAUBACH[5], LECLERC DU SABLON[6], KOCH[7] VOLKART[8], SPERLICH[9], and others, these parts are not essentially different in structure in all the Rhinantheae studied by them. From the investigation of SPERLICH we see that these three parts have each a function of its own and are important to the haustorium for its action as absorbing organ. But in the haustorium of *Siphonostegia* it is scarcely possible to distinguish these important parts distinctly. The cortex, which SPERLICH has been taken as a reservoir of carbohydrates, occupying the greater part of the haustorium, shows no peculiar structure that seems especially adapted to play such a function: it consists simply of loosely connected parenchymatous cells, as in the cortex of the mother-root itself, and serving as a reservoir of carbohydrates in a merely subordinate way.

[1] HEINRICHER, E., Cohn's Beitr. z. Biol. d. Pfl., Bd. VII, 1895, p. 315.

[2] KUSANO, S., Journ. of the Coll. of Sci., Vol. XVII, 10, 1902.

1. 1906.

[3] BARBER, C. A., Memoirs of the Dept. of Agric. in India. Bot. Series. Vol. I, No. 1., 1906.

[4] PITRA, A., Bot. Ztg., Bd. XIX, 1861.

[5] SOLMS LAUBACH, H. GRAF ZU, Jahrb. f. wiss. Bot., Bd. VI, 1868, p. 566.

[6] LECLERC DU SABLON, Ann. d. sci. natur., VII. Série, t. VI, 1887, p. 90.

[7] KOCH, L., Jahrb. f. wiss. Bot., Bd. XX, 1889, p. 1 and Bd. XXII, 1891, p. 1.

[8] VOLKART, A., Untersuchungen über den Parasitismus der Pediculasisarten, 1899. Zürich.

[9] SPERLICH, A., Beihefte zum Bot. Centlbl., Bd. XI, 1902, p. 437.

Remarkably divergent is the structure of the nucleus. Both from the illustrated descriptions of the same part by various authors and from my own observations in other Rhinantheae, we know that it shows the same characteristic in all the plants studied thus far in having a hyaline tissue of wide extent with a few tracheidal strands in its centre, and distinguished from the surrounding cortical parenchyma by having much smaller, larger-nucleated cells rich in plasm. To this tissue SPERLICH attributed the elaboration of the formative materials for the parasite. The tracheids here appear in longitudinal sections mostly in two or three rows extending from the mother-root to the host. In none of the haustoria of *Siphonostegia* examined by me have I been able to detect such a distinct hyaline tissue in a corresponding position. In its place there occur massively developed tracheids of just same extent. Numerous parenchymatous cells are found scattered here and there among the tracheids, which seem to correspond to those of the hyaline tissue of other species. The tracheids are irregular in form but appear to retain the form of the parenchymatous cells from which they have been differentiated.

Contrary to the great development of the tracheids in the nucleus we see in the neck, which lies between the haustorium and the mother-root, exceedingly few tracheids; so that the relative development of the characteristic tissues is the reverse of what we find in other Rhinantheae. They form in the neck a few isolated strings traversing the parenchyma and extending from the vascular strand of the mother-root to the nucleus of the haustorium. On the whole, the development of the tracheids in the haustorium is quite the same as in *Buckleya* (Fig. 5).

In the next place it is exceedingly difficult to point out a sharply defined part as sucker that usually represents the frontal portion of the haustorium in most of the parasites above quoted. In the adult haustorium attached to a slender host-root the frontal portion of the nucleus seems to come directly in contract with the living tissue of the host, accompanied by the inner layers of the cortical parenchyma (Figs. 2, 3).

The above account may hold true with a somewhat younger haustorium, though the relative size of the three parts differs slightly: the

nucleus is narrower with much more parenchymatous cells, and the frontal portion including the nucleus and a portion of the cortex penetrates more or less into the cortex of the host-root, and assumes the appearance of the so-called sucker.

To make the difference in structure between the haustorium of *Siphonostegia* and the other Rhinantheae still more clear I may take for comparison a figure of the haustorium of *Alectrolophus* (*Rhinanthus*) given by SOLMS-LAUBACH[1] (Fig. 4), with which the haustoria of all the other Rhinantheae essentially agree in structure so far as the main parts above mentioned are concerned. Here the hyaline tissue composing the nucleus is very prominent, and the tracheids which traverse its median portion, very few as they are, developed in just the sufficient and necessary amount to convey the water taken up by the sucker to the mother-root. The differentiation of the frontal portion into the sucker is also apparent. A similar disposition of parts is found also in the haustorium of some Orobanchaceae[2]. We can therefore distinguish the haustorium of *Siphonostegia* from that of other Rhinantheae by the following characters:—

1. The absence of the massive hyaline tissue in the nucleus which is sharply distinguishable from the cortex.

2. An abundant occurrence of the tracheids in the nucleus.

3. The occurrence of very few tracheids between the mother-root and the haustorium, as compared with the nucleus.

4. The obscure demarcation of the sucker from the other part of the haustorium.

These characters show that the haustorium of *Siphonostegia* presents a close resemblance in structure to the same organ of Santalaceae (Fig. 5).

Considering that the tracheids generally serve for the conduction of aqueous solutions, it will not be superfluous to add a few words here

[1] SOLMS-LAUBACH, *loc. cit.*, Pl. XXXIV. Fig. 2.

[2] HEINRICHER, *loc. cit.*

regarding the probable function which the massive tracheids occurring in the haustorium of *Siphonostegia* and Santalaceae must fulfil. For the mere purpose of conveying aqueous solutions from the host to the parasite their abundant occurrence in the nucleus strikes us at once as being disproportionate. As I have stated above in *Siphonostegia* and as I have mentioned elsewhere in Santalaceae[1], the passage of aqueous solutions between the host and the haustorium as well as between the haustorium and the mother-root takes place through a few strings of tracheids. The widely spread tracheidal mass in the nucleus is too conspicuous to be regarded as a mere continuation of the former. Considered from the merely anatomical standpoint we may with good reason look upon this structure as an example of "Speichertracheiden." This name was first proposed by HEINRICHER for the tracheids which are found in some xerophilous leaves[2] and in the scaly leaves of *Tozzia*. He regarded this tissue as a water-reservoir in a wide sense, and says, "Bei Pflanzen trockenen Standortes sind die Speichertracheiden als Vorrathsreservoire für Wasser angebracht, die eventuell eintretendem Wassermangel begegnen sollen. Bei *Tozzia* ist solch ein Mangel nicht zu befürchten, bei ihr handelt es sich darum, einem Zuviel an Wasser abzuhelfen. Die Speichertracheiden sind auch hier Wasserbehälter; aber nicht als für die Reserve wichtiges Material wird das Wasser in ihnen gesammelt, sondern um eine Erfüllung der Intercellularräume mit Wasser zu verhüten, wird es in den Speichertracheiden, die hier gewissermassen als Stauwerk dienen, untergebracht, wenn die wasserausscheidenden Organe, die Hydathoden, nicht schnell genug arbeiten sollten. Die Speichertracheiden erscheinen demnach bei *Tozzia* als ein die Hydathoden ergänzender Apparat"[3]. The same argument may be applied to the case of *Siphonostegia* and Santalaceae. Since the transpiring organ, the leaf, may have different structure in the host and parasite, it is obvious that the demand of water is not equal in the two, notwithstanding that

[1] KUSANO, S., *loc. cit.*

[2] HEINRICHER, E., Bot. Centbl., Bd. XXIII, 1885, p. 25.

[3] HEINRICHER, E., Jahrb. f. wiss Bot., Bd. XXXVI, 1901, p. 665.

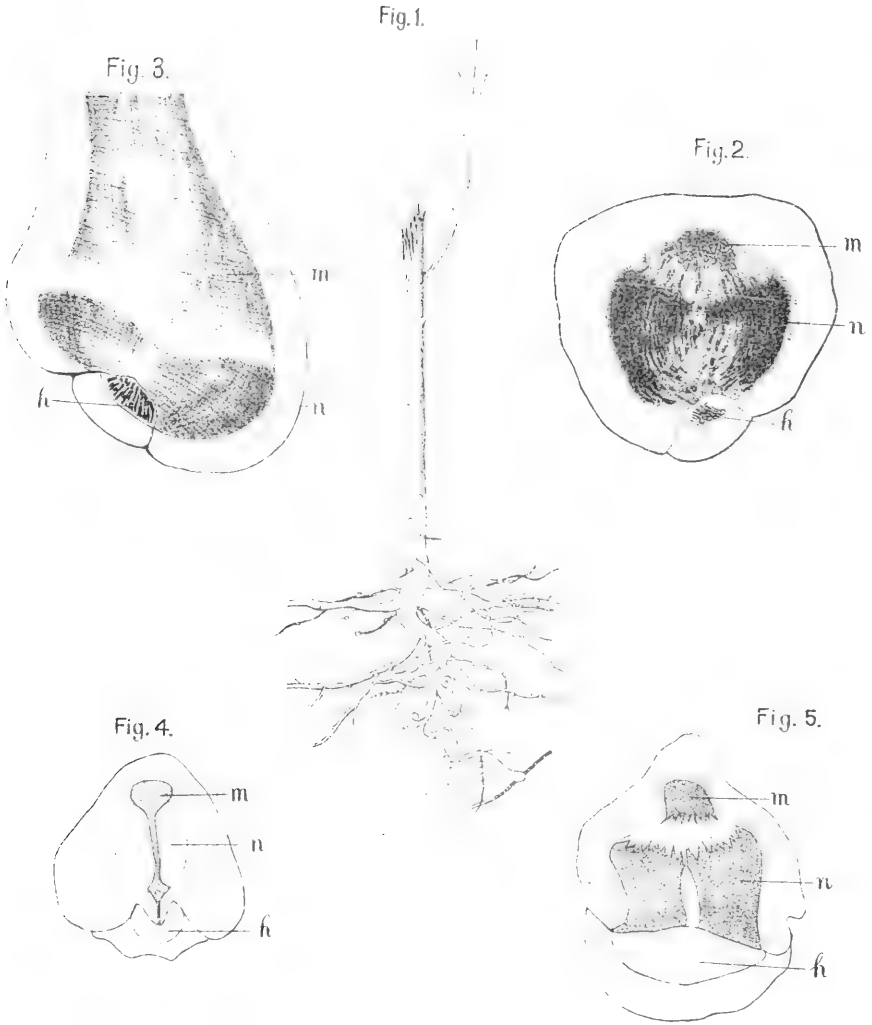

Fig. 1. Root-system of an adult *Siphonostegia*, bearing numerous haustoria. Nat. size.

Fig. 2. Longitudinal section of a lateral haustorium. *m*, mother-root; *h*, host-root. *n*, nucleus of the haustorium. ×50.

Fig. 3. Longitudinal section of a terminal haustorium. Mother-root is shown also in longitudinal section. *m*, *n*, *h*, as before. ×22.

Fig. 4. Longitudinal section of a haustorium of *Rhinanthus* (*Alectrolophus*). *m*, mother-root; *n*, hyaline tissue in the nucleus; *h*, host-root. (After SOLMS-Laubach).

Fig. 5. Longitudinal section of a haustorium of *Buckleya*. *m*, mother-root; *n*, tracheids in the nucleus; *h*, host-root. ×3 (After S. KUSANO).

it has to be supplied by the host-root alone. From the anatomical characters of the leaves and using "cobalt-test," we may be sure that the transpiration, for instance, in *Buckleya*[1] surpasses that in any of its host—conifers and some foliage trees; and its variation during the day and night, and under different conditions of environment, may be greater than in its host, or the supply of water by the host-root may not necessarily accompany its loss by the parasite. It may therefore occur that, under a condition which promotes transpiration in the parasite, the amount of water necessary to replace it may not be supplied by the host. At any rate it is certain that the demand and supply of water in the parasite is not always uniform under the different conditions of environment. Again, the anatomical characters of the leaves of *Siphonostegia* point out that it absorbs water from the host under similar condition as *Buckleya*. Therefore it is highly probable that in both parasites the massive tracheids in the nucleus of their haustoria may act as a reservoir of water, serving either to supply or retain it according to circumbstances; in short, they act as the "regulator" of water-supply for the parasites.

The problem here presented may apply to all the other phanerogamic holo-and hemi-parasites, and I think the development of tracheidal element in the haustorium may be connected with the mutual relation of the parasite and the host with particular regard to the amount of water which they require. To ascertain how far this view is correct, therefore, comparative and more extended studies of all of these parasites is necessary. Groom[2] has stated that parasites in moist soil have leaves so constructed as to enable them to get rid of any excess of water absorbed. We may here remark that the structure of leaves of the plants under consideration is not only correlated to the environment under which they grow, but is also intimately associated with their capacity of absorbing water which, in turn, depends upon the structure of the haustorium.

March, 1908.

[1] For the anatomical structure of its leave see Beim, Beiträge zur anatomischen Charakteristik der Santalaceen. Dissertation. 1895.

[2] Groom, P., Journ. Linn. Soc., Vol. XXX, 1895, p. 149; Ann. Bot., Vol. XI, 1897, p. 385.

Further Studies on *Aeginetia indica*.[1]

BY

S. Kusano.

With Plate VII.

In my former paper ('03) some accounts were given of the morphology, anatomy, and biology of *Aeginetia indica*. So far as my observations went, this parasite showed no special character in the manner of its development, which can be distinguished from that of *Orobanche* as thoroughly investigated by Koch ('83). When I undertook during the past year a further study of this parasite, particularly as regarded the germination of the seeds and the development of the seedlings, I could show that at an early stage of development *Aeginetia* displayed many peculiarities, some of which are perhaps unique. As the results obtained appear not only interesting in themselves, but also to contribute something to the knowledge of phanerogamic parasites, I think it advisable to give them briefly in the present paper.

Very little has as yet been published on the early stage of development of the Orobanchaceae. In *Lathraea* Heinricher ('94, '95) made some experiments on the germination of the seeds and the development of the seedlings. According to him, the seeds show no features during germination and further development that are worthy of special mention. The vegetative organs are very much reduced in form, but the embryo does not differ essentially in structure from that of most autophytic plants, being provided apparently with a pair of cotyledons and a radicle. In germination the radicle first grows into a filamentous root which soon branches into numerous rootlets. The rootlets then produce haustoria where they come in contact with the host-root (Heinricher, '94, p.

[1] Contribution from the Botanical Laboratory.

(128)). Further he ascertained that the seeds require in germination the presence of proper host-root which he believed to exert a chemical stimulus.

KOCH ('83) extended our knowledge on *Orobanche,* and succeeded in raising seedlings from the seeds laid on or near a proper host-root. In this plant the embryo is so much reduced in form as to appear like a younger stage of a dicotyledonous embryo (KOCH, '78, p. 259), being merely an oval cell-mass, and the changes that take place during germination show certain peculiarities. At first the radicular half of the embryo develops into a filamentous root (KOCH, '83, p. 189), while the plumular half remains throughout in the endosperm, acting as an absorbing organ. Differing from *Lathraea*-seedling the parasitism of this seedling is effected by the root-tip, provided it abuts on a host-root lying before its course. In his culture-experiments KOCH ('83) assumed that in germination the seeds required a chemical stimulus from the host-root. Such being all that we know, at present, about the early stage of development of the Orobanchaceae, it appears to me to be not the less interesting to extend our study on *Aeginetia* which exhibits a close resemblance to the last mentioned species of Orobanchaceae, on account of the structure of the seeds as well as the vegetative organs, and to ascertain how far what was found on the latter plant is applicable to the former.

While the present study was carried out with this end in view, I have never undervalued the problem about the condition which the seeds of such holoparasite require in germination. Although it has been ascertained by the above mentioned authors that the stimulus of the host-root is invariably necessary to germination in plants of this family, the nature of the stimulus has not yet been studied with accuracy. Concerning this point I can not yet express any definite view, but as it seems to me that the results of a few incidental experiments are suggestive for a further study on this subject, I will note them briefly in the present paper.

Methods.

The seed of *Aeginetia* being very fine and pulverous, a special treatment is required in observing its germination. In order to observe easily the successive stages of germination, and of the development of the

seedlings, I transplanted, a month or two previously, some vigorous host-plants in pots of 15-20 cm. in diameter. These being kept sufficiently moist, the plants began to produce after a while young rootlets mainly traversing between the wall of the pots and the soil inside. When a thick meshwork was thus formed by the rootlets, I lifted up carefully the plants from the pots, laid the seeds of *Aeginetia* upon the meshes, and then put the plants again in the pots as before. By taking the plants from time to time out of the pots without disturbing the arrangement of their root-system on which the seeds were laid, I was able to follow in detail the changes that took place during the germination and subsequently.

The seeds used in the experiment were collected in the preceding year and kept dry. Under favourable conditions they germinated within two weeks in the early summer. However I could observe no germination to take place in seeds preserved in a dry state for two years. It has not yet been ascertained how long the germinating power can be kept intact in seeds kept moist. This is a practically important matter in connection with the protection of the cultivated plants (KUSANO, '03), in case they should be invaded by this parasite.

Embryo.

The embryo is microscopically small and enclosed in the endosperm packed with starch. In order to take it *in toto* out of the endosperm the seed was treated a day or more with a concentrated solution of chloralhydrate. If such a seed be gently pressed under the cover glass, the endosperm would escape easily from the testa, and the embryo from the endosperm. The mature embryo thus taken out consists simply of a few isodiametric parenchymatous cells of nearly equal size. It is somewhat oval in form with its narrow end directed towards the micropylar end of the seed. No morphological differentiation into plumule, radicle or cotyledons being visible, it represents, as it were, the younger stage of an embryo of a phanerogamic plant. Optical section shows that at most two but often a single row of cells in the direction of the long axis of the embryo is enclosed by the epidermal cells (Figs. 1, 2). Very simple

as it may be in structure, still it is not difficult to point out both the radicular and plumular ends in the embryo. These become evident in a germinating seed; the narrow end, which often consists of smaller cells, corresponds to the radicle, while the other end represents the plumule. As a whole the embryo of *Aeginetia* has quite the same structure as that of *Orobanche* (Koch '78, Smith, '01, p. 118).

Seedling.

It is a noteworthy fact that in spite of a great similarity in structure of the seed in *Aeginetia* and *Orobanche* the mode of germination is very divergent. In *Orobanche* germination is brought by the multiplication of cells in the embryo, so that a filamentous seedling of 1-2 mm. in length is the result. Both the radicular and plumular ends are seen to consist in longitudinal section of four rows of cells enclosed by the epidermis (Koch, '78, Figs. 17-19). The connection of the seedling with the host is effected by the tip of the radicle. The tip on coming in contact with any host sends out its epidermal cells in the form of papillae (Koch, '83, p. 189), and the subjacent initial cells then commence to proliferate and produce the tissue of the primary haustorium. In *Aeginetia* the changes are quite different. In the first place we can scarcely recognise multiplication of cells or longitudinal growth in the seedling before it finds out the proper host, and in the second place the development of the radicular end is very characteristic. The first change that can be observed as the sign of germination only consists in that two or three, large, hyaline globular cells appear outside the testa at the micropylar end of the seed (Figs. 3, 4). These are highly turgescent with abundant cell-sap. The nuclei are large and conspicuous, and the cytoplasm radiates from them. At an advanced stage the globular cells increase in number generally up to 15 approximately (Fig. 12). As can be seen in Figs. 1, 2 and 6, these are not a new tissue, but only the epidermal cells of the radicle but swollen up to nearly 4 times the original diameter. Simultaneously with the change all the other cells swell up more or less, making the embryo much larger in size; and judging from the number of cells seen in an optical section of the

embryo before and after germination (compare Figs. 2 and 6), it is very improbable that a multiplication of cells may partly concerned in the increase of size. An accumulation of starch more especially in the tissue under the globular cells is perhaps connected not with the cell division in this place, but with the further development of the globular cells.

Now follows the outgrowth of the globular cells one by one. Their external wall protrudes so as to make them first conical and then papillar-like in form (Figs. 7, 9, 10). The outgrowths proceed further until they become slender hairs growing at times up to 1 mm. in length. The diameter of the hairs is much smaller than that of the globular cells, measuring 38 μ on the average while the latter measure generally 115 μ in diameter. Although they belong morphologically to the category of trichomes, yet they are not identical in structure and even in function with the typical root-hairs; they are often septate or even branched (Figs. 8, 9, 14), resembling rather the rhizoids of some cryptogamic plants (HABERLANDT, '04, p. 200). If undisturbed, they are all straight and radiate from the radicular end in all directions as shown in Fig. 8, but if one of them during its further prolongation should come in contact by its tip with a young host-root, it seems to attach itself firmly to the latter and then to coil or contract through its whole length, whereby the seedling is drawn closer to the host (Fig. 10). This is evidently a most advantageous contrivance for the parasite to facilitate its organic connection with the host, that is to say, the formation of the primary haustorium. In Fig. 9 is shown one of the hairs just adhering to a host-root, and about to bend itself, while in Figs. 10 and 12 are shown hairs in a much contracted condition with the radicular end brought much nearer to the host.

By what means the tip of the hair fixes itself to the host has not yet been made out exactly. It is not impossible that a cementing substance is secreted by the hair, but there has actually come under my observation such a case as is shown in Figs. 9 and 12, where the fixation was effected by a slight penetration of the tip of a hair between the epidermal cells. My observations, however, are not extended enough to justify the conclusion that this is a general case with *Aeginetia*.

So far as I know, such an organ has not hitherto been described in phanerogamic parasites. Analogous but not homologous cases may perhaps be found in the root-hairs that develop previous to the formation of haustoria on the typical root of some hemi-and holo-parasites, such as *Melampyrum* (Leclerc du Sablon, '87), *Lathraea* (Heinricher, '95, p. 381), *Santalum* (Barber, '06). In all these cases the root-hairs appear to serve simply for the fixation of the root of the parasite to the host. The cushion-cells in *Cuscuta* (Peirce, '93) may be considered to perform the similar function. In *Aeginètia* it is quite obvious, as already stated, that the hairs serve first of all as a "tentacle," and after contact with the host as a "prehensile organ," besides drawing the seedling closer to the host.

In function, therefore, they possess all the characters of a typical tendril (i.e., in Cucurbitaceae), and hence I venture to propose for them the name of "hair-tendrils."

In the root-system a similar function has already been known to appertain to the so-called root-tendrils (see Pfeffer, '04, p. 416). They are not, however, identical morphologically with the hair-tendrils; for, in typical root-tendrils the entire root plays the part of a tendril, while in hair-tendrils an appendage of the radicle comes into play. In origin again, the hair-tendrils may be homologous to the papilla-like cells at the tip of the radicle in the seedling of *Orobanche* (Koch '83, p. 189). However, in structure and function the latter organ seems to be different from the former, showing a rather close resemblance to the cushion-cells of *Cuscuta*.

The kind of stimuli required in causing the curvature of the tendrils remains still unknown. But on the basis of my culture-experiments it seems highly probable that, unlike the true root-hairs (see Pfeffer, '04, p. 459), mere contact with sand or soil particles remains quite ineffectual, but that some chemical stimulus must be concerned, to which the tip of the tendrils coming in contact with the host-root must respond. That normal tendrils may respond to chemical stimuli has already been ascertained by Correns ('96, p. 16).

In almost all cases the globular cells do not appear to develop all into the hair-tendrils: some of them remain unchanged, while some are arrested from further development after reaching the conical or papillae stage. As for the most probable ground of such variable development of the globular cells, my observations of a number of seedlings have led me to the conclusion that the number of tendrils that are formed in a seedling must depend more or less upon the chances of meeting with an appropriate host. In fact I have found that when a seedling came in contact with a host by a premature development of some tendrils, the remaining ones were more or less arrested from further development and the globular cells from forming further tendrils (Figs. 9, 10, 12); while when a seedling remained away from the host long enough, many tendrils were observed to develop at once and in full length, or many globular cells to give rise to tendrils (Fig. 7).

This fact makes it most probable that the seedling develops as many tendrils as possible in several directions until it finds out a host, thus securing as many chances to meet with a desired host-root, but that as soon as one of the tendrils comes in contact with it, the seedling does not need the development of further tendrils.

Usually only the apex of the tendril is responsive to the stimulus, but that the other portions may also react may be seen in Fig. 11, where a tendril is shown twining around a root-hair of a proper host-root (*Zingiber*).

The tendril on coming in contact with the host seems to be retarded in growth as in the typical tendril (FITTING, '03, p. 604), and it seems to wither and die away, if kept indefinitely away from a proper host.

In view of all these facts there can not be any doubt that the hair in *Aeginetia*-seedling is quite different both morphologically and physiologically from the true root-hair, and that it most closely resembles the typical tendril in its function.

While the changes described above are taking place at the radicular end, we can not find any notable change at the plumular end except for

a slight increase in size. The general form of the embryo at this stage is then as reproduced in Fig. 12. It is perhaps the last stage to which an embryo can develop without coming in contact with the host-root. Much starch-granules remain in the embryo and endosperm, and serve as the reserve material for the further development of the seedling.

Tubercle and Primary Haustorium.

When a seedling as above described comes in contact with a host-root by means of a hair-tendril, further development follows immediately. By a rapid multiplication of cells the seedling grows so as to become visible to the naked eye. The newly produced tissue gives rise, besides a primary haustorium, to a tubercle from which the shoot and root-system of the plant are afterwards formed. What is remarkable is that the multiplication of cells does not take place, unless the seedling becomes attached by one of the tendrils to the host. Since the seedling is otherwise entirely incapable of further development, in spite of the presence of the reserve material left in the endosperm, it follows that the further development of the seedling is associated with the stimulus of the host.

The multiplication of cells occurs under the tendril-cells. The parenchymatous tissue thus derived pushes and finally breaks the latter, and comes to lie in direct contact with the tissue of the host-plant. Until an organic connection becomes established between the seedling and the host-tissue, the multiplication of cells must be due to the reserve material in the seed. The maximal size to which the cell-mass can thus attain is less than 1 mm. in diameter, approximately the same as that to which the seedling of *Orobanche* can reach with the help of its endosperm alone (KOCH, '83, p. 189).

The cell-mass thus formed becomes a tubercle generally of a spherical or oval form (Fig. 13). It forms a large part of the seedling, making the plumular end, globular cells, and tendrils highly inconspicuous. The formation of the tubercle has already been observed in *Orobanche*, in which however only one fifth of the whole length of the seedling is transformed into it.

The frontal portion of the tubercle penetrates into the young cortex of the host-root and becomes differentiated into a primary haustorium which is completed by the formation of tracheids in direct connection with the conducting system of the host-root. On the completion of the haustorium the tubercle derives nourishment from the host, and there ensues a vigorous development. The further development of the tubercle —formation of the shoot and root-system—is quite the same as in *Orobanche* (Koch, '83).

Germination-Experiments.

As has been quoted above, there is no doubt that in the germination of the Orobanchaceae, as ascertained in *Orobanche* and *Lathraea*, a chemical stimulus comes into play. Still it has not been conclusively shown whether the stimulus in question is due to the character of the roots as such, or is entirely peculiar to the root of the proper host. Although Koch has expressed the opinion that, "die Samen der Orobanchen keimen nur im Anschluss an die Wurzel einer geeigneten Nährpflanze" (Koch, '83, p. 188), it seems to me that a sufficient number of plants has not been tested with this point in view. Heinricher ('94) succeeded in raising the seedling of *Lathraea* on the roots of a very few kinds of trees. From his experiments we can not conclude that the roots of all trees can stimulate the seed to germination. A further study is also needed to decide whether the seed germinates on the roots of herbaceous plants. But when we consider that these parasites thrive only on certain plants,[1] one might consider himself justified in assuming that the germination takes place only on these plants. Likewise, as only monocotyledonous plants are, at present, known as the hosts of *Aeginetia* in the field[2], one

1 Among more than 300 species enumerated by von Beck ('90) as hosts of *Orobanche* no monocotyledonous plant is mentioned decidely as the proper host.

