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THE

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THE ROYAL DUBLIN SOCIETY.

No. 1.

EXPERIMENTS ON THE ELECTRIFICATION PRODUCED BY BREAKING UP WATER, WITH SPECIAL APPLICATION TO SIMPSON'S THEORY OF THE ELECTRICITY OF THUNDERSTORMS.

By PROFESSOR J. J. NOLAN, M.A., D.Sc., and J. ENRIGHT, B.A., M.Sc.,
University College, Dublin.

[Read March 28. Printed June 1, 1922.]

Introduction.

The work described in the present paper is an extension of certain experiments carried out by one of us on the electrical charges produced by breaking up water. In the previous work[1] water was broken up in two ways, (1) by allowing water-drops to fall into a strong horizontal air-blast, and (2) by means of a sprayer. The water, as is well known, takes up a positive charge. This charge was measured, and it was found that the magnitude of the charge per c.c. depended on the size of the drops into which the water was broken, increasing as the size of the drops decreased. The result of a considerable number of observations tended to show that the charge per c.c. on the water was proportional to $1/r$, where r is the radius of the drops. It was pointed out that this could be interpreted by saying that the total charge given to the water was proportional to the area of new water-surface created. The charge per square cm. of new surface was calculated, and had a value of about 2 to 3×10^{-3} electrostatic units, both methods of breaking up the water giving the same result. The smallest drops tested were of radius $5{\cdot}9 \times 10^{-3}$ cm., and the corresponding charge (the highest found) was 1·36 e.s. units per c.c. The water used was the ordinary distilled water of the laboratory; no attempt was made to reach any higher standard of purity.

Apart from the theoretical interest in the investigation of this phenomenon, it has an important connexion with the theory of thunderstorms put forward by G. C. Simpson.[2] As is well known, this theory traces the electric separation which gives rise to the lightning discharge to the breaking up of rain-drops in the strong

[1] J. J. Nolan, Proc. Roy. Soc., A, vol. 90, 1914.
[2] G. C. Simpson, Phil. Trans. Roy. Soc., A, 209, 1909, and Phil. Mag., 30, 1915.

vertical air-currents which are a well-ascertained feature of the thunderstorm. There is no doubt that Simpson's theory holds the field, not only as being the sole theory which will bear a moment's examination in light of the known facts, but as offering a reasonable explanation of all the electrical phenomena, and being fully in accord with modern meteorological ideas as to the nature of these storms. On only one point may some hesitation be felt, and that is the question of magnitudes. It may be asked have we any evidence that sufficient breaking up of water-drops occurs to account for the observed charges on thunderstorm rain and for the potentials developed in the atmosphere ?

In this connexion the question also arises as to whether sufficient breaking up of drops occurs under non-thunderstorm conditions to account for the charges observed on ordinary rain. Simpson[1] quotes the experimental value (1·36 e.s. units per c.c.) mentioned above, and states that "if about $\frac{1}{10}$ of the rain was broken up once into fine drops the observed charge would be produced" (on ordinary rain). Under thunderstorm conditions, of course, repeated breaking up of the water can be assumed, and by imagining a sufficient number of repetitions of the process the charge density can be pushed as high as we please. But it must be realized that, on the evidence so far available, in order that any quantity of water may have a charge of the order of one e.s. unit per c.c., it must all be broken into *very fine* drops (radius circa 6×10^{-3} cm.). The amount of shattering which drops are likely to experience, even in the highly disturbed conditions associated with a thunderstorm, is very small as compared with this complete pulverization. Some writers go so far as to deny that shattering of the rain-drop can occur in nature.[2] While this, no doubt, is an extreme view, the experiments of Hochschwender, quoted by Lenard,[3] tend to show that, except in the case of the largest drops, a very considerable counter-acceleration is required to produce rupture. The question of magnitudes, therefore, remains a difficulty. There is a gap between the amount of charging produced in the laboratory corresponding to a certain degree of breaking up and the amount of charge observed on rain—thunderstorm and ordinary—considered in the view of the amount of breaking up it is likely to have experienced. The experiments now described were undertaken with a view to a more complete examination of the phenomenon in the hope of bridging this gap. The investigation was successful to this extent, that it indicated the possibility of obtaining charge densities of an order ten times as great as those previously reported corresponding to the same degree of breaking up of water.

Experimental Methods.

In work of this kind there is no difficulty about the electrical observations; the charges produced are high and easily measured. But, as indicated in the previous paper, the degree of breaking up of the water is always difficult of determination. Drops covering a wide range of sizes are present in the water from a sprayer, and while for some purposes of rough estimation it may be accurate enough to calculate an average size by estimating the total number of drops and the total volume of water, still it is clear that this method may in some cases involve very great errors. Before entering on the details of the work, a preliminary experiment which is of some interest may be described. It occurred to us that

[1] G. C. Simpson, Phil. Mag., *loc. cit.*
[2] See J. Rey, "L'ionisation de l'air par les chutes d'eau," p. 73. Gauthier-Villars, 1912.
[3] P. Lenard, Ann. d. Physik, 15, 1921.

water could be broken into drops of a regular and controllable size by forcing a jet through a narrow orifice. A jet of this sort will break up into drops of equal sizes in accordance with any frequency impressed on it. When the electrical test was made, however, it was found that the degree of charging produced was very minute in comparison with that in water broken up by a sprayer or by an air-blast. This result should perhaps have been anticipated, for it is well known that the electrical separation at the water-air surface takes place only when there is rupture of the water-surface with some degree of violence. In the formation of drops from the unstable water-jet, the only rupture that occurs is the final break at each end of the minute cylindrical neck that forms between two adjacent drops.

We reverted, therefore, to the sprayer as a means of breaking up the water. The sprayer, which was of metal, was fitted to a bottle containing the water to be sprayed. The sprayer was driven by air contained in a large vessel under pressure. The spray was projected horizontally, and was received on a shallow zinc vessel 120 cms. long and 60 cms. broad, which was placed horizontally 40 cms. below the level of the sprayer. This receiving vessel was insulated by paraffin supports. The spraying bottle was also insulated, an insulating section being inserted into the tubing connecting it with the high-pressure air-supply. The apparatus was surrounded by a screen of wire netting connected to earth.

In investigating the charge on the water, it is necessary to eliminate effects due to actions between the water and the nozzle of the sprayer. Thus there might be a "water-dropper" effect, though this is unlikely to cause any appreciable charging owing to the good screening. Frictional effects between the issuing water and the nozzle must also be considered. If the spraying bottle and the receiving vessel are connected together, all effects of this kind are automatically eliminated, provided that all the water is captured. The charging of the combined system will then be due to the genuine air-water effect, the opposite charge being carried away in the air as an excess of negative ions. This was the method of working employed in practically all cases. Care was taken to ensure that all the spray reached the receiving vessel, and both vessel and sprayer were connected to the Dolezalek electrometer, with which the charge was measured.

Various methods of estimating the size of the drops were tried. Some of these were on lines already indicated. Test plates were introduced, and the drops falling on a certain area in a known time were counted. A second experiment gave the weight of water falling on the same area in a known time under the same conditions. Thus the average volume of the drops was deduced. But, as has been pointed out, this