2 So far the following plants have been ascertained to serve as the host.

Canna indica L. (Dandoku).
C. Morrowi Boott (Kansuge.)
Imperata arundinacea Cyr. var. *Koenigii* (Benth.) Hack. (Chigusa).
Miscanthus sinensis (Anders.) (Susuki).
Oryza sativa L. (Upland form) (Okabo).
P. flumentaceus L. (Hie).
Setaria italica Kth. var. *germanica* Trin. (Awa).
Zingiber Mioga Rosc. (Myōga).
Carex lanceolata Boott. (Hikagesuge).
C. transversa Boott. (Ko-ogisuge).
M. sacchariflorus Hack. (Ogi).
Panicum miliaceum L. (Kibi).
Saccharum officinarum L. (Satōkibi).
Zea Mays L. (Tōmorokoshi).

might be led to the same assumption. This has, however, been proved to be quite incorrect by the germination-experiments now to be described. As these expriments were originally planned to verify what we had assumed, they were not so extended as were afterwards found desirable.

1. Germination of the seeds on pot-plants.

Aeginetia-seeds were laid on the roots of several pot-plants. The experiments were made in July, and the germination took place within two weeks. The plants used comprised several species of Phanerogams and Cryptogams, two pots being prepared for each.

Pteridophytes: *Selaginella involvens* Spring. (Iwahiba) and *Aspidium rhomboideum* Wall. (Kanawarabi) have rather weakly developed roots. After two weeks some of the seeds laid on them were seen to have produced a few globular cells outside the testa, but no futher development took place even after four weeks or more.

Gymnosperms: *Cryptomeria japonica* Don (Sugi) and *Thujopsis dolabrata* S. et Z. (Asunaro) were used. Although the roots are not very vigorously developed, yet a few of the seeds produced globular cells. Further development remained uncertain.

Monocotyledons: Keeping in mind that *Aeginetia* grows in the field exclusively on plants of this group, I have used for my purpose several species from various families, comprising also the well-known hosts for control.[1]

Juncaceae.

Luzula campestris DC. var. *capitata* Miq. (Suzumenohie).

Cyperaceae.

Carex japonica Thumb. var. *chlorostachys* (Don.) Kük. (Shirasuge).

* *C. Morrowi* Boott. (Kansuge).

Gramineae.

Arundinaria Simoni Riv. (Medake).

Calamagrostis arundinacea Roth. (Chigusa).

* *Miscanthus sinensis* (Anders.) (Susuki).

[1] The natural hosts are marked with an asterisk.

* *Oryza sativa* L. (Upland form) (Okabo).

* *Panicum miliaceum* L. (Kibi).

Setaria excurrens Miq. (Inuawa).

* *Zea Mays* L. (Tōmorokoshi).

Araceae.

Acorus gramineus Ait. (Sekishō).

Commelinaceae.

Pollia japonica Hornst. (Yabumyōga). Thick, soft and vigorous roots with densely developed root-hairs.

Rhoeo discolor Hce. (Murasakiomoto).

Liliaceae.

Allium fistulosum L. (Negi). Vigorous development of roots.

Hemerocallis fulva L. (Yabukwanzō).

Ophiopogon japonicus Ker. (Janohige). Roots dense but not vigorous.

Iridaceae.

Iris tectorum Max. (Ichihatsu).

Dioscoreaceae.

Dioscorea sativa L. (Marubadokoro). The development of roots far less vigorous than other plants.

Zingiberaceae.

* *Zingiber Mioga* Rose. (Myoga). Root very vigorous.

Cannaceae.

Canna indica L. (Dandoku).

With the exception of *Ophiopogon* all the plants above mentioned gave the required stimulus, and the seed attained after two weeks to a stage similar to that shown in Fig. 4. The percentage of germination seemed to be larger on plants which produced vigorous roots. In *Zingiber* and *Pollia* young roots were constantly and luxuriantly produced during the experiment, so that almost all the seeds laid on them came to germination. As for *Ophiopogon* the roots were not very active during the experiment, and the necessary stimulus, if present, seemed to have been too feeble.

Dicotyledons: Only a few plants were taken here. This was due to the circumstance that more plants had not been prepared as pot-plants for my purpose.

Plumbaginaceae.

Armeria maritima Willd.

Araliaceae.

Fatsia japonica Dene. et Plane. (Yatsude). The roots were very few and not vigorous.

Geraniaceae.

Pelargonium Zonalle Willd. (Montenjikuaoe).

Rosaceae.

Pirus Marus L. var. *tomentosa* Koch. (Ringo). Roots very few, not vigorous.

Prunus Mume S. et Z. (Mume). Roots very few, not vigorous.

Solanaceae.

Solanum tuberosum L. (Bareisho). Roots very scanty.

Leguminosae.

Pisum sativum L. (Endō).

Compositae.

Chrysanthemum sinense Sab. (Kiku).

Solidago occidentalis Torr. et Gray. (Ōawadachisō).

Taraxacum officinale Wigg. var. *glaucens* Koch. (Tanpopo). Roots very few.

Of these plants *Fatsia* and *Taraxacum* did not bring the seed to germination. This might perhaps be due to a comparatively weak development of the roots as above noticed. On the other hand, the seeds laid on all the other plants mostly germinated just as they did on Monocotyledons. It must, however, be remarked that the seedlings thus produced did not all develop so far as to produce the hair-tendrils: stopping at the stage shown in Fig. 4, they ultimately came to death, mainly owing to mould fungi or other microorganisms.

The foregoing experiments show, contrary to our natural expectation, that the stimulus necessary for the germination of *Aeginetia*-seed is not peculiar to particular species of plants, but is given by all vigorously developing roots, whether of Phanerogams or Vascular Cryptogams. If it be admitted that a chemical stimulus is concerned here, it is most probable that the stimulant is an excretion of the roots. The following experiments afford some evidence for this view.

2. Germination of the seeds wrapped in paper on pot-plants.

This experiment was undertaken to ascertain whether direct contact of the seed with the host-root is necessary for germination or not. The seeds were wrapped in one or several sheets of well-washed filter paper and laid among the root-meshes of the pot-plants. For control, seeds prepared in the same manner were kept at the same time in a moist chamber, and again unwrapped seeds were laid directly on the roots of the same pots. The seeds wrapped in 3-5 sheets of paper did not germinate about the time that the unwrapped seeds germinated vigorously. However, those wrapped in one sheet and laid on *Zingiber* and *Pollia* germinated partly. In the mean time the control seeds in the moist chamber remained entirely unchanged.

From this experiment we see that direct contact of the seeds with the host-root is by no means an indispensable condition in bringing them to germination, and that the germination is associated with a certain substance or substances excreted by the host-root and diffused into the surrounding medium.

That the percentage of germination is smaller in the case of the wrapped seeds than in those laid directly on the root, and that it becomes less with the increase of the sheets of paper are strong evidences that the amount of the diffusible substance depends upon the nature of medium through which it must pass to reach the seeds.

3. Germination of the seeds without host-root.

The seeds were kept in water (tap-water or distilled water) or in a moist chamber. They were also sown in soil without any visible plant. In either case I was not able to observe any sign of germination. If such

seeds were afterwards brought on the root of any plant, the germination took place easily. Hence it follows that the seed of *Aeginetia* always requires a stimulus from the roots for germination.

4. Germination of the seeds in chemicals.

This is only a preliminary experiment to find out a stimulating substance among chemicals, and only a few substances were tested.

Under a bell jar, one end of a piece of filter paper moistened previously with distilled water, on which the seeds were placed, was immersed in a given solution of the substance to be tested in a small vessel. By capillary action the given solution diffuses up the paper, so that the seeds are acted on by the substance in various degrees of concentration at different parts of the paper. For control, tap-water and distilled water were tested in the same manner. The results were entirely negative, and no germinating seeds were observed after two weeks or more. The seeds were attacked by mould fungi and destroyed.

The chemicals tested and concentration in the vessels were the following:

Hydrochloric acid	1/100 and 1/500 mol.
Phosphoric acid	1/100 and 1/500 mol.
Tartaric acid	1/100 and 1/500 mol.
Citric acid	1/100 mol.
Formic acid	1/100 and 1/500 mol.
Malic acid	1/10 and 1/500 mol.
Monopotassium phosphate .	1/100 mol.
Sodium hydroxide	1/100 and 1/1000 mol.

It would be of great interest and importance to extend the above experiments and to determine, if possible, a chemical or chemicals that would stimulate the *Aeginetia*-seed to germination. If such a substance be found out, it is highly probable that it is one of the excretions of the roots. Much difficulty must certainly lie in the way of such a study. It is generally known that roots excrete acidic substances (Czapek, '05, p. 873), and recently Schreiner and Reed ('07) have found out that a very slight amount of substances is excreted by roots, which act deleterious to their growth.[1] The amount is so exceedingly small that it

[1] For the literature on root-excretions see Schreiner and Reed, *loc. cit.*

can not be detected by chemical analysis, but its presence is revealed by the chemotropism of roots. The method proposed by the last named authors is very ingenious, and it leads us to think that our germination-experiments, if extended further, might perhaps be applicable to the investigation of root-excretions.

Development of Tubercle and Selection of Host.

Although it is clear from the foregoing accounts that all roots can stimulate *Aeginetia*-seeds to germination, still the facts obtained both from field-observation and culture-experiments clearly show that *Aeginetia* can not grow on all plants. This is proved by the germination-experiments. By careful examination at intervals of the pot-plants on which the seeds were laid, we could ascertain that the germinating seeds did not develop equally well on Cryptogams, Gymnosperms and Dicotyledons. Again, among Monocotyledons different plants acted very differently. The plants of this group that induced the seedlings to form tubercles were *Luzula*, both species of *Carex*, *Calamagrostis*, *Miscanthus*, *Setaria*, *Oryza*, *Panicum*, *Pollia*, *Zingiber*, and *Canna*, most of them being already known as natural hosts.[1] Further, the development of tubercles was not only very unequal on these plants, but even in the same species it was different on different individuals. After two weeks all the seedlings reached the size shown in Fig. 4, but the size of the tubercles during the next two weeks was very variable, some attaining to the size of poppy grains and others to that of the corn. The growth of the tubercles was especially vigorous on *Zingiber* and *Pollia*. It was also observed that the development of the parasite was less rapid on pot-plants than on those in the field: in September it was all in flower in the field, while the shoot scarcely appeared above ground in the pots. It follows that the growth of the parasite is most intimately connected with that of the host, and in particular with the activity of its roots.

[1] See foot-note in the preceding page (p. 67).

I have already remarked that the host-root induces no germination when too feebly developed. The same cause must not be assigned for the non-development of tubercles on some of the plants used in the experiment. For instance, *Allium*, *Iris*, *Acorus*, *Hemerocallis*, and others produced numerous vigorous rootlets and appeared always to be much more rapid in growth than some natural hosts such as *Carex* and *Miscanthus*. It is certain, therefore, that there are fit and unfit plants as the host of *Aeginetia*.

As for the intimate relation between the seedlings and the proper hosts, or plants unfit as the host, I have no evidence to bring forward. It may be that the roots of some plants are unfit for inducing the formation of the hair-tendrils, the primary haustoria, or perhaps the tubercles. Which of these assumptions holds true must be settled by further investigations. At present I can go no farther than to state that *the stimulus which causes the seeds to germinate and the stimulus which causes the seedlings to develop further are of a quite different nature.*

General Remarks and Summary.

On looking over what have been described above, we see that *Aeginetia* presents many remarkable characters which must be due to its parasitic life. In the first place, the formation of the hair-tendrils is a most specialised contrivance for finding out the host. It may be that in *Lathraea* and *Orobanche* the seedlings can not easily reach the host, unless the seeds are placed close to the host-root so that their radicles lie against the latter. Otherwise, the tip of the radicles may diverge from the host-root more and more, as they grow further and further so as to make the development of the seedlings impossible, just as the same organ of *Viscum* would do, if it should be insensitive to light, or the same organ of autophytic plants, if insensitive to light and gravity. In *Aeginetia* the formation of the hair-tendrils is alone sufficient to avoid such a danger.

In the second place, *Aeginetia* shows some transitional states between autophytic and the most advanced parasitic life. In most hemiparasites, or more strictly speaking, green parasites such as Santalaceae (KUSANO, '06, BARBER, '06, '07), Rhinantheae (HEINRICHER, '01, '02), and Loranthaceae, the germination is neither associated with the presence of the host, nor have they any marked tendency to select their host. But some holoparasites, or at least Orobanchaceae hitherto studied, have acquired the habit of not developing and even of not germinating without the presence of the roots of their proper host. While thus the intimate relation of the parasite and host-root is in this case restricted to certain limited species of plants, *Aeginetia* shows itself to be many-sided in this respect: in *Orobanche* and *Lathraea* the selection of the host takes place already at the period of germination, but it takes place in *Aeginetia* at a later period. Thus certain variations being observed to occur in the Orobanchaceae in their behaviour towards the host-roots, it may be remarked that a study of other species of the same family is very desirable.

The chief results of the experiments described in the foregoing pages may be summarised as follows:

1. The germination of *Aeginetia*-seed does not take place in water, moist chamber, or soil. It requires always the stimulus of the root of other plants.

2. The seed kept dry for two years loses its germinating power.

3. The plants which stimulate the seed to germination may be Vascular Cryptogams, Gymnosperms, or Angiosperms.

4. The stimulant is an unknown substance that is perhaps excreted by the active roots of all higher plants.

5. The development of the seedlings takes place only on certain species of Monocotyledons. Its conditions are entirely different from those that are necessary for the germination of the seeds, the former being fulfilled only by certain plants while the latter are found in the roots of all higher plants.

6. The first change that takes place during the germination is the swelling of the epidermal cells at the radicular end of the embryo and their transformation into the hair-tendrils.

7. The seedlings are much reduced in form, and before they are connected with the host no multiplication of cells takes place.

8. The seedlings develop, when attached to the host, spherical tubercles. They are formed by the meristematic tissue under the hair-tendrils.

9. For the multiplication of cells in the seedlings certain stimulus from the host-roots to which the hair-tendrils are sensitive seems to be required.

10. The tubercles become differentiated first into the primary haustorium at the frontal portion, and then into the stem and root-system at the other portions.

June, 1908.

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Explanation of Figures.

All figures except Figs. 13 and 14 are drawn with the aid of the camera lucida from the fresh materials and magnified 130 times.

Fig. 1. An adult embryo in a ripe seed.

Fig. 2. The same shown in optical section. Fragments of testa are attached to the radicular end.

Fig. 3. A seed at the beginning of germination, with some swollen epidermal cells at the radicular end appearing outside the testa.

Fig. 4. A seed at somewhat later stage.

Fig. 5. An embryo in the germinated seed as shown in Fig. 3.

Fig. 6. The same in the seed shown in Fig. 4. (Optical section). Starch-granules accumulate at the median portion.

Fig. 7. Radicular end of a seedling showing one of the globular cells protruded into a papilla.

Fig. 8. The same with full grown tentrils.

Fig. 9. The same showing one of the tentrils attached to a host (*Zingiber*). Its apex is penetrating between two epidermal cells of the host.

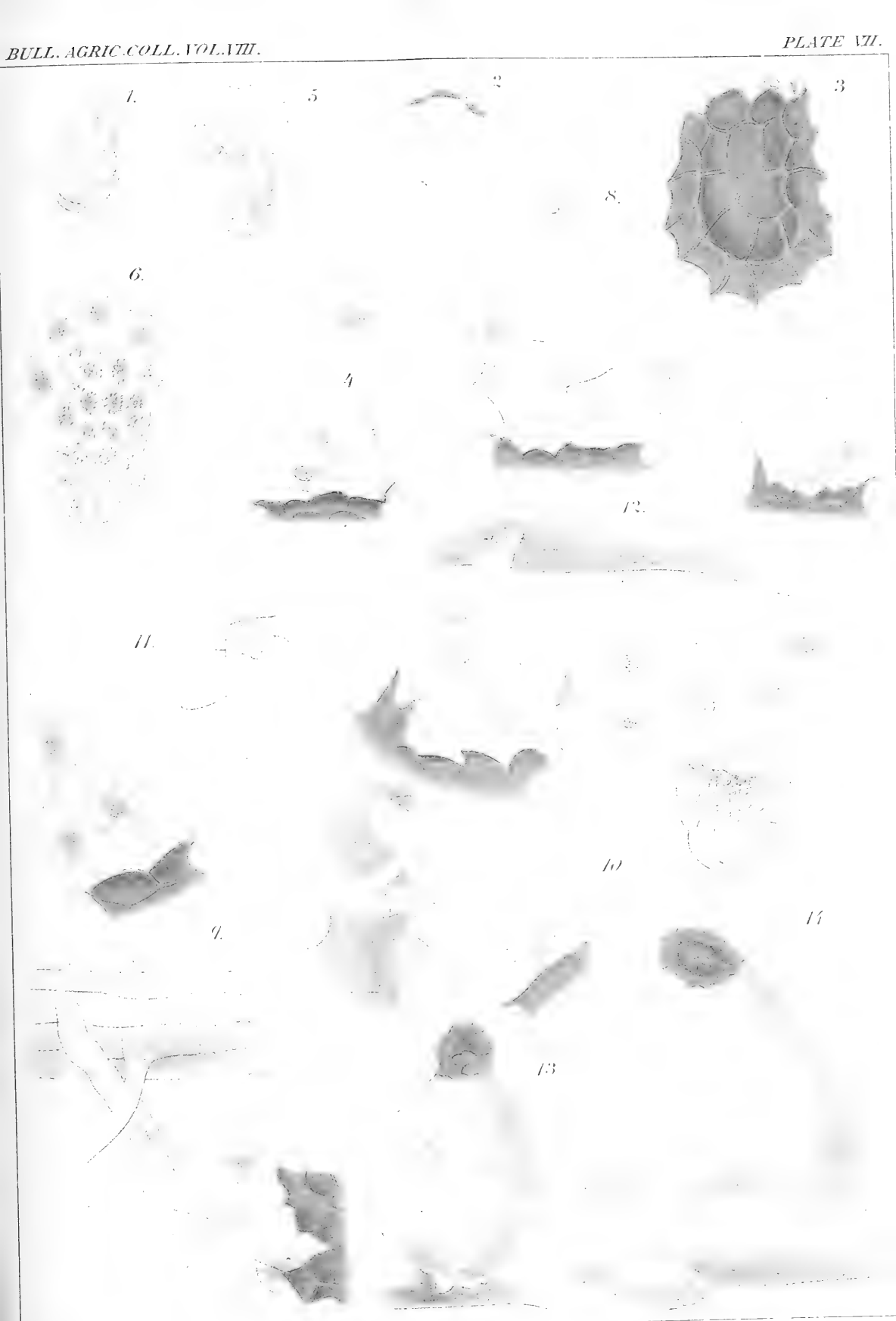
Fig. 10. The same with a much shrinked tendril.

Fig. 11. The same with a tendril twined round a root-hair of a host (*Zingiber*).

Fig. 12. Two seedlings at advanced stage. In the right is shown an entire embryo taken out of the endosperm.

Fig. 13. A tubercle on the root of *Zingiber*. ×*ca.* 40.

Fig. 14. The same at somewhat advanced stage. ×*ca.* 40.



S. Kusano del.

A Contribution to the Cytology of Synchytrium and its Hosts.

BY

S. Kusano.

With Plate VIII—XI.

CONTENTS.

I. Introduction.

In the "Centralblatt für Bakteriologie, etc., Bd. XIX, 2. Abt., 1907" (KUSANO, '07b) I have published a short account on *Synchytrium Puerariae* and *S. decipiens*. The results of observations set forth in that communication were chiefly concerned with certain cytological phenomena relating to their hosts. In the present article I intend to communicate the results of cytological studies on the fungus-bodies themselves, with a view to throw some light upon the structure of the nucleus and nuclear division in this group of fungi, which no one has heretofore exhaustively investigated. I may also be allowed to dwell upon the influence of these fungi upon the cytological phenomena of their hosts, of which certain points were not dealt with in detail in the former communication.

The authors who have worked upon the cytology of *Synchytrium* are not so numerous as those who have devoted themselves to studies in similar directions in other genera of Phycomycetes, and moreover none of them seems to have attempted to follow throughout the whole life history of the fungus. This is perhaps due to the small size of the nucleus and to the difficulty in getting serial stages of development. A comparatively full account of the successive stages of development was given by DANGEARD ('90) in *S. Taraxaci*, in which he pronounced the nuclear division to be mostly amitotic, while mitosis occurred in rather exceptional cases. Later,

ROSEN ('93) studied the same fungus and confirmed DANGEARD'S view as to the existence of amitotic division, remarking, however, that in the secondary nuclei mitosis occurred much more frequently than amitosis. Further, he noted that the amitotic division was quite different from the typical form: the chromatic threads thickened, the nucleolus divided into two halves, each migrating into one of the daughter-nuclei, and the nucleus constricted itself in the middle without the aid of the usual achromatic structure.

In his study on the cell-division in sporangia and asci, HARPER ('99) dwelt upon the mode of the sporangium-formation in *S. decipiens* and *S. Taraxaci*. He gave a very full account of the manner of cleavage-formation in the fungus-body, by which the protoplast was successively cut off into smaller and smaller portions till each portion contained finally a single nucleus in *S. decipiens*, or a few nuclei in *S. Taraxaci*, and thus he came to the conclusion against DANGEARD ('90) who maintained that the division of the primitive cell of *S. Taraxaci* to form sporangia was a process of simultaneous fragmentation, whereby the multinucleate protoplasmic mass was at once cut into a number of polyhedral multinucleate portions. As the object of HARPER'S work was to know the details of the formation of sporangia, no statements were made about the other cytological phenomena of the fungi.

A considerable advance of our knowledge of the nuclear division in *Synchytrium* was made by STEVENS ('03). He took the primary nucleus of *S. decipiens* as the object of his study, and concluded, contrary to DANGEARD and ROSEN, that the division was usually mitotic. Besides, he mentioned several characteristics of considerable interest in the nucleus, namely a sudden shrinkage of the notoriously large nucleus and marked diminution of chromatin previous to division, dissolution of the nuclear membrane into a granular persistent halo around the karyokinetic figure, a peculiar form of the spireme, etc. These results of his observations particularly attracted my attention and induced me to undertake the present work, intending on the one hand to verify such remarkable nuclear phenomena by my own observations in the same fungus, and on the other to ascertain whether they hold true also in allied species.

Papers referable more or less to the cytology of *Synchytrium* were published by LÖWENTHAL ('05 a, b) and recently by RYTZ ('07). The former

author described the structure of the nucleus in the resting condition in *S. Taraxaci* and in the resting cell of *S. Anemones*, while the latter author noted, during his morphological and biological studies on several species of *Synchytrium*, the structure of the resting nucleus and also briefly the formation of the sporangium in *S. Succisae*.

These are all that we know at present on the cytology of *Synchytrium*. The results obtained by the authors mentioned above, of course valuable as they are, seem somewhat fragmental, and consequently we can not by any means draw from them a definite conclusion, as for instance, about the karyokinetic figures in the fungi under consideration. Regarding other nuclear phenomena a more extended comparative study seems to be very much desirable.

In my present work special attention has been paid upon the behaviour of the nucleolus during the nuclear division, the formation of the nuclear membrane, and the ontogenetic origin of different elements in the daughter-nucleus. Relating to the nucleolar problem in general some interesting and important results have been obtained by WAGER ('04), M. v. DERSCHAU ('06), GEORGEVITCH ('08), and others, but regarding the origin of the nuclear membrane and the other nuclear elements which enter into the composition of the daughter-nucleus, there are several difficult questions still awaiting solution. These questions are certainly due to the difficulty of observing the finer details of the processes that take place during the reconstruction of the daughter-nucleus. Now in my material, I have been fortunate enough to follow very closely the changes of the nuclear elements from the telophase stage to that of the reconstruction of the daughter-nucleus. Some of the important results on the nuclear phenomena obtained at these stages were already given in my preliminary note ('07 a).

The authors who have studied Chytridiaceae have directed their attention more or less upon the cytology of the host-cell. MAGNUS ('97, '01, '02) reported several instances of the fact that *Urophlyctis* dissolves the cell-wall of the host. LÖWENTHAL ('05 b) mentioned the hyperchromic deformation of the nucleus in the host-cell infested by *Synchytrium Anemones*, while the hypertrophy and fragmentation of the nucleus took place, according to POIRAULT ('05), in the host-cell of *Physoderma*.

Among other intracellular parasites *Plasmodiophora* and *Dendrophagus*

show in their manner of development and in their nutritive relation to the host-cell many points of resemblance to *Synchytrium,* so that their pathological influence upon the host should be taken into consideration in the present study. NAWASCHIN ('99) came to the conclusion with *Plasmodiophora* that the cytoplasm of the host-cell somewhat increased in amount, the nucleus hypertrophied, and both were then consumed entirely by the parasite. At nearly the same time TOUMEY ('00) studied *Dendrophagus* and arrived at a similar conclusion.

The material of the present study—*Synchytrium Pueratiae* on *Pueraria Thunbergiana* and *S. decipiens* on *Amphicarpaea Edgeworthii* var. *japonica*—was collected mostly at Komaba in the spring of several years and at different times of the day. It was fixed chiefly in FLEMMING'S solution and KEISER'S acetic sublimate, directly in the field, or after keeping for a few hours in a moist chamber at 20-25°C. Most of the karyokinetic stages were obtained from the warmed material. The sections were stained either with FLEMMING'S triple stain, or with HEIDENHEIN'S iron haematoxylin. Sometimes fuchsin iodine-green was very satisfactory in differentiating the structure of small nuclei in sporangia.

In this place I express my deepest thanks to my friend Dr. K. MIYAKE, Lecturer in our College, for his kind advices and criticisms during the progress of the present work.

II. Mode of Infection of the Swarm-Spores in the Host-Cell.

Almost all the authors who have undertaken the study of *Synchytrium* agree in stating that the infection takes place exclusively on the epidermal cells of the host. However, according to the illustrations of some tubercles caused by the fungus by SCHROETER ('70), RYTZ ('07), and others, it appears that the fungus infects the subepidermal cells, since host-cell is shown as lying beneath the surface of the host. Notwithstanding, evidences to the contrary have been given by many observers who have studied the development of the tubercles. Hence, taking the mature tubercle caused by *S. decipiens* and *S. Pueratiae* into account, we have good reason to believe, as

its structure is in no wise different from that of the tubercles caused by other *Synchytrium*, that the host-cell belongs also to the epidermis. This view seems to be especially applicable to the tubercles caused by *S. decipiens* on the stems, petioles, and veins of the leaves; but the comparative studies on the development of the tubercles in different portions of the host, made on the two fungi under consideration, afford a strong evidence that in no case the swarm-spore enters the epidermal cell. If inoculation should take place on the epidermis, it must be admitted, unless the swarm-spores are able to penerate the thick cuticula, that the possibility of inoculation be limited to the younger stages of development of the host, when the cuticular layer is not yet produced. The fact that inoculation may take place on older parts of the host makes it most probable that the swarm-spores enter the host through the stomata, or water-pores. Chemotactic experiments made with *S. Puerariae* offer a strong evidence for this view (KUSANO, '08). I found that certain tissues of the host contain a strongly attractive substance; for instance, an adult but still living trichome, when its cut end is inserted in a drop of water in which the swarm-spores swim actively, attracts them most promptly at or near the given end, and also a piece of hyaline tissue exerts the same action upon the spores, while the green tissue repels them, or even acts injuriously. Though not so striking as in *S. Puerariae*, the attractive action of hyaline tissue upon the swarm-spores of *S. decipiens* is quite evident. Hence, the conclusion to be drawn from these facts relating to the mode of infection of the swarm-spores will be that a certain attractive substance is diffused out through the stomata, or water-pores, from the inner tissue to the fluid on the surface of the host, in which the swarm-spores are liberated; it stimulates them to approach the source of stimulant after leading them to an intercellular space under the stomata. As I have stated elsewhere ('07), the young fungus-body is always found in the hyaline cell, or the cell with very little chlorophyll, a fact entirely in accord with the result obtained by experiment that hyaline tissue attracts, while green tissue repels, the swarm-spores.

Let us now consider how the position of the host-cell is controlled by the development of the intercellular space. The size of the intercellular space has bearing upon the distribution of the swarm-spores after entering the subepidermal tissue. Generally, the host-cell of *S. decipiens* is found

in the hyaline mesophyll lying between the palisade and the spongy tissue, and in the hyaline tissue of the veins, petioles, and stems immediately below the epidermis, especially just under the stomata. So far as my observations go, the peripheral position of the host-cell, in all parts of the host-plant except the mesophyll, is correlated with the lesser development of intercellular space in the tissue of the given parts. For, it is evident that the swarm-spores, after reaching the wide intercellular space developed under the stomata, are not capable, on account of the compact arrangement of the cells, to proceed further in the subepidermal tissue, so that they are compelled to infect any available cell in the immediate vicinity (Fig. 97). We find in this case very characteristic structure of the tubercle to be developed, as a rule, in the infected spot by the enlargement of the host-cell due to the subsequent growth of the fungus-body inside and by multiplication of cells around the host-cell. The epidermis is forcibly upheaved and consequently stretched. The guard-cells of the stomata, being then separated from each other, may leave between them a wide space, and may bring the wall of the host-cell to direct exposure through this space on the surface of the host. Cross sections or surface views of the tubercle thus developed reveals no different feature from that developed when the epidermal cell is infected, as is the case in other *Synchytrium* (Fig. 98).

There is a remarkable difference in the structure of the tubercle caused by *S. Puerariae*. In the tubercle either in the mesophyll or other parts, we find in almost all cases many layers of cells between the epidermis and the host-cell, and no definite relation can be made out between the position of the host-cell and the stomata. This peculiarity of the tubercle is due, in my opinion, to the development of intercellular spaces in the subepidermal tissue, large enough to allow the swarm-spores more latitude of movement, and to give them more frequent occasions to attain the cells more removed from the stomata.

The conclusion to be drawn from the results of my observations is that the swarm-spores of both *S. decipiens* and *S. Puerariae* enter the subepidermal tissue through the stomata, being responsive to chemical stimuli, and infect those cells which contain the stimulating substance, the depth of infection depending upon the degree of development of the intercellular spaces in different parts of the host. In the mesophyll of the leaf the swarm-spores

of both fungi can with equal easiness reach the proper host-cell, since the intercellular spaces necessary for their forward movement is sufficiently developed in the spongy tissue, while in other parts of the leaf and in the stem, *S. decipiens* develops near the surface and *S. Puerariae* in the deeper tissue according as the intercellular spaces are more or less developed.

III. Cytology of Synchytrium Puerariae.

After the swarm-spore enters the host-cell, it begins to grow rapidly in the protoplast of the host till it becomes a spherical, orange yellow, semifluid mass easily recognizable with the naked eye. When the fungus attains its maximal size, the feeding action of the host seems to be closed, and now commences nuclear divisions in the fungus-body. The repeated divisions are followed by the formation of sporangia, in which the swarm-spores are formed by further successive nuclear divisions. Unlike other *Synchytrium*, no typical resting spore ("Dauerspore") is found in this fungus. For the sake of mere convenience we will designate the uninucleate stage the vegetative, and the subsequent stage the reproductive period.

A. VEGETATIVE PERIOD.

1. The Swarm-Spore.

For the sake of comparison with the youngest form of the fungus-body developed in the host-cell I shall take here first the swarm-spore into consideration. The general shape of the swarm-spore is oval, with a long flagellum at narrow end. At the broader portion of the body it has a few drops of orange yellowish oily matter, which stains intensely black with osmic acid (Fig. 80 *a*). The nucleus is comparatively small, on which account we can not recognize distinctly the detailed structure (Fig. 80, 81). In well-stained preparation we find two or three deeply stained granules, all adhering to the indistinct nuclear membrane, and a hyaline space in the interior of the nucleus. Of the granules the largest represents the nucleolus, and the other, chromatin. Compared with another stage of development the

relative size of the nucleolus at this stage is very small, being more or less flattened and not spherical as usual.

In the living state the nucleus is visible as a refractive speck. The refraction of light is due perhaps to the nuclear sap which makes up the main part of the nucleus, so that I can not agree with ZIMMERMANN ('96, p. 33) who considers the similar speck visible in the swarm-spore of Chytridiaceae as nucleolus, which SCHMITZ takes as chromatin-mass.

2. Youngest Form in the Host-Cell.

The youngest form of the fungus in the host-cell, as observed by myself, is shown in Fig. 1, and on the basis of culture experiment of the swarm-spores,[1] I estimate it to be nearly one day old after infection. In the given figure the fungus-body measures twice or more in diameter as the swarm-spore. The cytoplasm is dense and granular, being easily distinguished from the somewhat fibrillar protoplast of the host in which it lies imbedded. It is not always spherical, though usually so, and often it undergoes more or less deformation (Figs. 1-3). The visible change in the nucleus at such a stage is, above all, its increased size and the enlarged nucleolus, while the chromatin-granules remain as before in size and number, and the nuclear membrane is yet obscure.

The enlarged nucleolus is now spherical, homogeneous and deeply stained. As a whole, there is no essential change in the structure of the nucleus except the increase of nucleolus in size (Figs. 1, 2).

3. Growing Stage.

As the fungus undergoes further development, some changes can be seen in the growing nucleus. Accompanying its growth in size the chromatic substance increases gradually. It appears mostly as fine granules, lying close to the nuclear membrane. There occur moreover considerably

1. I was able to raise the swarm-spore to a certain stage of growth by inserting a capillary tube filled previously with the spores in the young shoot of the host, whereby the spores near the inserted end of the tube are nourished by the juice of the host, and after 24 hours they grew up to a spherical mass twice or more in diameter as compared with the spores at rest (KUSANO, '08).

large granules, or globules, studding the nucleolus, which appear to be made up of the chromatin judging from their staining reaction (Figs. 3, 4). The Achromatic substance also increases in amount. It does not form a typical reticulum, but forms fine granules without any definite structure, or sometimes of a faintly fibrous structure.

It is a very remarkable fact that the quantity as well as the structure of the achromatic substance are not constant according to the fixing fluids, even in nuclei of similar stages of development. Fixed in KEISER'S solution we always find a large amount of it in a finely granular (Figs. 31-37) or globular form (Fig. 5). When FLEMMING'S solution is used, it appears more or less fibrous and is very scanty (Figs. 4, 7, 26, 29). Careful observations appear to show that the globular mass produced by the former fixative occupies in the cavity of the nucleus the space left hyaline by the latter. This difference comes out constant whatever stains we may use. Hence, there is but little doubt that what we designate achromatin in this case on account of its staining quality comprises in reality two substances of quite different origin, one the true linin substance which is revealed distinctly by FLEMMING'S solution and the other a precipitate of the karyolimph, which is brought out more prominently by KEISER'S solution. The precipitation seems, I believe, to be due to the presence of certain protein substance. DAVIS ('98, p. 268) found a similar structure of achromatin in the nucleus of the mother-cell of the tetraspore of *Corallina,* which he also assumed as due to a soluble protein substance in the karyolimph. It seems, however, that the protein substance in question of *Synchytrium* is not of the same nature as that of *Corallina,* since in the latter it is precipitated by FLEMMING'S solution, which is not the case in the former. The lightly stainable, finely granular mass found by LÖWENTHAL ('05 b, Fig. 2) in the nucleus of *Synchytrium Anemones* is probably a precipitation product in the karyolimph closely similar to that of *S. Puerariae;* for, it was also obtained from a material fixed in a solution of sublimate (hot alcoholic solution).

Accompanying the increase of chromatin-granules in the cavity of the nucleus a remarkable change occurs in the enlarging nucleolus. Hitherto it has appeared as a compact mass, but now it becomes highly vacuolate. At first, a few comparatively large vacuoles occupy the centre, but finally

numerous small ones come to lie uniformly distributed in the peripheral portion.

The time of the first appearance of the vacuoles can not be definitely made out, since the nucleolus about such a stage stains always deeply. It is however to be observed that the comparatively small nucleolus, in spite of its compact appearance, betrays a vacuolate structure, if a very thin section through its peripheral portion be examined. Hence it is safe to conclude that some vacuoles are present at an early stage, as shown in Figs. 3 and 4, but are invisible on account of the deep staining of the ground substance of the nucleolus.

The vacuolation is a general occurrence in the nucleolus of plant as well as animal cells (WAGER, '04), and has been already ascertained in Chytridiaceae (ROSEN, '93, STEVENS, '03, LÖWENTHAL, '05 b, RYTZ, '07). In the latter family the most remarkable vacuolation was first noticed by STEVENS in *Synchytrium decipiens*. He distinguished two different structures imbedded in the granular matrix of the nucleolus in the primary nucleus—the homogeneous globules of varying size (dissolution product) and ordinary vacuoles, both of which become more numerous in later stages. As regards the enclosed globules he did not mention their fate with accuracy. As the vacuolation is more prominent in *S. Puerariae*, I can not only confirm the view of STEVENS, but possibly show the behaviour of the nucleolus, which appears to have some bearing upon the vacuolation.

a.—Vacuolation of the Nucleolus and the Formation of the Secondary Nucleoli.

The increase of vacuoles in the nucleolus is accompanied by increase of the extra-nucleolar substances in the cavity of the nucleus, of which the large globules (Figs. 3-5) show at once their ontogenetic connection with the nucleolus by their attachment to the latter, though this is not always the case. At their first appearance at a young stage of the nucleus, these globules are comparatively small and compact, and in stained preparations look like the usual chromatin-granules. It is safe to say that, if they are not entirely made up of chromatin, they contain at least much more chromatic substance than those appearing at later stages. As the nucleolus becomes larger and larger, the globules that are presumably to be derived from it increase also in size, as shown in Figs. 6 and 7. In these figures these

globules are very numerous and are mostly detached from the nucleolus. In materials fixed in KEISER's solution and stained with haematoxylin, they are always stained heavily like the nucleolus itself and are indistinguishable from the typical chromatin-granules that are also present at this stage (Fig. 6). But in materials fixed in FLEMMING's solution and stained with haematoxylin, they take up less stain than the chromatin-granules or smaller globules, just like the nucleolus. I therefore believe that the large globules do not differ materially from the nucleolus, so that I propose to call them "secondary nucleoli" in distinction from the parent-nucleolus which may now be called "primary nucleolus." The chromatin-lumps which STEVENS ('03, Figs. 3, 4) mentions as occurring in a similar stage in *S. decipiens* seem to be homologous to, if not exactly same with my secondary nucleoli. Although the lumps figured by him are somewhat irregular in shape, it does not seem that they are invariably so. For, we have easily convinced ourselves that the secondary nucleolus in *S. Puerariae,* though originally spherical, may become irregular in outline, when the chromatin of the nucleolus becomes condensed on its surface as granules, which are nevertheless hidden from view by reason of the deep stain. According to my own observations on *S. decipiens,* which will be given fully later on, the lumps represent secondary nucleoli. Otherwise, they represent perhaps deformed nucleoli instead of being true chromatin-masses.[1]

There is perhaps no need practically to dwell upon the problem whether the smaller globules are of strictly chromatic or more or less nucleolar nature, but with the larger ones it is a matter of importance to make clear their nature. If we take into consideration the occurrence of globules of considerable size, such as are represented in Fig. 9, it will be at once clear that they are certainly of nucleolar nature.

In staining reaction the secondary nucleoli differ in varying degree from the primay nucleolus. Usually the oxyphile element predominates in the former, in consequence of which the secondary nucleoli take up the blue colouring matter. Stained with FLEMMING's triple stain, therefore, they are violet, while the primary nucleolus is intensely red. Both nucleoli also

1. The differentiation of chromatin and achromatin in the secondary nucleolus appears less distinct with FLEMMING's triple stain than with iron haematoxylin.

behave differently towards haematoxylin: fixed in FLEMMING's solution the secondary nucleoli are stained more intensely than the primary, and *vice versa* when fixed in KEISER's solution (Fig. 13).

In plant cells the occurrence of secondary nucleolus has not hitherto been paid much attention to as in animal cells. As MONTGOMERY remarked already ('99, p. 515), "it is, in animal kingdom, quite frequent in many egg-cells, though it is infrequent in somatic cells of Metazoa."[1] In the plant kingdom an instance was given by FERGUSON ('01) in the egg-nucleus of *Pinus Strobus,* but no additional cases have, as far as I know, been brought forward, which are at all comparable to it, nor has the nature of the secondary nucleolus been so throughly studied as in animal cells.

Concerning the ontogenetic origin of the secondary nucleolus various views have been advanced by zoologists. BRAUER ('91), FLODERUS ('96), and MONTGOMERY ('99) believe that the small nucleoli are simply budded off from the surface of the larger one, while SCHNEIDER ('83), MARSHAL ('92), and MCGILL ('06) have brought forward evidences to show that the former are produced in the interior of the latter, and pass out through the pores in the cortical substance. FERGUSON confirmed in *Pinus* the latter view. In *Synchytrium* we can readily mention several facts to prove that the secondary nucleolus is derived from the primary one. Firstly some larger ones among the secondary nucleoli are frequently found attached to the surface of the primary nucleolus; secondly the more the secondary nucleoli increase in number, the more the vacuoles appear inside the primary nucleolus; thirdly the relative amount of chromatin in the secondary nucleoli is approximately equal to that contained in the primary nucleolus just at the time of their appearance; and lastly, when the primary nucleolus is highly vacuolate, the secondary nucleoli that are still attached to it are also equally vacuolate.

All these facts irresistibly suggest that the secondary nucleoli have a genetic connection with the primary nucleolus. As regards the manner of their derivation I believe that they are preformed inside the primary nucleolus. Two large vacuoles in Fig. 14, which are found somewhat projecting from

1. He called the secondary nucleolus "paranucleolus" and ordinary one, "true nucleolus." WILSON designates them "accessory" and "principal" nucleolus respectively.

the surface of the primary nucleolus, seem to me not to be the ordinary vacuoles, but to be globules representing the secondary nucleoli, only slightly stainable with haematoxylin at this stage. In Fig. 13 one of the globules is in the way of passing out through the pore on the surface of the primary nucleolus in the cavity of the nucleus. This augument is strengthened by a similar process to be observed in the nucleolus of the allied form *S. decipiens*. In Fig. 93 two fissures may be found on opposite sides on the primary nucleolus, which were perhaps produced by compression under the cover glass. Close to each fissure lies a large vacuolate secondary nucleolus, probably just pushed out through it. It is highly probable that the fissures were produced at the hollow space made after the emission of these secondary nucleoli. A large vacuole near the surface of the primary nucleolus shown in Fig. 92 would represent with certainty such a hollow space. The crescent-shaped depression on the surface of this vacuole is in all probability the place from which the secondary nucleolus had passed out. A quite similar figure was given by FERGUSON ('01, Fig. 36) in a nucleolus of *Pinus Strobus*, which, according to her, produces secondary nucleoli.

The secondary nucleoli lying near, or attached to, the parent-nucleolus are usually larger than those already detached, and the vacuolation which may be seen in the attached nucleolus disappears in the detached ones. From these facts it may be concluded that the substance of the secondary nucleoli is being gradually condensed, and becoming more compact. We may further assert that the composition of the secondary nucleoli are more or less similar to that of the parent-nucleolus; while the latter contains more chromatin at its younger stage, the secondary nucleolus derived from it shows a stronger chromatic character, but when the parent-nucleolus becomes vacuolate and poor in chromatin, it produces also vacuolate and less staining secondary nucleolus.

LÖWENTHAL and RYTZ have already mentioned multinucleolate nucleus in *Synchytrium*, but they did not notice the origin of the smaller nucleoli, or their relation to the larger one.

b.—Condensation of the Chromatin.

The chromatin-granules increase gradually, as the nucleus advances in growth, and they attain the maximal number in the full grown nuclelus in which numerous secondary nucleoli are present. From observations of nuclei

in several stages of development I have arrived at the conclusion that the chromatin-granules are mainly condensation products either of the secondary or primary nucleolus. Though many of these granules are freely distributed in the cavity of the nucleus when full grown or a little earlier, showing no apparent evidence of their genetic connection with the nucleoli, yet some granules are found always to stud the secondary nucleoli when a certain period of time has elapsed after their derivation from the primary nucleolus. That these granules are derived from the nucleolus is quite obvious when we observe the development of the latter. With all secondary nucleoli the staining reaction shows at once that at an early stage they contain chromatin uniformly distributed. The staining capacity by chromatic stains of the ground substance gradually decreases during the development of the nucleoli, while the peripheral portion becomes more heavily stainable. The irregular shape which is often observed in some secondary nucleoli is certainly due to the precipitation of chromatin-masses at the cortical layer of the nucleoli. An advanced step of such process is shown in Figs. 7 and 8, in which the chromatin-granules apparently adhere to the surface of the nucleoli whose ground substance has now lost considerably their staining capacity. The next step at which the granules are to be entirely detached from the nucleoli is represented in Fig. 9.

The same process comes out most clearly in the primary nucleolus which has discharged all its secondary nucleoli. Its ground substance appears very faint in stained preparations, and small vacuoles which were previously distinct is now scarcely visible. There is perhaps a residue of chromatin, and it now commences to condense at the peripheral layer and then appears as a distinct cortical layer of the nucleolus (Fig. 15) or small granules studding the surface (Fig. 7). Some observers have already expressed the opinion that in the nucleolus of higher plants the chromatin occupies the peripheral portion, while the interior is filled with plastin (cf. STRASBURGER, '00, p. 138, '07, p. 75 and KOERNICKE, '03, p. (111)). In *Synchytrium* it is, of course, not to be denied, from the facts above given, that the chromatin is contained in the nucleolus. As to its state of distribution in the nucleolar substance I can not yet advance any definite view as to whether it occupies always the peripheral portion or not. So far as the stained preparations

are concerned, the chromatin seems to be distributed uniformly in the plastin of the nucleolus in the younger stage of the nucleus.

The relation of the chromatin and nucleolus is thus clearly demonstrated in the later stages of development of the nuclei, but there is some doubt about it when the nuclei are at the beginning of growth. It is then not practically apparent whether the globules derived from the nucleolus are chromatin-granules or small secondary nucleoli rich in chromatic substance, in which the condensation process of the chromatin is not clearly exhibited. Perhaps in the smaller secondary nucleoli condensation process may be practically omitted, and they then originate as globules approximately of the nature of chromatin-granules.

When we take into account the nature of smaller and larger globules derived at various successive stages of the nucleolus, it may be seen that the designation of secondary nucleolus does not accurately express its definite nature as distinctive from the parent-nucleolus. As its staining character, for instance, is not constant in the smaller and larger, i.e. early and lately derived nucleoli, it can hardly be said that it corresponds strictly to the paranucleolus (WILSON, '00) or oxyphile nucleolus (MCGILL, '06).

On the question of the derivation of chromatin from the nucleolus there is an immense literature, to which I can not refer in detail here. The critical reviews are found in an elaborate memoir of MONTGOMERY ('99) on the papers published up to 1897. On the botanical side WAGER ('04) and v. DERSCHAU ('07) have dwelt upon this problem, and KOERNICKE ('03, p. (108-109)) and STRASBURGER ('07, p. 74-77) have published critical reviews. Instances of the case in question are increasing in the plant kingdom, and are known in the nuclei not only of the lower, but also of the higher plants (GEORGEVITCH, '08, NICHOLS, '08). We may now mention *Synchytrium* as affording an additional instance of the derivation of the chromatin from the nucleolus.

Together with these changes there appear fine granules thickly besetting the inner surface of the nuclear membrane. They increase in number as the nucleus grows. A great variation is found in their size and staining character; some are like chromatin-granules in size and staining reaction, while others are much smaller and less stainable (Figs. 3, 4, 7, 8). Regarding their origin I am of the view that they are derived directly from

either the primary or secondary nucleoli, or that they are dissolution-products of chromatin-granules. Their peripheral situation in the nucleus is in favour of the view that they are passing out in a dissolved form into the surrounding cytoplasm through the nuclear membrane. The gradual diminution of size and staining capacity is most probably an expression of the process of dissolution they are undergoing. Similar phenomena have already been known in animal egg-cells, in which the nucleolar substances are given off into the surrounding cytoplasm as deutoplasm or yolk-nuclei (WILSON, '00). In the present case the chromatin developed in the growing nucleus is certainly more than sufficient for the formation of the chromosomes during karyokinesis, which, as will be given below, are exceedingly small and few in number. This fact renders it most probable that there are, as in animal egg-cells, two different components in the so-called chromatin, of which the essential component forms the chromosomes, while the other undergoes some chemical change and subserves another function, perhaps something related to nutrition.

c.—The Nuclear Membrane.

Various views have been expressed as to the structure of the nuclear membrane in *Synchytrium*. In *S. Taraxaci* DANGEARD ('90) mentioned the granular structure. According to STEVENS, the wall of the primary nucleus in *S. decipiens* is sharply defined, and at the end of growth the wall becomes very thick and is gelatinized finally. LÖWENTHAL's figure ('05 b) of the nucleus of the resting spore of *S. Anemones* does not show the existence of a distinst membrane. Lately RYTZ ('07, p. 814) succeeded in observing a somewhat sharply defined membrane in certain species of *Synchytrium*.

The definite limitation of the nuclear membrane in *S. Puerariae* is easily observable in the full grown nucleus, when the fine nuclear granules are closely arranged along its inner surface. In surface view the presence of granules makes the membrane appear as granular (Fig. 8), as DANGEARD stated. The increase of the granules in later stages may easily lead us to think that the membrane is swelling into a granular form, but previous to the division of the nucleus these granules vanish entirely, while the membrane proper becomes indistinct and disappears ultimately. Though more or less obscure, the membrane consists invariably of intricated kinoplasmic fibres, and in no case have I been able to observe swelling at any stage. Neverthless there exists in fact a granular mass at certain stage of karyokinesis, exactly similar

to that of *S. decipiens* found by STEVENS as originating from the membrane. As to its origin I must, however, offer a different view from that of STEVENS, as will be given later on at full length.

d.—Dimension of the Nucleus.

STEVENS has already remarked that the primary nucleus of *Synchytrium decipiens* is exceptionally large (35 μ with a nucleolus 11 μ in diameter) among fungi, which are generally known to have exceedingly small nuclei. In *S. Pueratiae* the nucleus is still larger, measuring when full grown 70×52 μ, with a nucleolus 14μ in diameter. This is doubtless the largest nucleus known among fungi. Even in Phanerogams, so far as the vegetative cells are concerned, instances of such a large nucleus are perhaps very few. In sexual cells ZIMMERMANN ('96, p. 11) mentioned among Angiosperms the embryosac-cell of *Fritillaria imperialis* (50×25μ) as having the largest nucleus. The nucleus of the oosphere of Gymnosperms is much larger; thus according to IKENO ('98, p. 583), it measures in *Cycas revoluta* 370μ in length, and according to my own measurement in a preparation of Mr. K. MIYAKE, it is in *Zamia floridana* 610×426μ. If we except these sexual cells, or those vegetative cells which have filamentous nuclei (KÖRNICKE, '03, p. (132)) in some higher plants, *Synchytrium Pueratiae* may be considered as one of the plants which possess the largest nuclei.[1]

B. REPRODUCTIVE PERIOD.

1. Primary Mitosis.

In the full grown fungus-body nuclear divisions occur with great rapidity, and the fungus passes from the uninucleate to the multinucleate condition. This latter condition seems to be coincident morphologically with the general character of the vegetative thallus of Phycomycetes. However, at this stage the symplast of the host (KUSANO, '07 a, b), on which the fungus relies for its nutrition, is expanded into such an exceedingly thin membrane that its activity must be very nearly nil. Simultaneous with this change the fungus excretes around its body a hyaline membrane which apparently seems to

1. For the dimension of nuclei in animal cells consult CARNOY'S paper ('88, p. 270).

arrest the entrance of nutritive substances from the host into the fungus-body, and indicates perhaps the end of feeding action. Consequently we take this stage as the end of the vegetative, and the beginning of the reproductive period in accordance with HARPER'S ('99, p. 481) and STEVENS' ('03, p. 406) view.

As far as my observations go, the first nuclear division is really mitotic as in *S. decipiens*, and no amitotic division, such as was found by DANGEARD ('90) and confirmed by ROSEN ('93) in other *Synchytrium*, was at all observable.

In studying the primary mitosis I experienced a similar difficulty as STEVENS. The karyokinetic figures are exceedingly small in contrast to the considerable size of the nucleus in the resting condition. Moreover, serial stages relating to the division could not be found out complete in spite of the examination of a great many preparations. So that I shall describe here the successive processes of division up to the metaphase only, from which I can draw conclusions different from those arrived at by STEVENS in *S. decipiens*.

It is somewhat difficult to point out precisely the beginning of the nuclear changes preparatory to mitosis, since the nucleus in the vegetative period is not constant in structure, and there is found no spireme stage preceding the division to indicate the early prophase. However, the critical point, at which the resting stage terminates and division begins, may be practically determined by taking into account certain features in the nucleus, such as its maximal growth, the maximal increase in number of chromatin-granules as well as the secondary nucleoli, and the sudden diminution of these nuclear elements. I will denote therefore this last mentioned condition of the nucleus as an early prophase, corresponding to the spireme stage in the typical karyokinesis.

As the first step in the diminution of the nuclear elements, we see that the secondary nucleoli begin to disorganize, becoming faintly stained and showing only the achromatic ground substance. They proceed then to fade away in the cavity of the nucleus, setting free the attached chromatin-granules. Simultaneously with this change the chromatin-granules gradually diminish, apparently by dissolution, as may be seen from their becoming smaller in size and less stainable (Figs. 10, 11). When this process proceeds further,

the nucleus arrives at a stage with exceedingly few visible elements in its cavity and with a hardly recognizable wall. Fig. 12 shows one of the sections of a nucleus at such a stage. We see here only a few chromatin-granules yet remaining and a few secondary nucleoli in indefinite or somewhat globular form. The primary nucleolus still retains its original form, but its staining capacity seems to have exceedingly diminished. A similar remarkable diminution of chromatic element was already observed by STEVENS in the corresponding stage of *S. decipiens*. He says, "formerly coarse and lumpy, its globular masses become much more numerous and relatively smaller. They then appear to elongate, the numerous globules being replaced by rod crossed and tangled in inextricable confusion" (p. 410). His globular masses (his Fig. 4) correspond to my secondary nucleoli, as already stated, and the much smaller granules of the next stage (his Fig. 5), to the chromatin-granules proper now set free by the dissolution of the nucleoli to which they had adhered. This I can confirm by my own observation of the same fungus. Further, he mentioned that the chromatin-granules were replaced by the rods of chromatin. It seems to me that a further study is necessary to place this fate of the granules beyond doubt. For, as to the perfectly similar granules in *S. Puerariae* there is no doubt that they undergo a gradual dissolution.

The nuclear membrane now vanishes, and the large cavity of the nucleus is soon occupied by the surrounding cytoplasm. At this stage the nuclear contents undergo further dissolution. The primary nucleolus is deformed and produces pseudopodia-like processes through which the ground substance passes out into the cytoplasm. In Fig. 16 is shown a somewhat advanced stage of this process. Here we find numerous rod-shaped granular fibres radiating from the central achromatic mass which represents the residue of the primary nucleolus now in the process of further dissolution. Numerous chromatin-granules studding these fibres seem also to dissolve away together in the surrounding cytoplasm. In the next figure (Fig. 17) a still more advanced stage is shown, in which the primary nucleolus has lost its original form and been replaced entirely by the fibres. The general feature at this stage may be perhaps comparable to the spireme stage in an ordinary nucleus, the fibres corresponding to the nuclear threads; but as the arrangement of chromatin on the fibres is not so regular as on typical nuclear threads, we

may regard it rather as a result of the dissolution of both the chromatic and achromatic substances of the nucleus.

A similar change is known in the prophase stage of the vegetative cell of *Chara fragilis*. According to DEBSKI ('98, p. 635), soon after the nuclear membrane disappears, the "Knäuel" of the chromosomes takes its position in the centre of the nuclear cavity and striations appear radiating from it in all directions, often intersecting one another (see his Fig. 9). He thinks that they form the spindle (p. 642). In *S. Pucrariae* I can not find any genetic connection between these fibres and the spindle. The radiating fibres afterwards appear as granular striations, and a few chromatin-granules left at the focus of the striations enter into the composition of the chromosomes (Fig. 18). While the chromosomes are being built up, the radial striations become fainter and fainter, and acquire a more granular character (Fig. 19). In Fig. 20, in which the spindle is already formed, the striations have disappeared almost entirely and are replaced distinctly by a dense granular cytoplasmic mass. The origin of the spindle could not be clearly made out, but it seems certain that the achromatic residue of the ground substance of the primary nucleolus (Fig. 18) is concerned in the formation of the spindle-fibres.

The spindle is at first broadly oblong with rounded poles (Figs. 20, 22-25). The fibres are very fine but distinct, though at first it appears somewhat gelatinous. At the metaphase the spindle becomes pointed at the poles (Fig. 21). The chromosomes, apparently five in number, are generally spherical (Figs. 22, 25). They often assume during splitting into daughter-chromosomes an oblong form (Fig. 23).

I have not been able to obtain further stages of karyokinesis, and so the details of the reconstruction of the daughter-nuclei is still unknown. This gap, however, may be filled by the serial stages easily observable in the secondary nuclei, with which the main features of the desired stages in the primary nucleus probably agree.

The nucleolus does not always disappear in later stages of the primary mitosis, and its residue is found frequently near the spindle (Figs. 19, 22).

In the metaphase the spindle is surrounded by a halo of granular mass (Fig. 25). A similar figure was already given by STEVENS, but as to its origin I cannot agree with him. He came, after a careful study, to the

conclusion that it is a dissolution-product of the nuclear membrane. His view is supported by his figures which represent successive stages of this remarkable modification of the membrane. When we look over his illustrations, there is little doubt as to his interpretation. Turning, however, to my material no evidence whatever can be found of the genetic connection of the halo to the nuclear membrane. A glance at Figs. 16-25 will be sufficient to convince one that the halo has arisen from the dissolution-product of the nuclear elements. As stated above, these elements appear at first, when the mitosis begins, as radial striations which are gradually transformed into a granular mass round the spindle. This mass is originally large, but afterwards its peripheral portion fades away gradually and is occupied by the surrounding cytoplasm of alveolar structure, leaving only its inner portion round the spindle as a halo which, however, disappears subsequently. A good evidence for the intranucleolar origin of this granular mass will be found in Fig. 21. Here the mass is distinctly separated from the cytoplasm by a wide clear space. The space is in all probability the nuclear cavity, not yet wholly replaced by the cytoplasm. In view of the fact that the nuclear membrane is usually composed of kinoplasmic fibres, the gelatinous swelling of the membrane in *S. decipiens* must be a very strange fact, and it seems to me that for a confirmation of this unique phenomenon a reinvestigation is very much desirable.

The most noteworthy fact observed in the nuclear division is the fate of the chromatin. Most of the chromatin-granules which have attained their maximal amount at the end of the vegetative period are not utilised for the formation of the chromosomes, but are cast away into the cytoplasm. Consequently it is obvious, as already stated, that there are two different components of the chromatic substance, one the bearer of hereditary qualities and the other a certain nutritive substance.

2. The Secondary Nuclei and their Division.

Under this heading I shall consider together the nuclei at the second, tertiary, and successive nuclear generations, since their structure is essentially the same at corresponding stages and it is practically impossible to distinguish accurately one nuclear generation from the one just preceding or succeeding

it, though it is not difficult, provided the difference in size and number of nuclei in different individuals of the fungus-body is apparent, to tell roughly whether nuclear divisions have taken place more or less frequently.

The study of karyokinesis in the secondary nuclei is much easier than in the primary nucleus, so that I can now describe all the successive changes involved in the division.

a.—Resting Stage.

Compared with the primary nucleus the resting nuclei at the second and succeeding nuclear generations are considerably smaller. However, in structure they are essentially similar. The nucleolus, usually one, but often two, in number, is most conspicuous among the visible elements in the nuclear cavity. Later, there appear a few chromatin-granules and a small quantity of achromatic substance in a more or less reticulated condition (Figs. 26, 31). The chromatin-granules increase in number gradually, and meanwhile a few secondary nucleoli may appear. The slight difference in size existing between the primary nucleolus and the secondary nucleoli makes it probable that the manner of derivation of the secondary nucleoli is not exactly the same as in the primary nucleus. In favour of this view we find frequently that the smaller nucleolus is connected by means of a string to the larger one, perhaps indicating that the smaller one is being budded off, or cut off by constriction, from the larger nucleolus (Figs. 27*c*, 31) without preformation inside the latter.

The staining capacity of the nucleoli varies somewhat at different stages of the development. This is, as in the primary nucleus, due to the quantity of chromatin they contain. When the chromatin-granules are set free in larger numbers in the nuclear cavity, the nucleoli become less stainable, and it shows that the chromtain-granules are derived from the nucleoli (Figs. 26-27).

Though not remarkable, the vacuolation occurs in the primary nucleolus. The vacuoles are visible only in well-washed stained and unstained preparations. They become more numerous when the chromatin-granules increase much in number.

b.—Prophase and Metaphase.

The preparation for mitosis is, as a rule, quite similar to that in the primary nucleus. After producing numerous chromatin-granules or secondary

nucleoli, the primary nucleolus becomes more or less irregular in form and produces pseudopodia-like processes which appear to be transformed into the linin thread (Figs. 28, 29). Then the nuclear membrane disappears, whereas the surrounding cytoplasm begins to occupy the nuclear cavity, and the achromatic striations are directly connected with it (Fig. 30). In its whole aspect, this stage is strictly comparable to the prophase stage of the primary nucleus shown in Fig. 16. Though I have not been able to follow its further changes, yet I believe that the process of chromosomes- and spindle-formation is similar to that of the primary nucleus.

We may mention some variations from that type just described. These are illustrated in Figs. 32-36 which have been all obtained from the materials fixed in KEISER'S solution. In the majority of cases the chromatin is discharged from the nucleolus as comparatively large, variously formed masses, not so numerous as in the case given above (Fig. 32). The achromatic substance which is at first diffuse now commences to aggregate in the central portion of the nuclear cavity (Figs. 32 *a, b*). At a more advanced stage it becomes more definite and compact being mostly elliptical. In all probability this achromatic mass represents an early stage of the spindle. At this stage no other visible element can be recognized except the prominent nucleolus. It is noticeable that the nucleolus does not remarkably decrease in size or staining capacity, showing that it still contains much reserve material.

We observe frequently that comparatively large but few chromatin-granules appear on the surface of the nucleolus (Fig. 33), whereas the ground substance of the latter loses remarkably its staining capacity. It seems that the chromatin contained in the nucleolus is far less than in the case given above. I am not able to get later stage which shows the fate of such nucleolus. Perhaps the nucleolus undergoes dissolution, and its last remnants go into the formation of the spindle, while the chromatin-granules are transformed into the chromosomes without any considerable decrease in amount.

Another most conspicuous process of division is that the nucleolus is broken down, without giving off previously numerous chromatin-granules, or without any condensation of the chromatin on its peripheral portion, as last mentioned, in the form of a few large, irregularly shaped masses representing the chromatin-granules. In Fig. 34*a* the first step of such a process is shown, in

which the nucleolus, after producing two large globules of chromatin, is divided into two parts. Figs. 34*b*, *c* represent a further process of fragmentation of the nucleolus, by which the latter is now replaced by a certain number of chromatin-globules forming a clump. It follows, therefore, that in the course of this process the formation of the secondary nucleoli, or the condensation of chromatin on the surface of the nucleolus, is nearly or entirely omitted, and the nucleolus is directly transformed by fragmentation into a number of chromatin-masses, as in the case of the chromatin-nucleolus of many microorganisms and some higher plants. Neither the number of the chromatin-masses nor their size corresponds to that of the chromosomes (Fig. 35). Therefore, in forming the chromosomes, which are much smaller and five in number, these masses must undergo a further change—condensation or dissolution (Fig. 36). While the chromosomes are definitely formed, the nuclear membrane still persists unchanged. The presence of the membrane at this stage shows that the spindle-fibres are of intranuclear origin and are formed from the nuclear elements, as in the primary nucleus. The achromatic substance derived from the nucleolus, or from the residue of the nucleolus, may form a mass on which the irregularly distributed chromosomes assemble and arrange themselves in a definite position. The mass, elongating and taking on an elliptical shape, afterwards becomes the spindle. The fibrous structure of the spindle is not distinct in earlier stages.

In most spindle-figures, so far as observed, there is a residue of the nucleolus, but in some it is wanting. In the latter case the spindle-figures has probably been formed by the fragmentation of the nucleolus (Figs. 39, 44*a*).

Of the numerous instances which show the derivation of chromatin or chromosomes from the nucleolus the process described by WOLFE ('04) in *Nemalion* may be mentioned as closely resembling that of *S. Puerariae*. The nucleolus of *Nemalion* consists of a peripheral region of denser material surrounding a less deeply staining central portion. As mitosis approaches, the peripheral substance aggregates in heaps, which are, according to WOLFE, the units from which the chromosomes are to be formed (compare my Figs. 32-36). Again COKER ('03) observed a similar fragmentation of chromatin-

1. The literature on the origin of the spindle-fibres is enumerated by KOERNICKE ('03) and STRASBURGER ('07,' 00).

nucleolus in certain cells of *Taxodium*. His Figs. 77-80 correspond to what I have mentioned above (Figs. 34-36). Such process seems to be of general occurrence in the lower algæ like *Spirogyra* (MOLL, '93, MITZKEWITSCH, '98, BERGHS, '06, *Sphaeroplea* (GOLENKIN, '99, and others.

It is of great interest to note that the prominent nucleolus persists usually through division. At the stage we have followed so far, its staining capacity does not differ remarkably from that of the resting condition (Fig. 41), though in some cases its size may vary more or less. As a rule, the presence of nucleolar residue in the karyokinetic figures of fungi is quite frequent (MAIRE, '05, GUILLIERMOND, '05). As it seems that the persistence of the nucleolar residue is associated with a certain behaviour of the nucleolus, a full account will be given when a still later stage will be treated of.

The fibrous structure of the spindle is very clearly recognizable at an advanced stage, but a gelatinous structure is frequently observed at an early stage. The fact, in my opinion, points to the interpretation that the plastin in the nucleolus is directly transformed into the spindle-fibres.

At the poles of the spindle we can never detect any structure comparable to the radial rays, centrosomes, or centrospheres. From the beginning of the spindle stage to its end the pointed poles are distinctly separated from the surrounding cytoplasm by a hyaline space.

The chromosomes, as was already stated in the primary division, are mostly globular, and five in number. Keeping in mind that the chromosomes are generally even in number in the vegetative cells of plants, I have examined carefully numerous figures so far obtained, and finding that the maximal number does not exceed five in the prophase (Figs. 40, 41) and ten in the metaphase (Figs. 42, 43), I am justified to conclude that the number of chromosomes is exactly five.

After coming into the equatorial plane, the chromosomes begin to split into daughter-chromosomes. On account of their small size it is not easy to observe the manner of splitting. To say transverse or longitudinal splitting has as a matter of fact no meaning in chromosomes of a globular form. The oblong chromosomes that may be seen in the late prophase (Fig. 41) are the results of the beginning of division into daughter-chromosomes. They are then constricted at the middle and assume a dumbbell-shape (Fig. 42). We find frequently at the same stage thread- or rod-like chromosomes (Fig.

44); they do not represent any definitive form, but a stage in the formation of daughter-chromosomes. Sometimes we find unequal-sized chromosomes in certain spindle-figures (Fig. 45); they are probably abnormal.

c.—Anaphase.

The daughter-chromosomes, migrating from the equatorial plane towards the pole, soon unite together and form an irregular mass (Fig. 46). When this chromosome-mass approaches or arrives at the pole, the spindle begins to elongate and be stretched, becoming narrower at the middle portion. Seldom it happens that deeply stained masses of various forms are visible on the spindle-fibres after the migration of the chromosome-mass (Fig. 47). I can not decide whether they migrate towards the pole or fade away *in situ*. It is rather probable that they are not chromosomes, but much thickened portions of the spindle-fibres taking a deeper stain.

At the next stage the spindle becomes much narrower at its middle portion, being stretched out into a fine string (Fig. 49). Sometimes the spindle is much constricted at the middle without elongation, while the daughter-chromosomes are still half way to the poles, and thus forms, as it were, two daughter-spindles (Fig. 48). In the majority of cases the spindle is broader at the equator even after the chromosomes reach the poles (Fig. 47). However, at the late anaphase the spindle is always drawn out into a fine string, often more than twice as long as its original length (Fig. 50). The elongation of the spindle is the general occurrence in Ascomycetes (Maire, '05, Guilliermond, '05), other fungi (Uredineae: Blackman, '04), or other plants such as Myxomycetes (Harper, '00), *Hydrodictyon* (Timberlake, '01), etc.

d.—Telophase.

After the stretched spindle is broken down at the middle portion, the fibres of each half comes to fade away gradually, and thus the daughter-chromosome-mass is isolated freely in the cytoplasm (Fig. 51). As regards the fate of the spindle-fibres there is little doubt that they contract and are mainly resorbed in the chromosome-mass (Fig. 52). When the chromosome-mass assumes a spherical form and the hyaline space round it is becoming larger, the remnant of the spindle-fibres may be distinguished as a lightly staining globular process attached to the chromosome-mass (Figs. 54, 55). This is a striking fact which however has not as yet attracted the attention of observers in other plants. Harper ('95) mentioned in *Peziza* a somewhat similar remn-

ant of the spindle-fibres at a similar stage of karyokinesis, which however is not attached directly to the chromosomes or other intranuclear elements, and does not enter into the composition of the nuclear elements. According to MITZKEWITSCH'S ('98) investigation on the karyokinesis of *Spirogyra,* the daughter-chromosome-mass at the telophase (his Figs. 22, 42) is attended by a lightly staining process which I believe to be the residue of the spindle-fibres about to enter into the chromosome-mass.

While, at an advanced stage, the chromosome-mass increases in size with the corresponding growth of the hyaline space around it, the residual spindle-fibres appear to be gradually taken into the chromosome-mass (Fig. 55). This mass of chromosomes is now scarcely distinguishable by its form, staining reaction, and other respects, from the residue of the nucleolus of the parent-nucleus, which is found near the equatorial plane as a conspicuous spherical mass or masses (Fig. 55).

This last stage represents the youngest form of the daughter-nucleus which still lacks the nuclear membrane. The prominent spherical mass found in such a young nucleus, which has been derived, as just stated, from the daughter-chromosomes and a residue of the spindle-fibres, represents now a nucleolus. We can say therefore that a part of both the chromatin and achromatin of the parent-nucleus is incorporated into the daughter-nucleus, the two uniting to form a nucleolus.

So far as I know, the material continuity of the nucleolar substances from the parent- to the daughter-nucleus has not been accurately studied except in *Spirogyra* (MITZKEWITSCH, '98). In most of the nuclei with chromatin-nucleolus the continuity of the chromatic substance has been ascertained by several investigators, for instance, DANGEARD ('00) in *Amoeba,* GOLENKIN ('99) in *Sphaeroplea* and green algae, GUILLIERMOND ('03) in yeast, IKENO ('03) in *Taphrina,* WILLIAMS ('04*a*) in *Dictyota,* WOLFE ('04) in *Nemalion,* etc. WAGER ('04) also enumerated similar cases in many higher plants whose nucleolus contains more or less chromatin. The above authors do not, however, seem to have observed the continuity of the achromatic substance in the nucleolus. Indeed WAGER stated in studying the reconstruction of the daughter-nuclei in *Phaseolus,* "there is no indication whatever of any concentration of the spindle-threads to form nucleoli, as NEMEC states, although it is quite possible that a portion of them have

been absorbed into the daughter-nuclei, and may enter into the constitution of the prominent network with which the chromosomes are connected" (p. 48). While it is believed that the nucleolus of both *Synchytrium* and *Phaseolus* is plastin-chromatin-nucleolus, it seems very probable that the achromatic substance in the daughter-nucleolus originates together with the chromatin from the parent-nucleus.

c.—Formation of the Membrane.

The daughter-nucleolus now begins to increase in size, attended by the enlargement of the hyaline space round it, so that it becomes easily discernible from the residue of the parent-nucleolus. However, this stage does not as yet represent a complete nucleus because of the absence of the nuclear membrane; it appears simply to be a large vacuole in the cytoplasm containing a single spherical mass of chromatic nature, now to be called nucleolus. As there is considerable period of time between the completion of the daughter-nucleus and the arrival of the daughter-chromosomes at the poles of the spindle, I could easily trace step by step the finer details of changes that take place during the telophase stage. The most striking phenomenon is the process of membrane-formation, a phenomenon which has attracted very little attention in the general cytology of plants and animals.

The formation of the nuclear membrane is associated with the appearance of a peculiar structure in the cytoplasm. As will be seen from the preceding paragraph, there has occurred, up to this stage, no noticeable structure in the cytoplasm at the polar end of the spindle, a fact which can be demonstrated in the numerous karyokinetic figures found in well-stained preparations. But now our attention is drawn by the presence of a dense mass of cytoplasm close to the incomplete daughter-nuclei. Were it not for its subsequent development, it would certainly have escaped our attention, since it is apparently nothing but a portion of the cytoplasm devoid of the alveolar structure that characterises the other portions. As to its definite position in relation to the nucleus I can not make any authoritative statement; but, so far as can be observed, it seems to appear typically at the polar end (Fig. 55).

At the next stage this cytoplasmic mass is replaced by a system of prominent radial striations, or aster. It is perfectly similar in structure to a typical centrosome. At the focal region of the astral rays we find constantly one (Figs. 57, 58), two (Fig. 56*b*), or more granules (Fig. 56*a*, *c*).

With haematoxylin they are deeply stained, while the rays take less or no stain, appearing notwithstanding clear and sharp. Stained with FLEMMING's triple stain the central granules are intensely red, while the rays appear somewhat blue or violet. Hence we are probably justified in concluding that the aster is of the nature of the centrosome.

At first the aster and nucleus are separated by the intervening cytoplasm; afterwards they come closer together, and the spherical hyaline space of the nucleus, turned towards the aster, reaches its centre, becoming in the meanwhile pyriform (Fig. 58). When the two have thus come in intimate contact, there begins a sharp delimitation of the hyaline space of the nucleus from the surrounding cytoplasm. We first recognize the limiting membrane at the pointed portion of the given space, owing to the precipitation of the kinoplasmic striations of the aster. The process of delimitation proceeds laterally, and finally the hyaline space is completely enclosed by the kinoplasmic striations which now form the nuclear membrane. The evidence that the aster is concerned in the precipitation of the nuclear membrane is furnished by the facts that the astral rays gradually shorten and become less sharp, the focal granules disintegrate, and at the time the membrane is about to be completed the aster becomes very indistinct (Fig. 59). In short, the change occurring in the aster and membrane is quite reciprocal; the more distinct the membrane, the more indistinct becomes the aster. When the formation of the membrane is finished, we find in the place of the aster merely a somewhat denser portion of the cytoplasm, exactly like that present at the beginning of the aster-formation (Fig. 60). This portion becomes entirely indistinguishable as the daughter-nucleus is further developed. The fact is clear and definite, and no further explanation is necessary in drawing the conclusion that the aster is a temporary structure especially developed for the formation of the nuclear membrane.

The development of the nucleolus and the nuclear membrane proceeds independently. Before the appearance of the membrane the enlarged nucleolus may be deformed or constricted so as to bring about the condition of a binucleolate nucleus (Fig. 56). The condensation of the chromatin in the nucleolus may take place before or after the membrane begins to appear (Figs. 57, 58). Generally, at the time the membrane is completely formed, the nuclear contents show very little differentiation (Fig. 60), but some-

times it may proceed further before the completion of the membrane-formation (Fig. 59).

Our knowledge of the membrane-formation in the daughter-nucleus is very incomplete. In *Dictyota* WILLIAMS ('04b, p. 200) has expressed the hypothesis that the formation of the nuclear membrane is determined by the metabolic processes going on in the chromatin-mass. In the typical nucleus of the higher plants LAWSON ('03) is of the opinion that the nuclear membrane originates round the karyolymph, just like the tonoplast which is formed by the cytoplasm coming into contact with the cell-sap. This last view is not generally accepted, since the most prevalent opinion on the nature of the nuclear membrane is that it is of kinoplasmic nature. Although the evidence is not complete, most authors incline at present to the view that the kinoplasmic fibres at the poles of the karyokinetic figures in most of the lower as well as higher plants are concerned in the formation of the nuclear membrane (KOERNICKE, '03, p. (?8)). In our present case the relation of the kinoplasmic rays to the nuclear membrane is shown most clearly, and gives us a proof of the correctness of the last mentioned view as regards the origin of the nuclear membrane.

The transitory occurrence of a centrosome-like body has already been noted by many observers in *Pellia*, and CHAMBERLAIN ('03) has expressed his view concerning its behaviour during mitosis. In the germinating spore he found polar radiations in the mitotic figures. At the centre numerous granules were found, often appearing as centrioles, but he thought they were optical cross sections of the rays. So he called the structure a centrosphere in distinction from the typical centrosome. The centrosphere was not constantly present during the successive stages of mitosis: it disappeared at the end of the prophase and was absent during the metaphase as well as anaphase, but reappeared in the telophase. Concerning its redisappearance while the nuclear membrane was beginning to appear, he said, "the radiations during the telophase may be concerned in forming the nuclear membrane or a *Hautschicht* about the nuclear membrane" (p. 47). Although this observation of CHAMBERLAIN seems to strengthen my view as here presented, further proofs for a more intimate relation between the radiations and the membrane in *Pellia* seem necessary to bring the fact in complete accord with what I have observed in *Synchytrium Puerariae*.

An analogous manifestation of radiations was first announced in Erysipheae by HARPER ('97, '05) during free cell-formation, and it is now known as occurring in other Ascomycetes during the same phases of nuclear division. The nucleus of the ascus is constantly attended by a "central body" which becomes the centre of activity during spindle-formation, and which is transmitted by division from one nuclear generation to the next as a constant organ. Its peculiar function is manifested at the telophase during the formation of the nucleus in the ascospores. The central body with most prominent astral rays comes now to lie near the nucleus on a beak-like prolongation of the nuclear membrane, and the rays begin to form an umbrella-like plasmic membrane surrounding the cytoplasm between it and the nucleus, from which the wall of the ascospore finally arises. The manner of the formation of the plasmic membrane in this case is in precise accord with that of the nuclear membrane in *Synchytrium Puerariae,* the only difference of perhaps minor importance being that in the former the membrane develops in the cytoplasm, while in the latter it appears between the karyolymph and the cytoplasm surrounding it. It seems certain that in Ascomycetes this process is brought about by an accessory function involved in the central body, though in *Synchytrium* a particular organ is developed at the necessary stage. Studying this particular organ in *Synchytrium* a question arises in my mind as to the further function of the central body in Ascomycetes. It is highly probable that it is concerned in the formation of the nuclear membrane as well as in the formation of the cell-wall of the spores. On this point HARPER does not express his view, as he says simply, "a nuclear membrane is next formed, close to the surface of the chromosomes at first, but soon expanding so that more or less clear space appears around the mass" ('05, p. 46). However, I believe further study will prove the correctness of the view of CLAUSSEN that "die Polstrahlen sich an der Bildung der Membran für die Tochterkerne beteiligen" ('06, p. (31)). The case given above in *Synchytrium Puerariae* furnishes evidently an instance in favour of this view.

f.—Fate of the Nucleolus.

Several striking features of the nucleoli have already been described in the resting stage and during division of the nucleus. Still another fact to be noted here will bring out the nature of the nucleolus of the fungus

under consideration still more definitely. In most karyokinetic figures, so far studied, the nucleolus persists essentially unchanged at the telophase (Figs. 54, 55). It represents in all probability the substances left behind by the nucleolus after supplying a sufficient quantity of the important materials required for the nuclear division. As there is apparently a large amount of substances in this residual nucleolus, as is evident from its size, it can usually exist in later stages, in spite of its dissolution taking place without cessation. A similar fate of the nucleolus was already mentioned in Ascomycetes by GUILLIEMOND ('05); but in *Synchytrium* it exists, owing to the slowness of the dissolution process, not only at the time the daughter-nuclei are completely formed, but till the next spindle-formation is beginning (Figs. 38, 39). At this time it diminishes greatly in size and is generally divided into variously sized granules. Now, admitting that the nucleolus is a store house, at least in *S. Puerariae,* of all the nuclear elements or nutritive substances to be used during karyokinetic activity, it may be thought that it stores up too much materials to be entirely spent during the activity of the nucleus. Exudation of the greater part of the nucleolar substance in the primary division will give strong evidence in favour of this view.

g.—Abnormalities.

In a normal condition the numerous nuclei developed in a fungus-body are distributed uniformly in the cytoplasm, and their development advances with equal pace, so that exactly similar figures are found in all of them. Departures from this condition are, however, very frequent. First of all there occurs in some fungus-bodies a bunch, or bunches, of varying number of equal-sized nuclei. The nuclei appear closely adhering to one another and render it at once probable that they were formed by the simultaneous fragmentation of a nucleus, or by amitotic division (Fig. 82). Considering that amitosis is a general occurrence, according to DANGEARD ('90) and ROSEN ('93), in some species of *Synchytrium*, against which STEVENS ('03) did not express a contrary view, we would have concluded, unless any dividing stage was not found in nuclei under such condition, that the bunch of nuclei was a proof of the existence of amitosis. On close examination of numerous bunches I have found only one in the telophase stage (Fig. 83). The stretched spindle-fibres are arranged in all directions, intersecting at a common point. Sometimes we find only two nuclei, perhaps sister-nuclei, lying close together.

Such simplest case of the bunch is, I believe, associated with an abnormal division, in which the spindle is broken into two parts at the telophase without much elongation (see Fig. 48). In all cases the bunches may be considered as formed by the nuclei which produce by mitosis daughter-nuclei lying close together. Whether these aggregated nuclei can afterwards separate from each other or not, I can not say for certain. However, as the bunch is no longer found in the fungus a little previous to the formation of the sporangium, it seems probable that these nuclei are afterwards scattered in the cytoplasm.

There occur in some fungus-bodies unequal-sized nuclei often at different stages of development. This must certainly be related to the difference of frequency of division, the smaller having divided more frequently than the larger.

Some nuclei contain exceedinly few chromatin-granules previous to division, while the nucleolus becomes almost devoid of chromatic substance (Fig. 37). From these nuclei we obtain spindle-figures in which the chromosomes are very small in size and stain very faintly, and the spindle appearing gelatinous instead of being fibrous. How these nuclei develop further is at present unknown.

3. Formation of the Sporangium.

The rapid successive nuclear divisions bring the fungus-body soon into multinucleate condition. At the time the hyaline membrane, which afterwards becomes the wall of the sporangium-sorus, is formed round the whole mass of the cytoplasm, nuclear division ceases for a while, and the formation of the sporangium begins to take place. A full account of this process already given by HARPER ('99) in *Synchytrium decipiens* and *S. Taraxaci* has pointed out the existence of specific difference in the manner of sporangium-formation. According to him cleavage first occurs in *S. decipiens* in the peripheral portion of the fungus-body, and proceeds progressively inwards finally dividing the body into numerous multinucleate masses. Cleavage proceeds further so as to divide each mass into uninucleate portions. He called these last portions "protospores." The nuclei of the protospores come afterwards to divide successively, while the spores are increasing in size, and they are now represented as young sporangia. In *S. Puerariae* I can confirm, in certain points, the facts found by HARPER, but the process

is more complicated here than in *S. decipiens*. Certainly, the process is not similar throughout all fungus-bodies, and we may now distinguish practically two forms of this process.

In the first form, we find, before the appearance of the cleavage or fissure, one or more irregular bodies in the cytoplasm (Figs. 62, 63) or between the outer membrane and cytoplasm (Fig. 61). The central portion of these bodies is more or less vacuolate, and it is intensely black, when fixed in FLEMMING'S solution, and can hardly be bleached with hydrogen peroxide. The peripheral portion is on the other hand hyaline and apparently frothy. It is very probable that these bodies are derivatives of the cytoplasm—perhaps excretion-products. HARPER has spoken on the occurrence of similar bodies in *S. decipiens* at the time the cleavage is about to take place (p. 482, 484), saying that they may be oily matter thrown off together with water by the shrinking protoplasm, being blackened by osmic acid or staining deeply especially with orange G. It is quite certain, as HARPER thought, that such process is the preparation in forming fissure in the cytoplasm.[1] The cytoplasm, having much shrunk in this manner, begins to produce wide furrows proceeding from the periphery to the interior of the cytoplasmic mass, which divide the latter into a few large irregular segments. The loose arrangement of the segments within the outer membrane of the fungus-body shows apparently the occurrence of shrinkage. Generally the furrows are arranged radially, so that the segments are pyramidal in shape (Fig. 62). Each segment is then cut off into many smaller multinucleate parts of varying size (Fig. 63), but ultimately the whole fungus-body becomes segmented into numerous, equal-sized parts (Fig. 64). Their rounded outline and loose arrangement appear to prove that they float or are embedded in a liquid, as was pointed out by HARPER (p. 484). In *S. decipiens*, according to HARPER, the final segments floating in a liquid are all uninucleate, that is, they are protospores, but in *S. Puerariae* they are always multinucleate, and surely represent young sporangia. I was unable to find, after a careful study of numerous serial stages, any segment which at once reached the uninucleate stage. It follows, therefore, that *S. Puerariae* does not form protospores, as is the case with *S.*

1. The throwing off of water occurs generally in the sporangium-formation of Zygomycetes (HARPER, '99, p. 482).

decipiens (HARPER, '99 p. 488). As regards the further development I can only confirm the observations of HARPER in *S. decipiens*.

So far as my observations go, the process just described takes place only in those fungus-bodies that are capable of condensation by throwing off some of their contents. In many fungus-bodies, however, an excretion-substance can not be detected at all at the time the sorus-membrane is found or sporangium-formation seems to have approached. Such a condition induces the other form of sporangium-formation which I am directly going to describe. The compact cytoplasmic mass produces partitions between two adjacent nuclei, as TIMBERLAKE ('01) observed in the formation of the swarm-spores in *Hydrodictyon*. Although I did not succeed in observing the process step by step, the partition seems to appear progressively with great rapidity. The final result of this process is the formation of compactly arranged polyhedral segments (Fig. 65). The size of each segment is approximately equal, but generally far smaller than those of the first form. In this case the process of shrinkage of the fungus-body by throwing off water or oily matter is perhaps omitted before the segmentation takes place, on account of which the formation of the furrow in the cytoplasm is rendered highly difficult.

The polyhedral segments appear as if they represented an advanced stage of the protospores of *S. decipiens* (see HARPER's Fig. 7). However, evidences against such a view are clear and definite. If these were protospores the partitions between two segments would be formed by two membranes. In *S. Pucrariae* two distinct membranes can never be made out between two segments; at first the partition is very faint and it is impossible to separate one segment from the other without breaking off the cytoplasm adhering to the partition. According to HARPER the compact arrangement of the segments which fill up the inside of the outer membrane or the sorus-wall is due to the growth of the protospores which were previously arranged loosely. The same result is effected in *S. Pucrariae* not by the subsequent growth of each segment, but by the omission of shrinkage of the whole protoplasmic mass just preceding the segmentation. The other evidence for this view is that in each segment the nuclei lie at first always close to the partition, leaving a large vacuole at the centre of the segment (Figs. 68, 69).

Fig. 66 will also give an evidence for the existence of the second form of the sporangium-formation. At one side of the fungus-body the polyhedral

segments—young sporangia—are separated freely, and at the opposite side no partition is yet visible, while at the central portion the segments are most clearly shown in compact arrangement. This feature points out decidedly that the segmentation occurs progressively from one side of the fungus-body to the other. This may be, I believe, an abnormal case of the second form; for, usually the segments appear all at the same time, indicating that the partition develops simultaneously, or nearly so, through the whole mass of the cytoplasm. Abnormal as it may be, it is certain that the young sporangia are formed not by furrows cutting the cytoplasmic mass successively, but by the precipitation of partitions in the compact cytoplasmic mass.

Comparing HARPER's and my figures we find a remarkable difference relating to the period of the sporangium-formation. HARPER's figures (Figs. 2, 4) show that the number of nuclei to be found in the whole cytoplasmic mass at the time the cleavage is beginning to appear is far less than that of the similar stage in *S. Puerariae*. Practically, as regards the number of the nuclei in the whole fungus-body, the stage in *S. decipiens* at which the protospore has so far developed as to contain a few nuclei corresponds to the stage in *S. Puerariae* at which the segmentation is just taking place. It seems, therefore, that during the multiplication of the nuclei segmentation occurs later in *S. Puerariae* than in *S. decipiens*, so that the formation of the protospore is omitted in the former.

According to RYTZ's investigation the mode of sporangium-formation in *S. Succisae* (p. 817) agrees well with our case with respect to the omission of the protospore, but in other respects it is not in strict accord. He says that the cleavage may begin to appear while the nuclear division is not wholly ended, so that when the sporangia are cut off, the nuclei are more numerous than at the beginning of the cleavage. This is not the case in *S. Puerariae*; the nuclear division does not take place during the progress of the sporangium-formation, and we find always an approximately equal number of nuclei in the fungus-body before and just after the segmentation.

4. Development of the Sporangium and the Swarm-spore.

In the youngest form of sporangia the cytoplasm is comparatively little in amount, and so it assumes a vacuolate character (Figs. 69-71). As the

nuclei increase in number, the vacuolation is lessened, and towards the end of the multiplication of nuclei, when the nuclei are arranged close to one another in the sporangium, the cytoplasm appears more dense (Fig. 78). In fixed materials the sporangium gives one the impression of having shrunk, when young, by throwing off the soluble substance; for, at the end of segmentation the sporangia are pressed against each other, but now they lie free, assuming a somewhat rounded shape (Fig. 70). The isolated sporangium has a distinct wall, thin and not double contoured. After successive nuclear divisions the sporangia increase in size and come again to form together a compact mass, each sporangium becoming again polyhedral by compression. Further growth of the sporangia themselves brings the whole body of the fungus to a much larger size, and causes considerable compression of the surrounding cells of the host.

The sporangia may be said to have attained maturity, when nuclear divisions have advanced so far as to produce the nuclei of the swarm-spores (Fig. 78). About this time the compressed surrounding cells of the host suddenly become highly turgescent, and their strong swelling causes a sudden rapture of the tubercle in which the fungus develops. By this mean the pulverulent sporangia are forcibly discharged to the outside (KUSANO, '08, Figs. 5, 6). As I have stated elsewhere ('08), the time required for the maturation of sporangia is exceedingly long when the fungus develops on the stem, the sporangia being discharged in the spring of the next year, while the fungus developed on the leaves comes to maturity in three or four weeks. Consequently, we may assume practically the sporangia on the stem as the winter, and those on the leaves as the summer, form.

The structure of the nucleus and the features of nuclear division of the sporangia are essentially similar to those already given. Each successive division considerably reduces the size of the nuclei. This difference is perhaps to be associated with the condition of nutrition. In sporangia the nutritive substances are not perhaps abundant enough to allow the daughter-nuclei to grow before the next division begins. If the nucleolus be assumed to be a storehouse, as I have maintained before, the remarkable decrease of the nucleoli in size during the successive divisions of the sporangia gives us the impression of considerably diminished nutrition of the nuclei.

The youngest form of the resting nucleus in the sporangia consists, like

that of the preceding stages, of a prominent nucleolus surrounded by a large clear space (Fig. 68). One or two chromatin-granules then begin to appear (Figs. 69, 70), and they gradually become more numerous, accompanied by some linin-like achromatic substance connecting the nucleolus and the granules (Figs. 71-73). During the development of the nuclei there is no indication of the derivation of the secondary nucleoli such as we have usually found in nuclei before the sporangium-formation.

Some nuclear phenomena during division are shown in Figs. 74-76. Fig. 74 shows an early prophase, in which the chromatin and linin become highly prominent. Their maximal amount is attained in the next figure (Fig. 75). Here the nuclear membrane disappears entirely, and some portions of both chromatic and achromatic nuclear elements are going to pass out into the surrounding cytoplasm, leaving behind those portions that come into the composition of the chromosomes and spindle. Fig. 76 shows several mitotic figures mostly at the metaphase. In some of them five chromosomes are distinctly present and are generally accompanied by residual nucleoli, while in others daughter-chromosomes are seen to be in the process of separating from each other. It may be noted that the nucleolus is still present at this stage. I am not able to find the subsequent stages, but from what we know it may be inferred that the subsequent mitotic changes are essentially similar to those of the usual type, as observed in other nuclear generations.

From the number of nuclei in the primordial and mature sporangia we can roughly measure the number of the nuclear divisions occurring in the sporangium. Since the primordial sporangium contains as a rule from five to six nuclei, while the swarm-spores derived from a mature sporangium may number from 200 to 300 approximately, divisions must be repeated five or six times. The nuclei at the stage just preceding the final division are shown in Fig. 77. They are exceedingly small, but the nucleolus and chromatin-granules are still very distinct. Once more division is required to form the nuclei of the swarm-spores, which are shown in Fig. 78. In these nuclei we see that the nucleoli have become exceedingly small, hardly distinguishable from the chromatin-granules, and adhere closely to the nuclear membrane in a more or less flattened form.

The mature sporangia when taken out of the host liberate the swarm-spores in 1.5-2 hours in water. The main changes taking place during this

interval are the condensation into granules of the orange-yellowish oily matter previously distributed uniformly throughout the cytoplasm of the sporangia, the segmentation of the cytoplasm into number equal to that of nuclei, and the remarkable swelling of the sporangia. The direct observation of the sporangia during swarm-spore formation under the microscope shows that the swelling of the sporangia is accompanied by the apparent decrease of the orange-yellowish substance, simultaneously with its condensation, and it leads us to think that this substance dissolves into a highly osmotic substance, by means of which the sporangia can absorb so much water as to exert an internal pressure approximately equal to 3½ atomospheres (KUSANO, '08).

Owing to the small size of the resulting spores I can not observe directly the process of the cytoplasmic segmentation. Consequently, it has not been determined whether the spores are formed by successive or simultaneous divisions.

When the swelling reaches the critical point, the sporangia dehisce suddenly at one or two thinner portions of the sporangium-wall, and some of the swarm-spores are ejaculated into the surrounding medium. At the same time the remaining spores begin to jostle against each other most actively, and finding the pores on the sporangium-wall they come out one after the other. The swarm-spores in motion appear somewhat larger than those still enclosed in the sporangia. This is due to the swelling of their body by absorbing much water (Figs. 79, 80).

I have paid much attention to the possible occurrence of blepharoplast in connection with the formation of the flagellum of the swarm-spores. The result has, however, been entirely negative, perhaps owing to the small size of the nucleus.

IV. Cytology of Synchytrium Decipiens.

In the general aspect of its life-history *Synchytrium Puerariae* closely resembles *S. decipiens*. It is, therefore, to be expected that the results of the cytological studies of the two species will agree in the main points. The results of the studies of STEVENS ('03) on *S. decipiens*, however, differ much

from what I have observed in *S. Puerariae*. Although his figures seem to justify his conclusions, yet a glance at them also suggests a similarity in the two fungi at least in the general course of development of the nuclei. It seemed, therefore, necessary for me to investigate *S. decipiens* and compare the results of observations with those of *S. Puerariae*. With this view I have attempted to get as many serial stages of the former as were obtained in *S. Puerariae*, but since my efforts have so far not been very successful, I shall here give only an account of the nuclear phenomena observed in the uninucleate stage of the fungus, to which STEVENS has also confined himself.

The essential features of the primary nucleus which I have been able to obtain are represented in Figs. 87-94, some of which show a remarkable agreement with the figures given by STEVENS. However, taking together the successive changes observable in the nuclei I can not exactly agree with STEVENS in his interpretations. On the contrary I am able to bring forward some very striking phenomena against his view, namely the nature of the coarse and lumpy masses of somewhat chromatic character and the fate of the nuclear membrane during division. It will be remembered that essentially the same phenomena were observed in *S. Puerariae*.

In describing now the nuclei in various stages of development I am compelled to repeat the statements I have already made in *S. Puerariae*. In a young stage the prominent nucleolus is deeply stained and appears homogeneous. I have invariably found numerous globules of varying sizes studding it (Fig. 87). Some of them are too large to be regarded as chromatin-granules, so that I do not hesitate in stating that they are the secondary nucleoli derived from the primary nucleolus in the same manner as in *S. Puerariae*. The evidence is found in Fig. 87. A large, vacuolate, secondary nucleolus is now passing out of the cortical layer of the primary nucleolus, which subsequently diminishes in size and becomes a globular mass with much affinity for chromatic stains. In the next figure (Fig. 88) is shown a portion of a nucleus in which the same process has advanced further. The primary nucleolus is highly vacuolate and less stainable. The secondary nucleoli are very numerous, and one of them still adhering to the primary nucleolus is larger than others and still vacuolate. The process of derivation of the chromatin-granules from the primary and secondary nucleoli is exactly the same as in *S. Puerariae*. STEVENS noted that the nucleolus is surrounded

by a thick darkly staining wall which is probably largely composed of a layer of linin laden with chromatin (p. 408). I have also observed this fact myself in the primary nucleolus at all subsequent stages. This is surely an expression of the condensation process of the chromatin in the ground substance of the nucleolus, the process occurring in a nucleolus that has spent the greater part of its materials, mainly by giving off numerous secondary nucleoli, and has approached division (Figs. 88, 90-92). The assumption of a highly vacuolate condition of the primary nucleolus is also connected with the derivation of the secondary nucleoli. There are found, besides numerous fine vacuoles, a few conspicuously large ones. They seem to represent the spaces where the secondary nucleoli are preformed, and are arranged near the periphery of the nucleolus (Fig. 92), though afterwards found in a more central portion (Fig. 88). These vacuoles appear to become much smaller in later stages (Figs. 89, 90, 91), and I am therefore inclined to the view that they become divided into numerous small vacuoles which are distributed uniformly inside the nucleolus.

A noteworthy feature connected with STEVENS' statement is found in Figs. 89 and 93. Instead of spherical secondary nucleoli, or much smaller chromatin-granules, we find some coarse and lumpy masses arranged densely, exactly comparable to the similar masses given by STEVENS in his Figs. 3 and 4. As they are not all spherical, being rather irregular and at times constricted in the middle, they are possibly not comparable to the typical secondary nucleoli as above mentioned.

Although STEVENS assumed these masses with apparent good reason to be globules of chromatin, I have now good evidence against his opinion and in favour of my view already set forth. They are, in my opinion, somewhat deviating forms of secondary nucleoli. A careful examination of the nucleus, such as is given in Fig. 88 will reveal that the chromatin-granules stud the surface, or the chromatin is condensed on the periphral portion of these problematic masses, as is the case with typical secondary nucleoli. In Fig. 93 are shown two large typical vacuolate secondary nucleoli just derived from the primary nucleolus. From the occurrence of analogous changes in *S. Puerariae* (Fig. 7) and *S. decipiens* (Fig. 88) it is certain that these large nucleoli become after condensation much smaller irregularly shaped masses such as

are found lying close to them, all of which are nothing but advanced stages of the secondary nucleoli.

Let us now turn to the fate of the nuclear membrane, of which STEVENS has spoken most emphatically as being unique. According to him the wall thickens when the nucleus approaches division; at his spireme stage (his Figs. 6, 7) it becomes gelatinous; and at length it is represented as a thick granular "halo" which persists most distinctly around the spindle-figures (his Figs. 11-14). In spite of careful observations I have not been able to observe the swelling of the nuclear membrane at any stage. Only an apparent thickening of the membrane is seen when the latter is studded densely with numerous fine granules (Figs. 89, 94). However, as division approaches these granules disappear entirely, and then the membrane becomes scarcely visible (Fig. 90), as is also the case in *S. Puerariae*. As for the existence of the halo around the spindle I have clearly and distinctly stated in *S. Puerariae* that it is connected with the dissolution of the nuclear elements, and now concerning the similar halo in *S. decipiens* I am inclined to think that it has the same origin. It is obvious that the serial stages showing the development of the halo, which STEVENS has illustrated, led him to conclude that it arose from the membrane. Still it seems to me not quite impossible to make another interpretation on the same figures, viz., that the dissolution-products of the nuclear elementns are precipitated on the outer surface of the membrane as a granular mass, which increases in amount as the process of nuclear division advances further, and may even persist after the disappearance of the membrane. In animal egg-cells a similar mass, called deutoplasm, may often be present around the nucleus, and according to many zoologists, is derived from the nuclear elements. In my opinion, the granular mass found by STEVENS around the nucleus in *S. decipiens* is comparable to the deutoplasm in its nature and manner of derivation.

This is an interpretation which it is merely possible to form on STEVENS' figures. However, I am convinced that nothing can be observed in my own preparations that would support the explanation STEVENS has attempted; the nuclear phenomena at the given stage are exactly similar to those already described in *S. Puerariae*. After the secondary nucleoli undergo degeneration, the chromatin-granules float freely in the nuclear cavity (Fig. 90). It is then followed by a stage represented in Fig. 91, in which all the nuclear

elements have undergone conspicuous degeneration, leaving behind a fibrous achromatic substance, a few chromatin-granules, and a partially degenerated primary nucleolus. The achromatic substance that remains is generally more abundant than in *S. Puerariae*. It sometimes assumes more or less a reticular form, corresponding to STEVENS' spireme. In my opinion, it can not be taken as the spireme, since there is no regular arrangement of the chromatin and achromatin. STEVENS found at this stage the transformation of the nuclear membrane into a granular mass and a nearly complete dissolution of the nucleolus, but in my preparations it is certain that the membrane become indistinct by dissolution and the nucleolus persist although in a highly vacuolate condition. Although I have not been able to follow its further changes, it may be inferred from the strict similarity of the nuclear phenomena so far to those of *S. Puerariae*, that the prophase and subsequent stages will be found to differ from the description of STEVENS, and be similar to those of *S. Puerariae*.

Numerous secondary nuclei in the resting condition were easily available. Their structure does not essentially differ from that observed in *S. Puerariae*. Hence, it is highly probable that they follow in their divisions the same processes as in *S. Puerariae*.

V. Discussion.

In this discussion we will deal chiefly with the nature of the nucleolus and certain characteristics afforded by the nucleus during division in the fungi under consideration.

1. Nucleolus. It has been already admitted that the nature of the nucleolus in many lower microorganisms, in which the differentiation of nuclear elements is not carried so far as in the higher plants, being principally represented by a prominent nucleolus, is quite different from that in higher plants. The resting nucleus of *Synchytrium* is not so simple in structure as in other microorganisms, as there are well differentiated chromatin-granules, linin or achromatic substance, karyolymph, and nucleolus enclosed by a distinct nuclear membrane, just as in a typical nucleus of the higher plants. But the nucleolus does not behave exactly in the same manner as those of the

lower organisms or of the higher plants. The nucleolus is here an essential visible element, or more strictly a morphological centre, in the young nucleus. It produces during its growth both chromatin and achromatin, which may appear in the form of atypical reticulum. In this process it spends a considerable part of its contents, but it does not disappear entirely; on the other hand there remains usually a large amount of nucleolar substance even after the nuclear division.

These facts lead us to think that the nucleolus furnishes, besides the materials for the chromosomes and spindle-fibres, the food materials necessary for the activity of the nucleus, or to be utilised in the cytoplasm. Thus in the present case the nucleolus satisfies all our possible notions of its function, viz., (1) it contributes to the formation of spindle-fibres, (2) it represents reserve supplies of chromatin or chromosomes, and (3) it stores or elaborates the nutritive substances.

The relative amount of chromatin and plastin which the nucleoli contain may vary at different stages of their development, as already ascertained in many plant-cells (see WAGER, '04, p. 42-45) as well as certain animal cells (R. HERTWIG, '08). At an earlier stage in almost all nuclear generations, they are typical chromatin-nucleoli; at a later stage the plastin increases more and more, and they are represented as plastin-chromatin-nucleoli; and lastly, before the prophase stage nearly the whole of the chromatic substance is separated from them, and plastin-nucleoli are produced, which will represent the typical nucleoli of the higher plants. In the secondary nuclei we have often observed that the nucleoli are fragmented directly into chromosomes, and in such cases the nucleoli may be regarded as persisting in their original character, that of chromatin-nucleoli, throughout their development.

In plant-cells examples of the nucleoli containing chromatin in varying degrees are increasing more and more, and even in the higher plants the evidence that the nucleolus hitherto distinguished as plastin-nucleolus is concerned in the formation of the chromosomes is gradually accumulating (WAGER, '04, NICHOLS, '07, GEORGEVITCH, '08). The remarkable character of the nucleolus in *Synchytrium* above given seems to suggest that between the so-called chromatin-nucleolus and plastin-nucleolus there may be found, on further search in this direction throughout the plant-cells, nucleoli showing

every gradation in the relative quantities of chromatin and plastin, thus diminishing the sharp distinction between chromatin- and plastin-nucleolus.

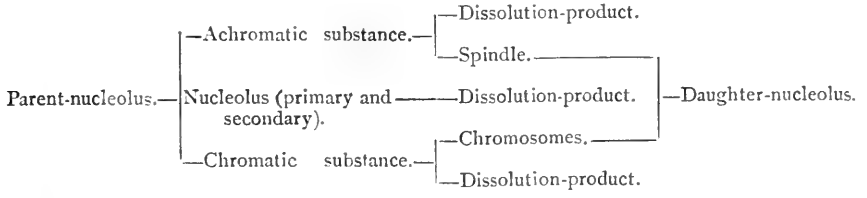
A few remarks must be made in this place about the composition of the gloubular masses which are derived successively from the nucleolus at different stages of its development. At an earlier stage of nuclear development there are much smaller globules, or properly granules, arising from the nucleolus and exhibiting perfectly the nature of chromatin-granules, but the globules appearing at a later stage increase in size and behave towards stains like the nucleolus, while its chromatin elements is further diminished with every granule that it gives off until the chromatin reach the minimum. It follows, therefore, that the globular masses undergo parallel change with the primary nucleolus. Although I previously called these nucleolar bodies conveniently secondary nucleoli., it must be now borne in mind, from the reaction of the globular masses and the primary nucleolus as just mentioned, that their functions are not totally different from those of the primary nucleolus. I am inclined to think that they are exudation-products of the substances stored up in the primary nucleolus during its activity, the exudation being more apparent in the much enlarged nucleolus of the primary nucleus than in exceedingly small nucleoli of the secondary nuclei.

It is a very striking fact that the nucleolus becomes smaller and smaller with each successive nuclear division, as compared with the chromatin-granules, and finally in the swarm-spore it becomes so exceedinly small as to be hardly distinguishable in all respects from the chromatin-granules beside it. Immediately after the swarm-spore begins its feeding action within the host-cell the nucleolus becomes larger again. This fact would seem to justify the conclusion that the nucleolus stores up the food-materials more or less according to the condition or activity of the fungus.

In the secondary nuclei the behaviour of the nucleolus during division differs somewhat from that in the primary nucleus. In the secondary nuclei one or more prominent nucleoli persist through the karyokinetic figures without considerable alteration in their size. This indicates that the exudation of the nucleolar substance just preceding the division is less intense than in the primary nucleus. The absence of the halo around the secondary spindles furnishes an evidence in favour of this conception, and again the same fact

points out that the nucleoli are not exhausted, or that the nuclei are not in activity so much as in the primary nucleus.

2. Ontogenetic origin of the nuclear elements. The study of the telophase stage brings our view on the origin of the constituent elements of the daughter-nuclei to a more definite issue in *Synchytrium* than in any other plant. The nucleolus of the daughter-nuclei owes its origin chiefly to the daughter-chromosomes associated with a residue of the spindle-fibres. The other visible nuclear elements within the nuclear membrane, namely chromatin-granules, linin, and secondary nucleoli, are all derived from the growing nucleolus. It is also clear that the chromosomes and spindle-fibres originate directly or indirectly from the nucleolus, as already accepted by STRASBURGER ('07, p. 75). Thus, the present study brings out very clearly the material continuity of the chromatin as well as the achromatin in the nucleoli from parent- to daughter-nuclei. Therefore, NEMEC'S ('99b) opinion that the spindle-fibres condense to become the nucleolus of the daughter-nucleus appears true in *Synchytrium*. It is a remarkable fact that the chromatin and achromatin are represented differently according to the different periods of the nuclear development; at a very early stage the two are combined in a nucleolus, in later resting stages they separate into chromatin-granules, linin, and nucleolus, and lastly, at the dividing stage they appear partly as chromosomes and spindle-fibres, these two combining again to form a nucleolus of the next nuclear generation, and partly in the form of dissolution-products to be extruded into the cytoplasm. Consequently, the relation between the chief nuclear elements may be represented as follows:



As to the nuclear cavity it is originally a vacuole in the cytoplasm developed so as to contain a certain quantity of karyolymph.

The origin of the nuclear membrane is also clear and definite from our observations. It is formed by the kinoplasmic rays specially developed like the centrosome, the nature of which I am going to discuss.

3. Membrane-forming body. The process of membrane-formation in the daughter-nuclei is certainly a most striking feature in the present study, unlike anything known in general cytology. Although in the foregoing paragraph I mentioned the aster as behaving like the centrosome because of its appearance, there is no doubt that in its function or its relation to nuclear division it does not show any resemblance to the typical centrosome. As is well known, the centrosome is a permanent organ during certain cell-generations, transmitted by division from nucleus to nucleus. It is, as a rule, the centre of activity during karyokinesis, and is especially concerned in the formation of the spindle. Likewise, it is not comparable to the centrosphere which may also play a similar rôle. Further, the polar rays appearing at the poles of the spindles in many higher plants seem to be closely related in function to the centrosome (NEMEC, '01), though their occurrence is quite transitory like the aster in question.

The nuclei of the ascus of many Ascomycetes are permanently attended by a centrosome, or HARPER's "central body," which is transmitted from one nuclear generation to the next. When the ascospore-formation is beginning, the centrosome acts as the centre in forming the "Hautschicht" of the ascospore; but the observations of several investigators have not brought to light any proof of its activity in the formation of the nuclear membrane. Even accepting CLAUSSEN's notion that it may be concerned in the formation of the membrane, we can not yet regard this body as exactly identical with the similar body in *Synchytrium*, since the period of occurrence is quite different in the two cases. Further, CHAMBERLAIN's centrosphere in *Pellia*, which appears to be associated with the nuclear membrane, is not comparable in structure to the centrosome-like body in *Synchytrium*.

The problematic body under consideration is characterised by its short existence, occurring only at the telophase. As noted above, it can not be distinguished morphologically from the typical centrosome or blepharoplast, but on account of its peculiar function it must be regarded as a body *sui generis*, and consequently I venture to call it by the name of "karyodermatoplast"[1] in distinction from other allied bodies.

The origin of the karyodermatoplast is not at present apparent. I am

1. καρυον = nut, δέρμα = skin, πλαστὸς = moulded.

sure that it is not a direct derivative of the nucleus; yet the staining reaction renders it probable that the central granules are of nucleolar origin. Making allowance for NEMEC's view ('99a) that there is a genetic connection between the kinoplasmic fibres and the extranuclear body at the poles of the spindle, I am inclined to think that the karyodermatoplast represents an extranuclear nucleolus which may be transformed wholly into the kinoplasmic fibres while forming the nuclear membrane. This notion agrees well with the prevalent view on the function of the intranuclear nucleolus that it contributes to the formation of the spindle-fibres. As already stated, much nucleolar substance is contained dissolved in the cytoplasm. The appearance of a dense granular mass preceding the formation of the karyodermatoplast indicates most probably the accumulation of this substance to reconstitute the extranuclear nucleolus. Admitting this view, we may say that the nuclear membrane is, like other nuclear elements, derived from the parent-nucleus.

4. Comparison with the ovum-cells of animals. Broadly speaking, the primary nucleus of *Synchytrium* has many points of resemblance to the nuclei of the ovum-cells of animals (WILSON, '00); (1) it is comparatively large, (2) it contains a large amount of karyolymph, (3) there occur, though not in exactly the same form, secondary nucleoli, (4) the greater part of the nuclear elements, mostly chromatin, are thrown off into the surrounding protoplasm, etc. These similarities are perhaps associated with similar behaviour of the nuclei during subsequent development. From the physiological point of view the uninucleate stage of the fungus is strictly comparable with the same condition of the ovum-cell: the fungus at this stage requires to store up or elaborate a large amount of nutritive substance for the subsequent rapid increase of the nuclei, a process in which the nucleolus plays an important rôle. A most remarkable resemblance lies perhaps in the existence of two different components of chromatin, one concerned in heredity and the other perhaps in nutrition. In the nuclei of the ovum-cells they are distinguished by some authors as "idiochromatin" and "tropochromatin" respectively (LUBOSCH, '02). The same designations may in my opinion be applied to the different kinds of chromatin found in *Synchytrium*.

VI. Cytology of the Host-Cell.

It is evident that in all intracellular parasites their development is most intimately dependent upon the condition of the host-cell in which they are enclosed. It is therefore advantageous for the parasites not to inflict direct injury on the host-cell, at least during their vegetative period. In this respect NAWASCHIN ('99) has already stated that the myxoamoeba of *Plasmodiophora Brassicae* is to a certain extent in symbiotic relation with the host, in proof of which it is pointed out that the parasite causes an accelerated development of the protoplast that harbours it. A similar relation between the parasite and the host-cell can be found in the case of *Synchytrium*. When a cell is infected at an early stage of its growth, it contain a larger amount of protoplasm than the unaffected neighbouring cells (Figs. 1-3), and its chloroplasts can accumulate at the same stage starch-granules like those of unaffected cells. While it is believed that *Synchytrium*, when full grown, exceeds considerably in size the ordinary cells of the host, it may be easily imagined that accompanying the enlargement of the fungus-body the protoplast encasing it must be expanded, unless a corresponding increase in amount takes place, to such an extent as to mechanically interfere with its normal activities and consequently to be disadvantageous for the further development of the fungus. However, in both *S. Puerariae* and *S. decipiens* the further development of the parasits is facilitated by the formation of symplasts, as I have stated elsewhere ('07b). The formation of symplasts brings about another advantageous effect upon the fungi. The wall of the cells entering into the formation of the symplast is dissolved by the action of the fungi, and a wide lysigenic chamber results, which affords sufficient space for the enlargement of the fungus-body. MAGNUS ('97, '01, '02), in his studies of *Urophlyctis*, has reported that such lysigenic chamber is produced in a quite similar manner, but it is not certain from his descriptions whether a symplast is formed at the same time, and it remains still undetermined whether the formation of the lysigenic chamber and symplast is of such a biological importance in *Urophlyctis* as in *Synchytrium*.

The group of cells of the host undergoing the dissolution is in almost all cases those that owe their origin to the stimulating action of the fungi.

The number of these cells is different in different parts of the host, or in different stages of development of the affected tissue. Generally the stem and both the petiole and veins of the leaf are more liable to produce such abnormal cells than the mesophyll of the leaf. Also the hypertrophy of the host-tissue is more remarkable when infection takes place at a younger than at a later stage. The varying size of the adult fungus-body is perhaps intimately related with this difference.

When the fungi are yet at an early stage of growth, the symplast is found as a somewhat thick coating around them. But further growth of the parasite causes the symplast to be much stretched (Fig. 95), and towards the end of its vegetative or growing period, the symplast becomes, as it were, an investing membrane for the parasite (Figs. 61, 85). At this stage the activities of the symplast are certainly brought to a standstill, and related to this condition there appears at this time a hyaline membrane around the fungus-body, making the interchange of substances between the symplast and the fungus possibly difficult or quite impossible. The above fact shows that the fungus does not consume the symplast at the end of its vegetative period, and that the disappearance of the symplast, which takes place after the fungus has further advanced in development to form mature sporangia, is most probably due to selfdisorganization. I take here the hyaline membrane appearing between the symplast and the fungus as a mark separating the vegetative from the reproductive period. It is on the strength of this assumption that I maintain that any subsequent change occurring in the symplast after the appearance of the hyaline membrane is not under the direct action of the fungus. According to NAWASCHIN and TOUMEY, the protoplast of the host-cell they studied is wholly consumed by the parasite. To determine whether selfdisorganization is concerned in this case in the disappearance of the protoplast or not, is practically impossible owing to the absence of any clear mark separating the vegetative from the reproductive period, though I think it likely that selfdisorganization takes place. Noteworthy is the behaviour of the parasite towards the nuclei of the host. It is not that the action is visible in all the nuclei of the tubercle, but it is more or less apparent in the nucleus of the host-cell or nuclei of the symplast. At the beginning these nuclei appear perfectly normal, but the growth of the parasite brings about a pronounced hypertrophy of the nuclei, accompanied frequently by deformation

(Figs. 84, 95, 96). However, the internal structure—the relative amounts of linin and chromatin—seems to be unchanged during the above modification, so that I can detect neither a decrease of chromatin, as was observed by NAWASCHIN and TOUMEY in their fungus-materials, nor an increase, as was reported by LÖWENTHAL in *Synchytrium*.

At the time the symplast is stretched into a thin membrane, its nuclei become strikingly flattened so as to be disc-shape (Figs. 85, 95). They are then often stained uniformly and deeply, showing the process of disorganization. Sometimes conspicuous vacuolation takes place before they become compressed (Fig. 86): the chromatin is divided into prominent globules, while the network structure appears somewhat indistinctly.

The number of nuclei of a symplast is variable according to the number of the composing cells: at times more than 20 nuclei may be easily counted. Frequently some nuclei lie close together, appearing as if they were produced by the amitotic division or fragmentation of a nucleus. However, any evidence for the occurrence of amitosis or even mitosis was not been found, and at present I am of the view that the nuclei of the symplast are nothing but the nuclei of the cells that have entered into the formation of the symplast.

The multiplication of cells around the diseased spot of the host takes place by means of typical mitosis and the occurrence of amitosis is exceedingly doubtful.

VII. Summary.

The chief results of my observations on *Synchytrium Pueariae* may be summarised as follows. But it must be remarked that nearly the same results have also been obtained in *S. decipiens*, so far as my observations have gone.

1. The swarm-spore infects not an epidermal cell, but always a subepidermal cell containing very little or no chlorophyll, by responding to the chemical stimulus exerted by the latter.

2. The fungus-body in the uninucleate condition resembles in many points, but especially in the structure of the nucleus, the ovum-cell of animals.

3. The youngest nucleus contains a single prominent chromatin-nucleolus as the only visible element within the membrane. Chromatin-granules and linin-threads that appear later are derivatives of it.

4. Secondary nucleoli are present, and are especially more numerous in the primary nucleus. They are, at least in the primary nucleus, preformed in the interior of the primary nucleolus and successively pass out through the cortical layer of the latter. The relative amount of chromatin and plastin contained in them varies according to the periods at which they are derived, but it is always approximately same as that of the primary nucleolus in which the relative amount of chromatin varies according to the period of development.

5. During the growth of the nucleus the primary nucleolus becomes more and more vacuolate; and the secondary nucleolus derived from the primary nucleolus in such a condition is also equally vacuolate. However, condensation process may soon take place on the latter, by which it becomes smaller and more compact and loses the vacuolate character.

6. The primary nucleolus is the morphological centre of the nucleus. It gives rise to chromatin-granules and linin-threads or achromatic substance. Its ontogenetic origin is the daughter-chromosomes together with residue of spindle-fibres. Thus it shows most clearly the continuity of the chromatic and achromatic substances in the successive nuclear generations, so that WAGER's statement about the behaviour of the nucleolus is applicable here without any modification.

7. The composition of the primary nucleolus is not constant throughout the successive stages of one nuclear generation, or throughout different nuclear generations. Generally, at the youngest stage or sometimes throughout the whole stage of development at certain secondary nuclear generations, it may be regarded approximately as chromatin-nucleolus. At an advanced stage it is, as a rule, a plastin-chromatin-nucleolus, and previous to nuclear division it becomes a plastin-nucleolus.

8. The chromatic substance, which is at first uniformly distributed in the primary as well as secondary nucleoli, condenses at a later stage at the periphery, and is ultimately set free as granules in the nuclear cavity.

9. During nuclear division the greater part of the chromatin is thrown off in a dissolved form into the surrounding cytoplasm, and the residue goes into the composition of the chromosomes.

10. The karyolymph contains certain soluble albuminous substances easily precipitated as fine granules or globules by corrosive sublimate.

11. The prophase stage of mitosis is atypical. No typical spireme is formed, and most of the nuclear elements dissolve away and the final remnants constitute a spindle.

12. In the secondary nuclei there takes place exudation of the nuclear elements, in consequence of which the spindle-figures are formed in various ways.

13. The dissolution-product of the primary nucleus persists as a dense granular mass around the spindle. At a later stage its peripheral portion fades away into the surrounding cytoplasm and then it assumes the form of a halo around the spindle. The halo is not present during the secondary mitosis.

14. At the telophase stage there appears suddenly a centrosome-like body, called here "karyodermatoplast," which is concerned in the formation of the nuclear membrane. It disappears when the membrane is completely formed.

15. The chromosomes are generally spherical and definitely five in number.

16. Generally, the nucleolus persists without any remarkable decrease in size during the secondary divisions. It then undergoes a gradual dissolution, but does not entirely disappear even at the time of the next division.

17. The primordial sporangium is formed by progressive cleavage-formation, when the cytoplasm of the fungus-body shrinks by throwing off water or oily matter. Otherwise, it is formed by approximately simultaneous formation of partitions among the cytoplasm.

18. The primordial sporangium contains a few nuclei and no uninucleate protospore is formed previously.

19. The fungus develops at first in a single host-cell; but its subsequent considerable enlargement causes the walls of the surrounding cells of the host to dissolve, and a wide lysigenic intercellular chamber and symplast are formed.

20. The symplast may be active so long as the vegetative or feeding period of the fungus lasts.

21. The number of nuclei in the symplast indicates just the number of the cells brought into fusion. They are remarkably deformed and enlarged.

Postscript.

While the manuscript of the present article was lying in its final shape, I received a most interesting paper of STEVENS, entitled "Some Remarkable Nuclear Structure in *Synchytrium*" (Annales Mycologici, Bd V, No. 6, 1907, p. 480), in which he gives many remarkable phenomena in the secondary nuclei of *Synchytrium decipiens*. In the accompanying plate he seems to have illustrated exhaustively and comprehensively the most important changes he was able to observe, and his interpretations of the numerous problematic phenomena seem to be based mainly on the figures of that plate. The facts noted by him and to be seen from his figures do not invalidate the conclusions drawn by me in *S. Puerariae*. Fortunately enough his figures are quite similar to those given by me in *S. Puerariae*, so that they strengthen my conclusions on the latter fungus, and also confirm my anticipations relating to the nuclear phenomena in *S. decipiens*. Notwithstanding, there are some differences between us, which lead me to make some remarks upon his paper. They do not, of course, touch the value of his preparations, but have reference to his opinions on those points upon which I have also advanced my views. In this connection it must be noted that he remarked previously in setting forth his opinions, "the greatest difficulty in answering these questions is the lack of any definite structure to use as a measure of the period of development to know whether a certain view is younger or older than another view" (p. 482). In *S. Puerariae*, however, I can say that I have succeeded in determining the period of development more accurately by using a larger number of serial stages than given by STEVENS. Although it must be admitted that there are generally specific differences on various points in different objects, I can not abstain from proposing my own interpretations of the several remarkable structures mentioned by STEVENS, on the basis of the results obtained in *S. Puerariae*.

Confining ourselves for the present to matters of fact, STEVENS' figures agree perfectly with my expectations. For, I have already expressed my view in my preliminary note ('07a) that the details of nuclear division in *S. decipiens* would be found almost identical with those in *S. Puerariae* (p. 121). It is, therefore, very interesting to compare his figures with my own

of *S. Puerariae,* to ascertain how far my statement is justified.

1. STEVENS has given in his Fig. 1 a primary nucleus of anomalous structure, entirely devoid of membrane and consisting of the chromatin and a large nucleolus. Admitting that the nuclear structure given in his first paper ('03) as normal, it is but natural to take such structure here figured as anomalous. It is, however, questionable whether the structure regarded by STEVENS as normal is really so or not. To judge from my own observations on *S. Puerariae* and partly on the same species as STEVENS', the gelatinous swelling of the nuclear membrane regarded by him as a normal occurrence during mitosis is highly questionable. In *S. decipiens,* so far as observed by me, the nucleus previous to division is provided with a scarcely recognizable membrane (Fig. 91); and in *S. Puerariae* I could observe with great definiteness the disappearance of the membrane and the consequent coming in contact of the surrounding cytoplasm with the central irregular mass of residual nuclear elements (Fig. 16). STEVENS' figure seems to represent this process of nuclear division, showing a stage just previous to that shown in my Fig. 16 in *S. Puerariae,* or just following that shown in Fig. 91 in *S. decipiens.* So far as I know, this is a normal process during mitosis in both species, and consequently I can not agree with him in taking the karyokinetic figures mentioned in his first paper as normal. This view is strengthened by the fact that these figures have many peculiarities of structure not generally observable.

2. In the multinucleate condition of the fungus he mentions some large homogenous nuclear bodies in the protoplasm surrounded each by a clear space with or without a limiting wall (Fig. 2), and also a few small isolated nucleolar bodies (Figs. 3, 11). All these bodies have been observed by me in *S. Puerariae.* The large nuclear body of STEVENS corresponds to the youngest stage of the daughter-nucleus, those with membrane being the later stages of those without it. The body itself represents the nucleolus arising from the chromosomes and a residue of the spindle-fibres. Later the nucleolus gives rise to the chromatin as well as the linin, which both appear in the form of a network in the adult nucleus (his Figs. 13-17). As to the isolated nucleolar body, what I have stated in *S. Puerariae* is applicable here; they are the nucleolar residue of the parent-nucleus now going to be dissolved. In the metaphase he says, "no nucleoli are certainly discernible" (p. 483).

Judging from my figures, however, this point requires reinvestigation, since in my preparations the absence of the nucleoli during karyokinesis, is not very frequent, and in the majority of cases the spindle is attended by one or two prominent nucleoli, persisting through the telophase and afterwards as extranuclear nucleoli for a short time.

3. A centrosome- or blepharoplast-like body was found by STEVENS in the multinucleate state of the fungus. As to its origin he state, "whether these asters are isolated or are connected with nuclei lying in other planes, and which are therefore not visible from the present viewpoint, is not certain. From their abundance it seems rather more probable that many of them are independent of any nuclear connection" (p. 481). However, he mentions in other places certain connection of the aster with the nucleus, saying, "the rays seem to shorten until the centre of the aster touches the nuclear membrane" (p. 481), and "the influence of the rays upon the shape of the nuclear wall is apparent" (p. 481). These observations do not appear to have led him to form a definite view on the significance of the aster, but they are enough to show the essential similarity of phenomena in his and my materials, and the presence of what I have called "karyodermatoplast." The aster in my case appears always close to the daughter-nucleus at the late telophase, among a dense cytoplasmic mass, and is replaced, when the membrane is completed, by a dense cytoplasmic mass again.

If we bear in mind the connection of the aster with the nuclear membrane, many characteristic phenomena relating to the aster, mentioned by STEVENS as yet problematic, will be explained most easily. Firstly, the deformation of the nucleus, which he thought to be due to the action of the astral rays, points out that the aster is going to form the membrane. Secondly, the shortening of the rays is an expression of the progress of the membrane-formation, and lastly, a dense cytoplasmic mass in the place of the aster is nothing but its remnant. His figures become more intelligible, if we arrange them according to the serial stages of this process as follows: Fig. 3, Fig. 4, Figs. 5, 7, Fig. 6, and Fig. 9. In all of these figures the membrane is represented very sharply, and this fact seems to conflict with my conclusion. However, it must be borne in mind that certain features may be liable to be exaggerated in drawing according to the peculiar view of the observer. In my preparations of *S. Puerariae* the membrane is not yet formed in the

nucleus at the time the aster begins to appear, and likewise a reexamination of the preparations of *S. decipiens* will reveal, I believe, the absence of the membrane at the same stage.

As to the multiple asters and blepharoplast-like body (his Figs. 11, 12) I can not express any definite view, since such structures were not found in *S. Puerariae*. They may possibly be abnormal forms of my karyodermatoplast whose function is never arrested.

A large cytoplasmic mass which appears close to an adult nucleus is for me a problematic body (his Figs. 13, 16, 17). Owing to its large size it is not comparable to the remnant of the ordinary aster, though its position and structure appear to suggest a close resemblance to the latter. STEVENS considered it to be the remnant of a nucleolus or its dissolution-product. However, if, as stated above, the remnant of the parent-nucleolus in *S. decipiens* is represented by the numerous extranuclear nucleoli scattered in the cytoplasm, the problematic mass does not seem to have any connection with the nucleolus. I am inclined to think that it indicates an abnormal remnant of aster.

4. STEVENS figures several clusters of nuclei as abnormal. Similar clusters occur quite frequently in *S. Puerariae*. I could not follow the fate of these nuclei, but from the fact that no clusters are found in the fungus-body previous to cleavage-formation, I agree with STEVENS' view that the nuclei of these clusters separate afterwards.

Thus, on the whole, his figures are so completely reproduced that I can draw from them the same conclusions as in *S. Puerariae*. I am emboldened to do so, chiefly supported by the fact that there are many points of resemblance in the two fungi, not only in the nuclear phenomena in the uninucleate condition, but also the morphological and biological aspects, as made clear in the present article. At any rate it is true that STEVENS' paper has confirmed the presence of my karyodermatoplast in another species of *Synchytrium*, or at least it has shown that the aster is by no means of an accidental occurrence, or an artefact, like the similarly appearing structures in the karyokinetic figures of the higher plants, on which conflicting opinions are still in vogue.

Shortly after the appearance of STEVENS' paper, a paper of GRIGGS entitled "On the Cytology of *Synchytrium* III. The Role of the Centrosomes in the Reconstruction of the Nucleus" (The Ohio Naturalist, VIII, March, 1908, p. 277) was published. His study was undertaken with STEVENS' material of *S. decipiens*, alcoholic specimen, paraffine cakes, slides, and notes of observations on the slides—perhaps the same slides from which STEVENS obtained the results just reviewed. It is, therefore, evident that the present study is an embodiment of the reinvestigation of the same subject and partly of the same preparations, as those of STEVENS. GRIGGS' present article has brought out many characteristic phenomena occurring in the secondary nuclei of *S. decipiens* more clearly and definitely. In particular, he has confirmed the absence of the nuclear membrane at the telophase, on account of which he has succeeded in demonstrating the connection between the centrosome-like body and the nuclear membrane in quite the same manner as I have already described briefly in the preliminary note ('07) and more fully discussed in the present paper.

He has further described other nuclear phenomena, partly confirming and partly opposing STEVENS' statement. As they are all intimately related to my own study on *S. Puerariae* I shall add here some remarks on his paper.

From both STEVENS and GRIGGS' investigations there is no doubt that the centrosome-like body in *S. decipiens* presents great irregularities in structure. Yet judged from the evidence in *S. Puerariae*, I may ask whether the absence of the central granules or granule in the aster is a constant character or not. In *S. Puerariae* I am of the view that the granules in the focus of the aster is the centre of activity in relation to the formation of the nuclear membrane. The granules become invisible when the membrane-formation has much advanced, while they are constantly present in the earlier stages. Hence it seems probable that the presence or absence of the central granules in the aster is not constant throughout the whole period of its activity.

According to GRIGGS the daughter-nuclei at the telophase contain a certain number of separate chromosomes. This is not in agreement with my observations on *S. Puerariae* nor with the results of STEVENS. In *S. Puerariae* the daughter-chromosomes fuse together already at the anaphase, and when the mass of the fused chromosomes reach the pole it represents

itself the nucleolus of the daughter-nucleus. The nuclei in STEVENS' Fig. 2 ('07) are represented each by a large globular mass surrounded by a clear space. Compared with the corresponding figures of mine his figures are, I believe, quite correct as showing the telophase stage. Thus STEVENS and I agree in concluding that at an early stage of development the daughter-nucleus has a very simple structure, and about the time the nuclear membrane is completely formed it becomes more complicated in structure, producing linin and chromatin-granules. According to GRIGGS' figures we may think that the daughter-nucleus is more complicated in structure at an early stage than at a later. On these points STEVENS and GRIGGS' observations on the same materials have brought forth discordant results. It therefore requires further study to make clear up this points.

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Explanation of Figures.

All the figures are drawn with the aid of ABBE's drawing apparatus using, except Figs. 61-66, the apochromatic 2mm. objective of ZEISS mostly with compensating ocular 12 (Figs. 1-20, 84-94) and 18 (Figs. 22-60, 67, 81).

F, fixed in FLEMMING's solution; K, fixed in KEISER's solution; T, FLEMMING's triple stain; H, HEIDENHEIN's haematoxylin stain; J, Stained with fuchsin iodine-green.

Synchytrium Puerariae.

Plate VIII.

Primary Nucleus.

Fig. 1. Youngest fungus-body in a host-cell, partly hidden from view by the nucleus of the host. (F.T.)

Fig. 2. A little later stage of the same. The nucleolus is homogeneous and only three chromatin-granules are visible. (F.T.)

Fig. 3. Advanced stage of the same. Chromatin-granules increase in number, achromatic substance becomes visible. Three large globules studding the nucleolus. (F.T.)

Figs. 4-12. One of several sections of nucleus.

Fig. 4. Nucleus somewhat advanced in growth, with many chromatin-granules and globules along the inner surface of the nuclear membrane and on the surface of the nucleolus. (F.T.)

Fig. 5. Nucleus more advanced in growth, with two chromatin-globules and a vacuolate secondary nucleolus attached to the vacuolate primary nucleolus. A large amount of achromatic substance is represented as faint globules. (K.H.)

Fig. 6. Nearly full grown nucleus, with a much more vacuolate primary nucleolus (dia-

grammatically shown) and a few secondary nucleoli, and producing a large amount of chromatic and achromatic substance. (K.H.)

Fig. 7. Full grown nucleus with numerous secondary nucleoli carrying chromatin-granules on their surface. Fine chromatin-granules stud the surface of the primary nucleolus whose ground substance has nearly lost affinity for stains. (F.H.)

Fig. 8. Surface view of the membrane of the nucleus at the same stage. Fine granular substances arranged close together. Immediately beneath the membrane are represented numerous secondary nucleoli. (F.H.)

Fig. 9. A portion of a full grown nucleus with exceedingly large secondary nucleoli emitting large globules of chromatin. The primary nucleolus is nearly empty of chromatic substance. (F.H.)

Fig. 10. First process of dissolution of secondary nucleoli and chromatin-granules. (F.H.)

Fig. 11. Somewhat advanced stage. (F.H.)

Fig. 12. Nucleus approaching division. Most of the secondary nucleoli and chromatin-granules have been dissolved. (F.H.)

Figs. 13-15. One of several sections of primary nucleoli at different stages.

Fig. 13. Peripheral portion of a vacuolate nucleolus at an early stage. A large and numerous small vacuoles are visible among the deeply staining ground substance, and a large vacuolate secondary nucleolus is now passing out from the primary nucleolus. (K.H.)

Fig. 14. Highly vacuolate nucleolus at later stage, poor in chromatin. (F.H.)

Fig. 15. Nucleolus at still later stage. Chromatin condenses on its peripheral portion, assuming a complete ring form in section. (F.H.)

Figs. 16-20. Prophase stage of the first mitotic division.

Fig. 16. Primary nucleolus in dissolution, producing pseudopodia-like striations. (K.H.)

Fig. 17. Striations completely replacing the nucleolus. (K. H.)

Fig. 18. Dissolved form of nuclear elements represented as radial striations with a small quantity of chromatic and achromatic substances in the focus. (K.H.)

Fig. 19. Striations becoming fainter, and chromosomes and residue of the nucleolus becoming differentiated. (K.H.)

Fig. 20. Striations being transformed into granular mass and spindle completed. The spindle is heavily strained and chromosomes are indistinct. (K.T.)

Fig. 21. Dissolution-product of nucleolar elements distinctly separated by large vacuoles from the surrounding cytoplasm. (F.H.) $\times 920$.

Fig. 22. The end of prophase stage with chromosomes at equatorial plane and a residue of nucleolus. Striations are entirely transformed into granular mass. (K.H.)

Fig. 23. Early stage of metaphase with broad spindle. (K.T.)

Fig. 24. The same stage with narrow spindle. (K.T.)

Fig. 25. Somewhat later stage of the same, some of the chromosomes splitted and the dissolution-product of nuclear elements diminished in amount forming a halo around the spindle. (K.T.)

Plate IX.

Secondary Nucleus.

Fig. 26. Earliest stage of resting nuclei with very few chromatin. *a*, with two nucleoli *b*, with prominent linin. (F.H.)

Fig. 27. More advanced stage of the same with much increased chromatin. *a*, with prominent linin reticulum; *b*, *c*, with two nucleoli; *d*, *e*, with secondary nucleoli. (F.H.)

Fig. 28. First stage of dissolution of nucleoli. (F.H.)

Fig. 29. Somewhat advanced stage. (F.H.)

Fig. 30. Disappearance of nuclear membrane and emission of the nuclear elements into the surrounding cytoplasm in the same manner as shown in Figs. 16, 17. (F.H.)

Figs. 31-37. Other modes of development of nuclei. (K.H.)

Fig. 31. Earliest stage of resting nuclei, with heavily stained globular mass budding out from the nucleolus.

Fig. 32. Emission of chromatin-granules from nucleoli preparatory to division. *a*, with achromatic substance arranged diffusely; *b*, with achromatic substance condensing in the central portion of the nuclear cavity; *c*, with achromatic substance about to form spindle.

Fig. 33. Chromatin-granules condensed on the peripheral portion of nucleolus.

Fig. 34. Direct transformation of nucleolus into a number of chromatin-granules. *a*, *b*, first step in which the outline of nucleolus is still visible; *c*, *d*, nucleolus becomes indistinct.

Fig. 35. Chromatin-granules about to form chromosomes.

Fig. 36. Advanced stage with definite number of chromosomes.

Fig. 37. Abnormal nucleus approaching division, with exceedingly less chromatin.

Fig. 38. Two spindles in the same fungus-body at second division. A few granules lying between them represent the residue of the nucleolus of the primary nucleus. (F.H.)

Fig. 39. A pair of spindles at third division, showing the residue of nucleolus between them. In the spindle of the left side some of the chromosomes have been divided into daughter-chromosomes, while in that of the right side five mother chromosomes are distinctly shown in polar view. (F.H.)

Fig. 40. Spindle with chromosomes at the equatorial plane. *a*, side view; *b*, polar view; *c*, oblique view. (K.H.)

Fig. 41. Early metaphase with elongated chromosomes, perhaps about to split. *a*, side view; *b*, oblique view. (K.H.)

Fig. 42. Formation of daughter-chromosomes. *a*, side view; *b*, polar view. (K.H.)

Fig. 43. Metakinesis accomplished. *a*, side view; *b*, oblique view. (K.H.)

Fig. 44. Chromosomes appearing thread— or rod—like, perhaps at the beginning of metakinesis. *a*, side view; *b*, polar view. (K.H.)

Fig. 45. Heteromorphic chromosomes at metakinesis. (K.H.)

Figs. 46-51. Anaphase stage.

Fig. 46. Early anaphase with daughter-chromosomes going to fuse together. (K.H.)

Fig. 47. A little later stage. (K.H.)

Fig. 48 Spindle constricted at the middle before the daughter-chromosomes reach the poles. (K.H.)

Fig. 49. Elongated spindle with rounded poles. (K.H.)

Fig. 50. Elongated spindle going to break at the median portion. (F.T.)

Fig. 51. The same. (K.H.)

Figs. 52-55. Telophase stage.

Fig. 52. Early stage with large nucleolar residue and spindle-fibre about to be drawn in the daughter-chromosomes. (K.H.)

Fig. 53. Development of hyaline space around daughter-chromosomes. (K.H.)

Fig. 54. Chromosome-mass becoming globular, with remnant of spindle-fibres attached to it. (K.H.)

Fig. 55. Growth of chromosome-mass and hyaline space around it. The chromosome-mass has now become the nucleolus of the daughter-nucleus, and the residue of parent-nucleolus diminishes in size. (K.H.)

Figs. 56-60. Formation of nuclear membrane.

Fig. 56. Karyodermatoplast appearing near daughter-nucleolus. *a*, with numerous granules in the focus of rays, and nucleolus constricted and deformed; *b*, with two granules in the focus, and a uniformly stained nucleolus; *c*, with numerous granules in the focus, and a nucleolus. (F.H.)

Fig. 57. The same stage with a single granule in the focus. *a*, with nucleolus having chromatic and achromatic substanses distinctly separated; *b*, with chromatin-grobules condensed on the surface of the nucleolus. (F.H.)

Fig. 58. Astral rays beginning to form the membrane around the hyaline space of daughter-nucleus. *a*, with nucleolus producing a single chromatin-granule; *b*, with nucleolus before production of chromatin-granules. (F.T.)

Fig. 59. A stage with membrane nearly complete. The rays become shorter and fainter, and the granules in the focus becomes indistinct. (F.H.)

Fig. 60. Daughter-nucleus completely reconstructed, with residue of karyodermatoplast near it as dense plasmic mass. (F.T.)

Plate X.

Sporangia, swarm-spores, etc.

Figs. 61-66. Sporangium-formation. (F.H.) × 300.

Fig. 61. Multinucleate fungus-body just before cleavage, with excretion-product between the cortex and protoplast.

Fig. 62. Formation of radial cleavage by which the protoplast is divided into numerous pyramidal masses.

Fig. 63. Further stage of cleavage formation in which the protoplast is divided into irregular masses.

Fig. 64. More advanced stage of the same.

Fig. 65. Formation of primordial sporangia by nearly simultaneous partition of whole protoplasmic mass.

Fig. 66. Progressive formation of primordial sporangia.

Figs. 67-79. Development of sporangia and formation of swarm-spores.

Fig. 67. A part of the fungus body just before the appearance of cleavage. (F.T.)

Fig. 68. The same at the beginning of cleavage-formation. The nuclei are at younger stage than before. (F.T.)

Fig. 69. Primordial sporangium just formed (compare Fig. 65). (F.H.)

Fig. 70. Later stage of the same. (F.J.)

Fig. 71. The same with nuclei at a more advanced stage. (K.J.)

Figs. 72 73. The same having much more chromatin-granules. (K.J.)

Fig. 74. Nuclei at early prophase. (F.J.)

Fig. 75. Nuclei in which the membrane has disappeared and nucleolus become smaller while chromatin-granules are more numerous. (F.J.)

Fig. 76. Nuclei at metaphases. In Some nuclei duaghter-chromosomes are not yet formed. (F.J.)

Fig. 77. Multinucleate sporangium with sporangium-wall completed and mother-nuclei of swarm-spores. (K.J.)

Fig. 78. Mature sporangium with nuclei of swarm-spores. (F.H.)

Fig. 79. Formation of swarm-spores in the sporangium in water. (K.J.)

Fig. 80. Adult swarm-spores liberated from sporangium. *a*, with oil drops blackened by osmic acid; *b*, with the drops bleached. (F.T.)

Fig. 81. Swarm-spores at rest. (F.T.)

Fig. 82. Aggregated nuclei at resting stage in a multinucleate fungus-body. (K.H.) ×920.

Fig. 83. Anaphase of aggregated nuclei. (K.H.) ×920.

Fig. 84. Nuclei of symplast and surrounding host-cell. The fungus is yet at uninucleate stage. (F.T.)

Fig. 85. Compressed nucleus of symplast at the stage the fungus is about to form cleavage. (F.T.)

Fig. 86. Vacuolated nucleus of symplast approaching disorganization. (F.T.)

Synchytrium decipiens.

Plate XI.

Fig. 87. Young fungus-body with nucleolus emitting secondary nucleoli. (F.T.)

Figs. 88-91. One of several sections of nuclei.

Fig. 88. A part of nucleus showing primary nucleolus much vacuolated and with numerous secondary nucleoli. (F.T.)

Fig. 89. Full grown nucleus with numerous secondary nucleoli and chromatin-granules along the nuclear membrane. The primary nucleolus is shown diagrammatically. (F.T.)

Fig. 90. Dissolution of secondary nucleoli leaving behind chromatin-granules freely. (F.T.)

Fig. 91. Nucleus approaching to division. Secondary nucleoli and most of the chromatin-granules disintegrate leaving in this section only four chromatin-granules and a single secondary nucleolus still attached to the primary nucleolus. Somewhat fibrous achromatic substance is perhaps the dissolution-product of the secondary nucleoli. (F.T.)

Fig. 92. A highly vacuolate primary nucleolus with a large vacuole near its peripheral layer. (F.T.)

Fig. 93. Primary and irregular secondary nucleoli. Two large vacuolate secondary nucleoli have just passed out at the opposite side of the primary nucleolus leaving clefts behind. (F.T.)

Fig. 94. Surface view of nuclear membrane. (F.T.)

Fig. 95. Nucleus of the symplast and of the cell of the host surrounding the lysigenic hamber. The fungus is at this stage uninucleated. (F.T.)

Fig. 96. Hypertrophied and much deformed nucleus of the symplast. (F.T.)

Fig. 97. Section of a tubercle on the vein of a leaf, showing the fungus developing just below the stomata. The intercellular space under the stomata is compressed. ×400.

Fig. 98. Another section of the same. ×400.

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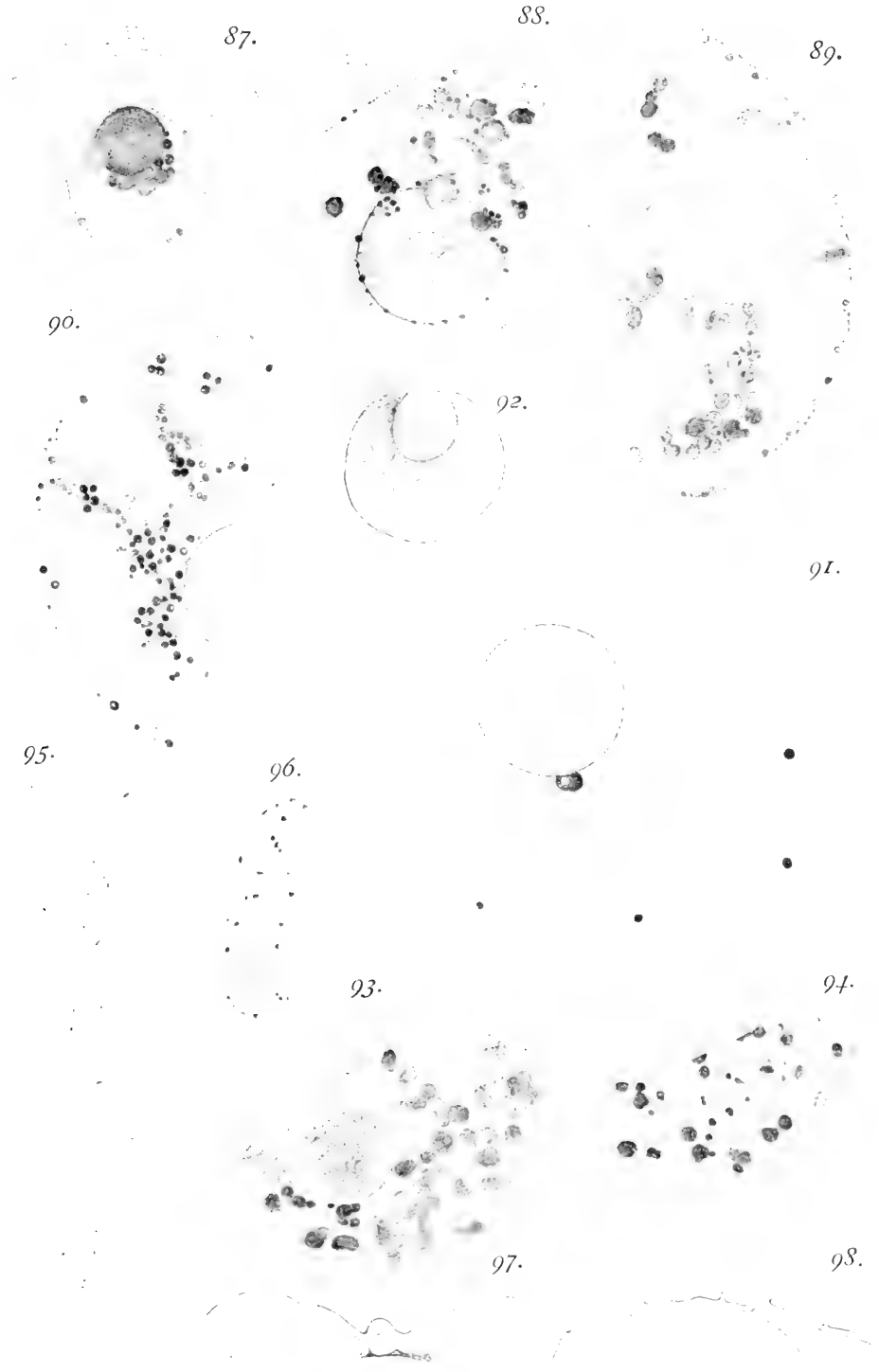


S. KUSANO DEL.

Print. by K. Ogawa.

Description of a New Species of the Genus Latirostrum, with Remarks on the Generic Character and the Significance of its Long Palpi.

BY

T. Miyake.

With one Figure in the Text.

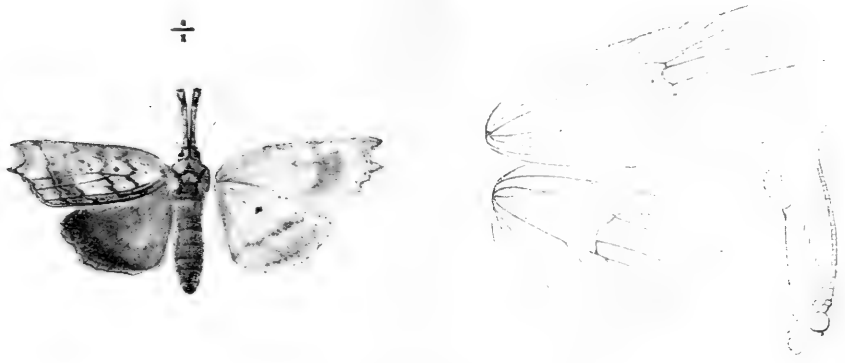
The genus *Latirostrum*, first erected by Sir, G. F. HAMPSON and described in his 'Fauna of British India, Moths,' vol. III, p. 68 (1895), has a peculiar and interesting character in having very long labial palpi. The only species is HAMPSON's genotype *Latirostrum bisacutum* of the Himalayas, described in the work just cited; and no species has, so far as I know, been added since.

Recently Baron N. TAKACHIHO of the Imperial Agricultural Experiment Station at Nishigahara brought me a moth for identification. On a thorough examination it has turned out to be a new second species of the present genus, and the first Japanese species to be described. I give it the name *Latirostrum japonicum* (Japanese name: *Tengu-atsuba*).

Latirostrum japonicum nov. spec.

♂. Ochraceous brown. Head traversed by a median black line extending along the dorsal edges of palpi to their extremity; antennae fuscous-brown; tegulae edged with bluish-black; inner margins of patagia and tops of meso- and metathorax irrorated with bluish-black; legs and under-side of body pale ochraceous; abdomen except basal segment suffused with fuscous; extremity with tuft of fuscous black hairs.

Fore-wing with a bluish-black patch at base; a dentate subbasal purplish-black line angled outwards on subcostal and inwards on median nervure; a



Latirostrum japonicum n. sp. ♂.

like-wise coloured antemedial line angled inwards on subcostal and outwards on median nervure; both lines somewhat ill-defined towards costa; a small very prominent purplish-black spot in cell between the above-mentioned lines; a very small rather indistinct spot at end of cell; a rather indistinct but posteriorly well-defined purplish-black postmedial line highly excurved beyond cell; a well-defined almost straight subterminal line edged internally with a narrow silvery streak, which is shaded internally and posteriorly with a fuscous-brown line; outer margin defined with purplish-black; inner margin suffused with black along vein 1. Hind-wing suffused with fuscous along outer margin, with the cilia yellow. Under-side paler; fore-wing with a curved brownish postmedial line, which is outwardly irrorated with brown; a small discoidal spot; hind-wing with a brownish rather straight postmedial line, externally with slight brown suffusion; an indistinct subterminal series of brownish spot; a brown spot at the end of cell.

Expanse of wing 42 mm; length of body 19 mm; length of palpi 9 mm.

A single male specimen captured by Baron TAKACHIHO, on Hikosan, Kiushiu, July, 1906.

This species is quite different from the *L. bisacutum* of HAMPSON and nothing need be said on this point. But the two species are to a certain extent allied, as the two spots in and at the end of cell, the antemedial dentate line and the post-medial and subterminal lines are situated topographically alike in both.

I have slightly modified the generic characters as given by HAMPSON; namely vein 6 of the fore-wing is not obsolete but distinct like the other veins, while HAMPSON says vein 5 is "almost obsolete." In his figure of the venation, vein 8 terminates at the margin before the apex, i.e. on the outer margin, whereas in the present species, it terminates beyond the apex, i.e. on the costal margin (see the figure).

Baron TAKACHIHO captured the moth in a forest on Mt. Hikosan, one of the highest mountains in Kiushiu. He says that the moth was resting on the leaf of a certain tree, with its long palpi extended forwards so as to imitate a spine in a very perfect manner, and he supposes that when it settles on a branch of a tree it may pass unobserved even by keen eyes, showing us the significance of the long palpi of this species.

November, 1908.

A Revision of the Arctianae of Japan.

BY

T. Miyake.

With six Figures in the Text.

The ARCTIANÆ, one of the subfamilies of ARCTIADÆ, include some insects injurious both to our farm crops and forests, especially to the mulberry-tree. Indeed the latter harbours seven species of the present subfamily; these and other injurious species are as follows:—

NAME OF SPECIES.	NAME OF VEGETABLE AFFECTED.
Diacrisia lubricipeda.	Mulberry-, cherry-tree, &c.
Diacrisia bifasciata.	Mulberry-tree.
Diacrisia obliqua.	Mulberry-tree.
Diacrisia subcarnea.	Mulberry-tree.
Diacrisia flammeola.	Persimmon-tree.
Diacrisia imparilis.	Mulberry-, peach-, pear-, plum-, cherry-, apple-tree, and many others.
Diacrisia infernalis.	Mulberry-, peach-, pear-, plum-, cherry-, apple-tree; *Quercus serrata*, *Q. glandulifera*; &c.
Amsacta lactinea.	Maize, soja bean, &c.
Arctia caja.	Hemp, rape, mulberry-tree; *Ribes grossularioides*; and many others.
Camptoloma interioratum.	*Quercus serrata*; *Q. glandulifera.*

Japanese Arctianae have been studied, like other groups of Lepidoptera, by several foreign entomologists such as BUTLER,[1] LEECH,[2] PRYER,[3] HAMPSON,[4]

1. Ann. Mag. Nat. Hist., (4) XX (1879); Cist. Ent., iii (1885); Trans. Ent. Soc. Lond., 1881; Ill. Typ. Lep. Het., ii, iii (1878–9).
2. Proc. Zool. Soc. Lond., 1888; Trans. Ent. Soc. Lond., 1899.
3. Trans. Asiat. Soc. Jap., vol. XX (1885).
4. Cat. Lep. Phal., iii, (1901).

and many others, and I myself have also published a systematic review of the group (Tōga-akwa ni kwansuru kenkyu hōkoku) in the 'Extra-reports from the Imperial Central Agricultural Experiment Station No. 22 (1906),' and there is but little room for anything in the way of its systematic. However since then a few species have been discovered, some of which I consider to be new to science and some to be new to Japan. I therefore propose to describe them in this paper and I may also improve this opportunity by adding a few remarks on some other species and describing the larvae of some.

Of this subfamily I recognize 32 species, of which only 8 species are peculiar to Japan. Of the 8 species 5 are found in Japan proper, and the other three are limited to Formosa. The other species are either palaearctic or oriental or both palaearctic and oriental. They are divided as follows:—

Palaearctic...	14	
Oriental	7	24
Common to both regions...	3	

10 of our species are common with Corea, 17 with China, 10 with Amurland, 1 with Siberia, 9 with Europe, 9 with India, and 4 with the Malay Peninsula and the adjacent islands.

The distribution among the main Japanese islands and the relationship to other regions may be summarised as follows:—

Species occuring in North Japan (Hokkaido)	Palaearctic	7	11
	Oriental	1	
	Common to both regions	3	
Species occuring in Central Japan (Honto)...	Palaearctic	14	19
	Oriental	2	
	Common to both regions	3	
Species occuring in South Japan (Kiushu)...	Palaearctic	7	10
	Oriental	2	
	Common to both regions	1	
Species occuring in Formosa (inc. Riukiu)...	Palaearctic	1	8
	Oriental	5	
	Common to both regions	2	

In order to bring out a few more details, I have drawn up the following table showing the distribution of the species in the principal parts of our Empire, Hokkaido, Honto, Kiushiu and Formosa, as well as in other countries or regions which are more or less related faunistically to ours. In preparing this table I have relied on my own knowledge so far as Japan was concerned, but for foreign countries I have mostly followed the statements of such authorities as HAMPSON, BUTLER, LEECH, KIRBY, ELWES, STAUDINGER &c. Within the limits of Japan my experience enables me to tell the occurrence of the species with great accuracy, although it is not uncommon that a species known to occur in a limited district may be found in a quite detached place.

The classification adopted in this work is most commonly followed to one of HAMPSON, as set forth in his "Catalogue of Lepidoptera Phalaenae vol. III (1901)."

For convenience, I have included the genus *Camptoloma*, mentioned in his "Fauna of British India, Moths, vol. II (1894)" but omitted in his catalogue, just referred, as a genus probably to be referred to another family.

	JAPAN				COREA	CHINA	AMURLAND	SIBERIA	EUROPE	INDIA	MALAY
	Hokkaido	Honto	Kiushu	Formosa							
1. *Phragmatobia fuliginosa.*	*	*					*		*		
2. *Diacrisia nivea.*	*	*	*		*	*	*				
3. *D. punctaria.*	*	*	*		*	*					
4. *D. lubricipeda.*	*	*	*	*	*	*			*	*	
5. *D. Lewisi.*		*	*			*					
6. *D. bifasciata.*		*	*								
7. *D. inaequalis.*		*				*					
8. *D. seriatopunctata.*	*	*	*		*		*				
9. *D. obliqua.*	*	*	*	*	*	*				*	
10. *D. subcarnea.*	*	*	*	*	*	*					
11. *D. purpurata.*		*			*		*		*		
12. *D. amurensis.*	*	*				*	*	*			
13. *D. simanensis.*		*									
14. *D. nebulosa.*	*	*									
15. *D. metalkana.*	*	*			*		*		*		
16. *D. sannio.*		*			*		*		*		
17. *D. flammeola.*		*	*			*					
18. *D. Moltrechti.*				*							
19. *D. imparilis.*	*	*	*								
20. *D. infernalis.*	*	*									
21. *D. caesarea.*		*	*			*	*		*		
22. *Amsacta lactinea.*		*	*	*							*
23. *Creatonotus transiens.*				*						*	*
24. *Creatonotus gangis.*				*		*				*	*
25. *Creatonotus Koni.*				*						*	
26. *Pericallia picta.*				Riu-kiu		*		Altai	*		
27. *Parasemia plantaginis.*		*					*		*	*	
28. *Arctia caja.*	*	*			*		*		*		
29. *Utethesia pulchella.*	*	*	?	*		*				*	*
30. *Rhodogastria astreas.*				*		*				*	
31. *Camptoloma interioratum.*		*				*				*	
32. *Nicaea (?) formosana.*				*							

(FAMILY ARCTIADAE).

SUBFAMILY ARACTIANAE.

(The literature which are not accessible to me are indicated with " ")

Genus **Phragmatobia** Steph.

Hampson, Cat. Lep. Phal., III, p. 233 (1901).

1. **Phragmatobia fuliginosa** L. *(Amahitori).*

Bombyx fuliginosa L., "Syst. Nat., I. p. 509 (1758)."

Arctia rubricosa Harr., "Ins. Mass., p. 253 (1841)."

Spilosoma fuliginosa Leech, Proc. Zool. Soc. Lond., 1888, p. 618.

Phragmatobia fuliginosa Leech, Trans. Ent. Soc. Lond., p. 1899, p. 162; Hampson, Cat. Lep. Phal., III, p. 243 (1901).

Rather rare but occasionaly found in Tokyo and the northern part of Honto. Very common in Hokkaido.

Genus **Diacrisia** Hübn.

Hampson, Cat. Lep. Phal., III, p. 256 (1901).

2. **Diacrisia nivea** Mén. *(Shiro-hitori).*

Dionychopus niveus Mén., Schrenk's Reise. Amur., Lep., p. 52, pl. IV, fig. 6 (1859); Leech, Proc. Zool. Soc. Lond., 1888, p. 620.

Spilosoma niveus Leech, Trans. Ent. Soc. Lond., 1899, p. 151.

Diacrisia nivea Hampson, Cat. Lep. Phal., III, p. 267 (1901).

Very common in Honto and Hokkaido. Crimson patches and black spots on abdomen extremely variable; in some specimens they are almost obsolete. Some specimens have sublateral series of black spots on abdomen in addition to the usual markings.

3. **Diacrisia punctaria** Cram. *(Akahara-gomadara-hitori).*

Bombyx punctaria Cram., "Pap. Exot., IV, p. 233, pl. CCCXC VIII, fig. D (1782)."

Arctia punctigera Motsch., "Etudes Ent., IX, p. 31 (1860)."

Spilosoma rosciventer Voll., "Tjidsk. V. Ent., XI, p. 143 (1893)."

Spilisoma dornesii Oberth., "Diagnoses, pl. 6 (1879)."

Spilosoma doerriesi Oberth., "Etud. d'Ent., V, p. 31, pl. I, fig. 7 (1881)."
Spilosoma punctaria Leech, Trans. Ent. Soc. Lond., 1899, p. 150.
Diacrisia punctaria Hampson, Cat. Lep. Phal., III, p. 268 (1901).

Common. The number of points in wings are very variable; when scanty, there are a few subterminal points in fore-wing and a discoidal spot in hind-wing. LEECH and STAUDINGER considered *D. punctaria* and *D. lubricipeda* to be synonymous or at least one to be a variety of the other. HAMPSON separates in his valuable work "Catalogue of Lepidoptera Phalaenae" vol. III the two species. Mr. NIWA of the Tokyo Sericultural Institute assurs me that repeated breedings of larvae of *lubricipeda* has not given rise to a moth that can be referred to *punctaria*. This is a strong confirmation of HAMPSON's view that the two species are distinct.

4. **Diacrisia lubricipeda** L. *(Kihara-gomadara-hitori).*

Bombyx lubricipeda L., "Syst. Nat., I. p. 506 (1758)."
Phalaena lepus Retz., "Gen. Spec. Ins., p. 37 (1783)."
Bombyx menthastri Esp., "Schmett., III, p. 334, pl. LXVI, fig. 6-10 (1786)."
Bombyx mendica Rossi, "Fanud. Etrusc., II, p. 174 (1790)."
Phalaena erminea Marsh., "Trans. Linn. Soc., I, p. 70, pl. 1, fig. 1 (1791)."
Chelonia luxerii Godt., "Lép. Fr., IV, p. 360, pl. 37, fig. 4 (1822)."
Spilosoma sangaica Walk., Butl., Ill. Typ. Lep. Het., III, p. 5 pl. XLII, fig. 5 (1879).
Spilosoma lubricipeda Leech, Proc. Zool Soc. Lond., 1888, p. 619; Trans. Ent. Soc. Lond., 1899, p. 147.
Diacrisia lubricipeda Hampson, Cat. Lep. Phal., III, p. 271 (1901).

Very common. Wing-spots extremely variable and the variation quite similar to that of *punctaria*.

For the synonymy of *lubricipeda* and *menthastri*, I have simply followed HAMPSON's statement, because the original descriptions of the both species are not accessible to me.

Larva. Purplish brown, with fuscous hairs; head fuscous black; abdomen with yellow dorsal line; tubercles greyish white. Food-plants: mulberry-tree, cherry-tree, etc.

5. **Diacrisia Lewisi** Butl. *(Kurofu-shiro-hitori).*

Seriarctia Lewisi Butl., "Cist. Ent., III, p. 115 (1885)"; Leech, Proc. Zool. Soc. Lond., 1888, p. 620.

Alphaea Lewisi Leech, Trans. Ent. Soc. Lond., 1899, p. 162.

Diacrisia Lewisi Hampson, Cat. Lep. Phal., III, p. 274, pl. XLIV, fig. 23 (1901).

Rare; but I have received several specimens from various parts of Honto. I myself have captured two males in Kiushiu. Colour and dosal serial points occasionally vary; in some specimens, they are obscure and in some very pronounced.

6. **Diacrisia bifasciata** Butl. *(Futasuji-hitori).*

Spilarctia bifasciata Butl., Trans. Ent. Soc. Lond., 1881, p. 7; Leech, Proc. Zool. Soc. Lond., 1888, p. 618.

Spilosoma bifasciata Leech, Trans. Ent. Soc. Lond., 1899, p. 153.

Diacrisia bifasciata Hampson, Cat. Lep. Phal., III, p. 284, pl. XLV, fig. 12 (1901).

Fig. 1. *Diacrisia bifasciata Butl.* aberrant form. ♀. ÷.

This species, which is known to occur only in Japan (exc. Formosa), is rather common and therefore injurious to some degree to the mulberry. An aberrant form found in the collection of 'Ogawa Natural History Store,' without date of capture and locality, has the black mark of fore-wing developed towards tornus, but differently in right and left wings, as here figured. Outer margin is also edged with black. Outer half of wing suffused with brown. Hind-wing immaculate. The ground colour deepened towards the margin. Abdomen buff, slightly tinged with scarlet and without any spot. A female specimen. Expanse 55 mm.

Larva. Purplish fuscous, with long black hairs: in earlier stages it has a dorsal orange line, which disappears in later stages; head and legs blackish fuscous; a reddish ochraceous lateral line: tubercles bluish black. Foodplant: mulberry-tree.

7. **Diacrisia inaequalis** Butl. *(Kakumon-hitori).*

Spilarctia inaequalis Butl., Ann. Mag. Nat. Hist., (5) IV, p. 351 (1879).
Spilosoma inaequalis Leech, Proc. Zool. Soc. Lond., 1888, p. 619.
Thyrgorina inaequalis Leech, Trans. Ent. Soc. Lond., 1899, p. 159.
Diacrisia inaequalis Hampson, Cat. Lep. Phal., III, p. 288, pl. XLV, fig 9 (1901).

Rather rare: I know only one specimen captured at Okitsu, in the collection of the Nishigahara Agricultural Experiment Station.

8. **Diacrisia seriatopunctata** Mots. *(Sujimon-hitori).*

Arctia seriatopunctata Mots., "Etud. Ent., IX, p. 32 (1860)."
Spilarctia seriatopunctata Kirby, "Cat. Lep. Het., I, p. 230 (1892)."
Spilarctia rosacea Butl., Ann. Mag. Nat. Hist., (5) IV, p. 352 (1879).
Spilarctia basilimbata Butl., Trans. Ent. Soc. Lond., 1881, p. 6.
Spilosoma seriatopunctata Leech, Proc. Zool. Soc. Lond., 1888, p. 618; Trans. Ent. Soc. Lond., 1899, p. 147.
Diacrisia seriatopunctata Hampson, Cat. Lep. Phal., III, p. 285 (1901).

Very common and extremely variable, some of this species are often referred to *D. obliqua*. *D. seriatopunctata* was unknown from Kiushiu but I have captured a fine series at Goka in Kiushiu, 1908.

9. **Diacrisia obliqua** Walk. *(Usu-sujimon-hitori).*

Spilosoma obliqua Walk., "Cat. Lep., III, 679 (1855)."
Spilosoma todara Moore, Proc. Zool. Soc. Lond., 1872, p. 574; Hampson, Fauna Brit. Ind., Moths, II, p. 7 (1892).
Spilarctia nydia Butl., Ill. Typ. Lep. Het., V, p. 32, pl. LXXXV, fig. 12 (1881).
Spilarctia ione Butl., Ill. Typ. Lep. Het., III, p. 6, pl. XLII fig. 6 (1879).

Spilarctia confusa Butl., Ill. Typ. Lep. Het., V, p. 33, pl. LXXXV, fig. 13 (1881).

Spilarctia mollicula Butl., Ann. Mag. Nat. Hist., (4) XX, p. 395 (1877); Ill. Typ. Lep. Het., III, p. 6, pl. XLII, fig. 7 (1879).

Spilosoma mollicula Leech, Proc. Zool. Soc. Lond., 1888, p. 619; Trans. Ent. Soc. Lond., 1899, p. 149.

Spilosoma mandarina Moore, Ann. Mag. Nat. Hist., (4) XX, p. 88 (1877).

Spilosoma howqua Moore, Ann. Mag. Nat. Hist., (4) XX, p. 88 (1877); Butl., Ill. Typ. Lep. Het., VII. p. 28, pl. CXXII, fig. 3 (1889).

Spilarctia howra Moore, Lep. Atk., p. 40 (1879).

Spilarctia dalbergiae Moore, Proc. Zool. Soc. Lond., 1888, p. 394; Butl., Ill. Typ. Lep. Het., VII, p. 28, pl. CXXII, fig. 2 (1889).

Spilosoma dalbergiae Hampson, Fauna Brit. Ind., Moths, II, p. 4 (1894).

Spilarctia bifascia Hampson, Ill. Typ. Lep. Het., VIII, p. 55, pl. CXL, fig. 21 (1891).

Spilosoma bifasciatum Hampson, Fauna Brit. Ind., Moths, II, p. 9 (1894).

Spilosoma bisecta Leech, Proc. Zool. Soc. Lond., 1888, p. 618, pl. XXXI, fig. 3; Trans. Ent. Soc. Lond., 1899, p. 148.

Diacrisia obliqua Hampson, Cat. Lep. Phal., III, p. 289 (1901).

Extremely variable not only in coloration and markings but also in the relative length of wings. In some specimens fore-wing is much longer than hind-wing.

Larva. Greyish fuscous, with brownish fuscous hairs; a subdorsal yellowish line; head and legs brownish ochraceous; tubercles greyish. Food-plant: mulberry-tree.

10. **Diacrisia subcarnea** Walk. *(Hara-aka-hitori).*

Spilosoma subcarnea Walk., "Cat. Lep. Het., III, p. 675 (1855)"; Leech, Proc. Zool. Soc. Lond., 1888, p. 619; Trans. Ent. Soc. Lond., 1899, p. 149.

Spilarctia subcarnea Butl., Ill. Typ. Lep. Het., III, p. 6, pl. XLII, fig. 8 (1879).

Aloa bifrons Walk., "Cat. Lep. Het., III, p. 705 (1855)."
Spilosoma bifrons Leech, Trans. Ent. Soc. Lond., 1899, p. 149.
Spilosoma erubescens Moore, Ann. Mag. Nat. Hist., (4) XX, p. 89 (1877).
Spilactia erubescens Kirby, "Cat. Lep. Het., I, p. 231 (1892)."
Spilosoma rybakowi Alph., Rom. sur Lep., IX, p. 171, pl. X, fig. 9 (1897).
Hyarias oberthuri Semp., "Schmett. Phil., II, p. 489 (1899)."
Diacrisia subcarnea Hampson, Cat. Lep. Phal., III, p. 315 (1901).

Very common and variable. Females always yellowish white, males commonly tinged with crimson, occasionally however whitish like females. In the spring brood the crimson colour of the male rather weak, in the summer it becomes very deep, especially in the hind-wing. In very rare case the moth has a black point on each patagium.

A female specimen captured by Mr. NIWA of the Tokyo sericultural Institute is closely allied to the female of this species, but its abdomen is orange-yellow instead of red. Whether this is another species or not can not be determined. I call it *D. subcarnea* var. *flavoventris*.

Larva. Ochraceous yellow with long ochraceous hairs; head and legs fulvous black; a brownish subdorsal line; tubercles greyish white. Food-plant: mulberry-tree.

11. **Diacrisia purpurata** L. *(Goma-benishita-hitori).*

Bombyx purpurata L., "Syst. Nat., I, (2) p. 828 (1769)."
Bombyx purprea L., "Syst. Nat., I, (2) p. 505 (1758)."
Rhyparia purpurata Leech, Trans. Ent. Soc. Lond., 1899, p. 154.
Diacrisia purpurata Hampson, Cat. Lep. Phal., III, p. 298 (1901).
Arctia purpurata Kirby, Butt. Moth, Europ., p. 110, pl. 25, fig. a b (1903).

Rather uncommon, but I have received several examples from various localities of Honto and have myself captured some specimens at Nasuno (Tochigi-ken). The moth displayed in its flight a very beautiful red colour very similar to that of *argynnis* butterfly.

12. **Diacrisia amurensis** Brem. *(Hoshi-beni-shita-hitori).*

Chelonia rubescens var. *amurensis* Brem., Lep. Ost-Sib., p. 39, pl. III, fig. 16 (1864).

Rhyparioides rubescens (part) Leech, Proc. Zool. Soc. Lond., 1888, p. 616; Trans. Ent. Soc. Lond., 1899, p. 155.

Diacrisia amurensis Hampson, Cat. Lep. Phal., III, p. 298 (1901).

Rather common; I have received many specimens from Honto and Hokkaido. I myself have also captured a series of examples which came to the lamp at Kurodahara in July of this year. Some are marked as is stated in HAMPSON'S work, and some as in the original figure of BREMER and again some are almost immaculate. Females are comparatively very few, while in the previously mentioned *D. purpurata* the females seem to be very numerous. In the fine series of specimens examined by me, all except one have orange abdomen as is stated in BREMER'S figures and not scarlet as is stated by HAMPSON. Only one female specimen which I consider to be an aberrant form has scarlet abdomen.

13. **Diacrisia simanensis** nov. sp. *(Kogata-hoshi-benishita-hitori).*

♂. Head and thorax fulvous yellow; palpi black, the basal part scarlet below; antennae biserrate, brownish black; pectus crimson; legs brownish black, with the femora crimson above; abdomen scarlet, with dorsal and lateral series of black spots.

Fore-wing fulvous yellow; inner half tinged with pink; basal part of costal edge brownish black; two antemedial black points below costa; an oblique indistinct medial line from median nervure to inner margin; three

Fig. 2. *Diacrisia simanensis* n. sp. ♂. $\frac{1}{1}$.

black subterminal spots below costa. Hind-wing scarlet, an antemedial black spot below median nervure, and a small spot on vein 1. Underside of wings scarlet; fore-wing with a black spot near the base and in the middle of cell; a spot below origin of vein 2 and discoidal lunule; hind-wing immaculate.

♀. More fulvous yellow; underside of wings with more pronounced black spots; fore-wing with subterminal black spots; hind-wing spotted as above, only the conjoined subterminal spots absent.

Expanse 37-39 mm.

Two males captured by the author at Mionoseki, Shimane-ken, on 28th August 1906, and a female specimen in the collection of the Agricultural College, obtained at Zeze, Shiga-ken.

This species is allied to *D. nebulosa* and *D. amurensis*. But it differs from the former by the absence of the black suffusion of the inner half of the fore-wing, and from the latter by the coloration of the fore-wing and abdomen and the biserrate structure of the antennae of the male. Besides, this species is smaller than either of the two species.

14. **Diacrisia nebulosa** Butl. *(Benishita-hitori)*.

Rhyparioides nebulosa Butl., Ann. Mag. Nat. Hist., (4) XX, p. 396 (1877); Ill. Typ. Lep. Het., II, p. 5, pl. XXIII, fig. 2 (1878); Leech, Trans. Ent. Soc. Lond., 1899, p. 156.

Rhyparioides simplicior Butl., Trans. Ent. Soc. Lond., 1881, p. 6.

Rhyparioides rubescens (part) Leech, Proc. Zool. Soc. Lond., 1888, p. 616.

Diacrisia nebulosa Hampson, Cat. Lep. Phal., III, p. 316 (1901).

Rather uncommon in Honto but common in Hokkaido; I have not yet captured the species in Tokyo, where FENTON is said to have obtained some examples. The dark suffusion of the fore-wing is very variable, in some very obscure as in *D. amurensis*, and in some very strong so that the fore-wing is almost entirely dark-coloured. Abdomen is without exception coloured with crimson.

15. **Diacrisia metalkana** Led. *(Ko-benishita-hitori)*.

Nemeophila metalkana Led., "Wien. Mon., V, p. 162, pl. III, fig. 12 (1861)"; Leech, Proc. Zool. Soc. Lond., 1888, p. 616.

Chelonia flavida Brem., Lep. Ost-Sib., p. 39, pl. IV, fig. 4 (1864).

Rhyparioides metalkana Leech, Trans. Ent. Soc. Lond., 1899, p. 155.

Diacrisia metalkana Hampson, Cat. Lep. Phal., III, p. 299 (1901).

Parasemia metalkana Kirby, Butt. Moths Europ., p. 109, pl. 23, fig. 16 (1903).

Very rare. I have only one male specimen obtained by Mr. OGUMA in Tokyo. The specimen seem to have no remarkable difference from the European form.

16. **Diacrisia sannio** L. *(Mon-heriaka-hitori).*

Bombyx sannio L., "Syst. Nat., I, p. 506 (1758)."

Bombyx russula L., "Syst. Nat., I, p. 510 (1758)."

Diacrisia irene Butl., Trans. Ent. Soc. Lond., 1881, p. 6; Leech, Trans. Ent. Soc. Lond., 1899, p. 157.

Diacrisia russula Leech, Proc. Zool. Soc. Lond., 1888, p. 615; Trans. Ent. Soc. Lond., 1899, p. 156.

Diacrisia sannio Hampson, Cat. Lep. Phal., III, p. 209 (1901).

Parasemia sannio Kirby, Butt. Moth. Europ., p. 109, pl. 23, fig. 15ab (1903).

Uncommon. I have two females from Suwa, Shinano and a male from Hiroshima.

17. **Diacrisia flammeola** Moore. *(Aka-hitori).*

Alpenus flammeolus Moore, Ann. Mag. Nat. Hist., (4) XX, p. 89 (1877); Leech, Proc. Zool. Soc. Lond., 1888, p. 617.

Spilosoma flammeolus Leech, Trans. Ent. Soc. Lond., 1899, p. 154.

Diacrisia flammeola Hampson, Cat. Lep. Phal., III, p. 301, pl. XLV, fig. 10 (1901).

I have a male specimen, probably captured in Honto, from Mr. NAWA, and three males from Mr. YANO obtained in Kiushiu. One of the specimens

Fig. 3. *Diacrisia flammeola* Moore. ♂. ÷.

before me has, as is figured, a series of postmedial spots not mentioned by MOORE or HAMPSON. Mr. YANO says that the larvae feed on persimmon-tree and therefore they are more or less injurious to the plant.

18. **Diacrisia Moltrechti** nov. sp. *(Chairo-hitori).*

Brownish ochraceous; head and thorax ochraceous; palpi brownish black; antennae bipectinate, black; legs brownish, with the femora ochraceous above;

Fig. 4. *Diacrisia Moltrechti* n. sp. ♂. -|-.

abdomen bright ochraceous yellow, the basal joints of which are paler on the ventral side, with dorsal, lateral and sublateral series of black spots.

Fore-wing brownish ochraceous suffused with black; a medial series of six black points from costa to inner margin, slightly angled outwards at the median nervure. A postmedial series of five spots, of which two are excurved from below costa to vein 3, situated in the end of discoidal cell, then incurved to meet the above-mentioned medial series above vein 1; of the five spots, one, situated between vein 2 and 3, is oblique and somewhat elongated; another small spot between vein 5 and 6 just beyond the discoidal cell. Hind-wing ochraceous orange, with two discoidal spots and one spot just beyond the discocellulars; an indistinct patch at vein 1 near the base. Under surface ochraceous without blackish suffusion, similarly marked as above.

Expanse 32 mm.

A male specimen in the collection of Mr. Kox of Vladivostok, captured by Dr. Moltrecht on Mt. Arisan in Formosa, 1908.

This species is to a certain extent allied to *Diacrisia flammeola* Moore, *D. biseriata* Moore, *D. flavens* Moore and *D. eugraphica* Walk., but can readily be distinguished on many points and is doubtless a distinct species.

19. **Diacrisia imparilis** Butl. *(Kuwa-gomadara-hitori).*

Spilarctia imparilis Butl., Ann. Mag. Nat. Hist., (4) XX, p. 394 (1877); Ill. Typ. Lep. Het., II, p. 4, pl. XXII, fig. 4 (1878); Ann. Mag. Nat. Hist., p. (5) IV, p. 351 (1879); Leech, Proc. Zool. Soc. Lond., 1888, p. 620.

Spilosoma imparilis Leech, Trans. Ent. Soc. Lond., 1899, p. 153.

Diacrisia imparilis Hampson, Cat. Lep. Phal., III, p. 308 (1901).

Very common in Honto; I have also received a series of specimens from Hokkaido. The larvae are very common on various plants in Tokyo.

Larva. Purplish fuscous, with hairs of greyish white and greyish black; head and legs greyish fuscous; a dorsal and subdorsal series of greyish yellow spots; tubercles mostly ochraceous brown, some of 6-12 somites metallic blue; prothoracic shield metallic blue.

Food-plants: mulberry-, peach-, pear-, plum-, cherry-, apple-tree and many others.

20. **Diacrisia infernalis** Butl. *(Kurohane-hitori).*

Thanatarctia infernalis Butl., Ann. Mag. Nat. Hist., (4) XX, p. 395 (1877); Ill. Typ. Lep. Het., III, p. 7, pl. XLIII, fig. 9 (1879); Leech, Proc. Zool. Soc. Lond., 1888, p. 617; Trans. Ent. Soc. Lond., 1899, p. 160.

Diacrisia infernalis Hampson, Cat. Lep. Phal., III, p. 312 (1901).

Not very rare in Hokkaido and Honto: I have received some specimens captured in Tokyo.

Larva. Purplish fuscous with mixed hairs of whitish and blackish; head ochraceous brown; legs brownish; a yellowish dorsal line with some indistinct irregular lateral lines; tubercles of dosal half metallic blue: lateral ones ochraceous brown. Food-plants: mulberry-, peach-, pear-, cherry-, apple-tree; *Quercus serrata; Q. glandulifera;* &c.

21. **Diacrisia caesarea** Geoze. *(Kibara-hitori).*

Bombyx caesarea Geoze, "Ent. Beytr., III, (3) p. 63 (1781)."

Bombyx luctifera Esq., "Schmett., III, p. 222, pl. XLIII, figs. 1-5 (1784)."

Atolmis japonica Walk., "Cat Lep. Het. Suppl., I, p. 223 (1864)."

Spilosoma luctifera Leech, Proc. Zool. Soc. Lond., 1888, p. 618.

Estigmene moerens Butl., "Cist. Ent., III, p. 114 (1885)."

Arctinia caesaria Kirby, "Cat. Lep. Het., I, p. 276 (1892)"; Leech, Trans. Ent. Soc. Lond., 1899, p. 160.

Diacrisia caesarea Hampson, Cat. Lep. Phal., III, p. 313 (1901).

Phragmatobia caesarea Kirby, Butt. Moth. Europ., p. 113, pl. 25, fig. 7 (1903).

Very rare. I obtained a male specimen at Sasago (Yamanashi-ken) by the lamp in May 1906.

Genus **Amsacta** Walk.

Hampson, Cat. Lep. Phal., III, p. 322 (1901).

22. **Amsacta lactinea** Cram. *(Kyō-jorō).*

Bombyx (Aloa, Phalaena) lactinea Cram., "Pap. Exat., II, p. 58, pl. CXXXIII, fig. 0 (1777)."

Bombyx sanguinolenta Fabr., "Ent. Syst., III, p. 473 (1793)."

Aloa lactinea Walk., "Cat. Lep. Het., III, p. 702 (1855)"; Leech, Proc. Zool. Soc. Lond., 1888, p. 620.

Rhodogastria lactinea Leech, Trans. Ent. Soc. Lond., 1889, p. 124.

Creatonotus lactineus Hampson, Fauna Brit. Ind., Moths, II, p. 27 (1894); Leech, Trans. Ent. Soc. Lond., 1899, p. 163.

Amsacta lactinea Hampson, Cat. Lep. Phal., III, .p 328 (1901).

Common in Honto.

Larva. Head brownish black; tubercles on subdorsal, supra- and subspilacular lines, with tufts of longer or shorter hairs of black and reddish brown; skin and legs brownish black. Food-plants: maize, soja bean, &c.—*Prof. Sasaki.*

Genus **Creatonotus** Hübn.

Hampson, Cat. Lep. Phal., III, p. 331 (1901).

23. **Creatonotus transiens** Walk. *(Hai-iro-hitori).*

Spilosoma transiens Walk., "Cat. Lep. Het., III, p. 675 (1855)."

Amphissa vacillans Walk., "Cat. Lep. Het., III, p. 675 (1855)."

Aloa isabellina Walk., "Cat. Lep. Het., III, p. 705 (1855)."

Phissama vacillians Butl., Ill. Typ. Lep. Het., III, p. 5, pl. XCII, fig. 4 (1879); Hampson, Fauna Brit. Ind., Moths, II, p. 29 (1894); Leech, Trans. Ent. Soc. Lond., 1899, p. 164.

Creatonotus transiens Hampson, Cat. Lep. Phal., III, p. 334 (1901).

There is a series of specimens in the collection of the Science College from Riukiu.

24. **Creatonotus gangis** Linn. *(Kurosuji-hitori).*

Phalaena gangis Linn., "Amoen. Acad., VI, p. 410 (1764)."
Phalaena (Noctua) interrupta Linn., Syst. Nat. I, (2), p. 840 (1767).
Bombyx francisca Fabr., "Mant. Ins., II, p. 131 (1787)."
Creatonotus continuatus Moore, Ann. Mag. Nat. Hist., (4) XX, p. 344 (1877).
Creatonotus interruptus Hampson, Fauna Brit. Ind., Moths, II, p. 26 (1894); Leech, Trans. Ent. Soc. Lond., 1899, p. 163.
Creatonotus gangis Hampson, Cat. Lep. Phal., III, p. 333 (1901).
A male specimen in the collection of Mr. KINOSHITA from Formosa.

25. **Creatonotus Koni** nov. sp. *(Arisan-hitori).*

Head and thorax ochraceous white, very slightly tinged with pink; palpi black; legs pale brown, the femora orange above, white below; abdomen orange above, the extremity of anal tuft and ventral surface white; dorsal, lateral and sublateral series of small black spots. Fore-wing ochraceous white with

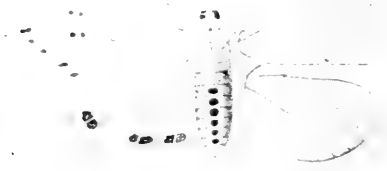


Fig. 5. *Creatonotus Koni* n. sp. ♀. ⅓.

slight pinkinsh tinge; small black points in and beyond upper and lower angles of discoidal cell; a subterminal series of five small black spots from above vein 5 to vein 1. Hind-wing slightly tinged with ochraceous brown, with three rather elongate black subterminal spots.

Expanse 58 mm.

A single female specimen in the collection of Mr. KON captured on Mt. Arisan in Formosa by Dr. MOLTRECHT. Mr. KON was very kind to show and lend me the specimen for study and I have a great pleasure in naming the specific name after him.

Genus **Pericallia** Hübn.

HAMPSON, Cat. Lep. Phal., III, p. 350 (1901).

26. **Pericallia picta** Walk. *(Akasuji-hitori).*

Deiopeia picta Walk., "Cat. Lep. Het., XXXI, 263 (1864)."

Tatargina formosa Butl., Trans. Ent. Soc. Lond., 1877, p. 366; Ill. Typ. Lep. Het., III, p. 8, pl. XLIII, fig. 1 (1879).

Tatargina picta Hampson, Fauna Brit. Ind., Moths, II, p. 54 (1894).

Pericallia picta Hampson, Cat. Lep. Phal., III, p. 353 (1901).

Some specimens in the collection of the Sapporo Agricultural College from Riukiu.

Genus **Parasemia** Hübn.

Hampson, Cat. Lep. Phal., III, p. 458 (1901).

27. **Parasemia plantaginis** L. *(Hime-kishita-hitori).*

Bombyx plantaginis L., "Syst. Nat., I, p. 501 (1758)."

Bombyx alpicola Scop., "Ent. Carn., p. 205 (1763)."

Bombyx hospita Den. and Schiff., "Wien. Verz., p. 310 (1776)."

Bombyx matronalis Freyer, "Neu. Beitr., V, p. 37, pl. 405 (1843)."

Chelonia caucasica Herr.-Schäff. "Schmett. Eur., II, p. 147, figs. 42-44 (1845)."

Nemeophila petrosa Walk., "Cat. Lep. Het., III, 626 (1855)."

Lithosia nicticans Mén., Schrenck's Reise. Amur., Lep., p. 50, pl. 4, fig. 4 (1859).

Platarctia modesta Pack., "Proc. Ent. Soc. Philad., III, p. 113 (1864)."

Platarctia scudderii Pack., "Proc. Ent. Soc. Philad., III, p. 113 (1864)."

Eupsychoma geometrica Grote, "Proc. Ent. Soc. Philad., IV, p. 318, pl. 2, fig. 1 (1865)."

Nemeophila caespitis Grote and Rob., "Trans. Am. Ent. Soc., I, p. 337, pl. 6, fig. 43 (1868)."

Nemeophila cichorii Grote and Rob., Trans. Am. Ent. Soc., I, p. 338, pl. 6, fig. 44 (1868)."

Nemeophila macromera Butl., Trans. Ent. Soc. Lond., 1881, p. 5.

Nemeophila macromera var. *leucomera* Butl., Trans. Ent. Soc., 1881, p. 5.

Nemerophila macromera var. *melanomera* Butl., Trans. Ent. Soc. Lond., 1881, p. 5.

Nemeophila geddesi Neum., "Papilio, III, p. 137 (1884)."

Nemeophila selwynii H. Edw., "Can. Ent., XVII, p. 65 (1885)."

Nemeophila plantaginis Leech, Trans. Ent. Soc. Lond., 1899, p. 157.

Parasemia plantaginis Hampson, Cat. Lep. Phal., III, p. 458 (1901).

The Japanese forms included by BUTLER under the names of *Nemeophila macromera*, *N. leucomera* and *N. melanomera* are undoubtedly to be considered as varieties of *P. plantaginis*. The characteristics mentioned by BUTLER, "the white spot in the discodal cell apparantly never touching the costal margin, the subapical sigmoidal stripe not united to the λ-shaped marking," are not constant in our species. As may be seen from the figures, in the form represented by fig. b, the above mentioned points are exactly as in the European form, whereas in the form represented by fig. a, the white spot in the discoidal cell touches the costal margin and in the form or fig. c the λ-shaped marking is united to the subapical sigmoidal stripe.

The figure a belongs to the form *leucomera* and c to *macromera*, and I have not yet received any example of *melanomera*. Fig. b, which is, as

Fig. 6. Parasemia plantaginis. ÷.

a. Form *leucomera*. ♂. b. European from. ♀. c. Form *macromera* ♂.

stated above, exactly similar to the European form, is a female in which the hind-wing is orange coloured.

Two males and a female were kindly sent to me by Mr. M. SUZUKI of Kyoto, which were captured on Mt. Asama in the summer of 1907.

Genus **Arctia** Schrank.

HAMPSON, Cat. Lep. Phal., III, p. 463 (1901).

28. **Arctia caja** L. *(Hitori-ga).*

Bombyx caja L., "Syst. Nat., I, p. 500 (1758)."

Phalaena crinacea Retz., "Gen. Spec. Ins., p. 36 (1783)."

Euprepia phaeosoma Butl., Ann. Mag. Nat. Hist., (4) XX, p. 395 (1877);

Ill. Typ. Lep. Het., III. p. 7, pl. XLII, fig. 10 (1879).

Euprepia phaeosoma var. *auripennis* Butl., Trans. Ent. Soc. Lond., 1881, p. 7.

Hypercompa phaeosoma Kirby, Trans. Ent. Soc. Lond., 1881, p. 259.

Euprepia caja Leech, Proc. Zool. Soc. Lond., 1888, p. 617.

Arctia orientalis Moore, Ann. Mag. Nat. Hist., (5) I, p. 230 (1878); Hampson, Fauna Brit. Ind., Moths, II, p. 16 (1892).

Arctia caja Hampson, Cat. Lep. Phal., III, p. 463 (1901); Leech, Trans. Ent. Soc. Lond., 1899, p. 159.

Rather rare in Honto but very abundant in Hokkaido. The whitish markings of fore-wing are extremely variable. The ground colour and black spots of the hind-wing also variable, the former being usually scarlet, but often orange and rarely yellow. The moths often fly about in the day-time.

Larva. Head black with reddish-brown spot at sides; body black; each body-segment with two deep-black tubercles on subdorsal line, one on supra-, subspiracular and basal lines; tubercles on subdorsal and subspiracular lines thickly covered with longer or shorter light greyish yellow hairs; tubercles on subspiracular and basal lines with short reddish brown hairs; thoracic legs black; abdominal legs dark brown. Food-plants: hemp, rape, mulberry-tree, *Ribes grossularioides.—Prof. Sasaki.*

Genus **Utethesia** Hübn.

Hampson, Cat. Lep. Phal., III, p. 480.

29. **Utethesia pulchella** L. *(Beni-gomadara-hitori).*

Tinea pulchella L., "Syst. Nat., I, 2, p. 884 (1767)."

Noctua pulchra Den. and Schiff., "Wien. Verz., p. 68 (1776)."

Geometra lotrix Cram., "Pap. Exot., II, pl. 109, E, F (1779)."

Utethesia pulchella Kirby, "Cat. Lep. Het., I, p. 346 (1892)"; Hampson, Cat. Lep. Phal., III, p. 483 (1901).

Leiopeia pulchella Hampson, Fauna Brit. Ind., Moths, II, p. 55 (1894); Leech, Trans. Ent. Soc. Lond., 1899, p. 170.

Described from Hokkaido, Kiushiu and Riukiu. I have received some specimens from Formosa. There is also a series of specimens in the collection of the Agricultural College.

Genus **Rhodogastria** Hübn.

HAMPSON, Cat. Lep. Phal., III, p. 498 (1901).

30. **Rhodogastria astreas** Drury. *(Tsumaguro-sukashi-hitori).*

Glaucopis (Sphinx) astreas Drury, "Ins. II. pl. XXVIII, fig. 4 (1773)."

Sphinx melanthus Cram., Pap. Exot., III, pl. 286, B (1780).

Rhodogastria astraea Moore, "Lep. Ceyl., II, p. 76, pl. CVIII, figs. 1, 1a (1882)."

Noctua eugenia Fabr., "Syst. Ent., 3, II, p. 19 (1794)."

Chelonia madagascariensis Boisd., "Delegorgue, Voy. Afr. Autr., II, p. 598 (1847)."

Amerila rhodopa Walk., "Cat. Lep. Het., XXXI, p. 305 (1864)."

Creatsnotus communis Walk., "Cat. Lep. Het., XXX, p. 283 (1864)."

Amerila vitrea Plotz, "Stett. Ent. Zeit., XLI, p. 84 (1880)."

Rhodogastria astraea Moore, "Lep. Ceyl., II, p. 76, pl. CVIII, figs. 1, 1a (1882)."

Amerila bauri Möschl., "Verh. Zool.-bot. Ges. Wien, XXXIII, p. 289, pl. 16, fig. 2 (1884)."

Pelochyta astrea Hampson, Fauna Brit. Ind., Moths, II, p. 38 (1894); Leech, Trans. Ent. Soc. Lond., 1899, p. 167.

Rhodogastria astreas Hampson, Cat. Lep. Phal., III, p. 505 (1901).

I have not seen any specimen of this species nor have I been informed of its occurrence by any of our correspondents. LEECH and HAMPSON have described the species from Formosa.

Genus **Camptoloma** Feld.

HAMPSON, Fauna Brit. Ind., Moths, II, p. 31 (1894).

31. **Camptoloma interioratum** Walk. *(Sarasa-hitori).*

Numenes interiorata Walk., "Cat. Lep. Het., Suppl., I, p. 290 (1864)"; Leech, Proc. Zool. Soc. Lond., 1888, p. 615.

Camptoloma interioratum Kirby, "Cat. Lep. Het., I, p. 359 (1892)"; Hampson, Fauna Brit. Ind., Moths, II, p. 31 (1894); Leech, Trans. Ent. Soc. Lond., 1899, p. 1654.

Very common in Honto. The moth emits a peculiar sound by its body segments.

Larva. Body greyish yellow except head, the dosal shield of first body segment, legs and last segment, which are blackish in colour; ventral side of body orange; six brownish black longitudinal streaks between dorsal and ventral lines; six brownish spots with white hairs, from dorsal to basal line in each segment. Food-plants: *Quercus serrata*, *Q. glandulifera*.—*Prof. Sasaki.*

Genus **Nicaea** Moore.

Lep. Atk., p. 11 (1879); HAMPSON, Cat. Lep. Phal., III, p. 218 (1901).

32. **Nicaea** (?) **formosana** Miyake. *(Kiboshi-hitori).*

Nicaea (?) *formosana* Miyake, Ann. Zool. Jap., vol. VI, part 2, p. 8 (1907).

A female specimen captured at Jukirin, Formosa, in the collection of the Science College.

Jan. 1909.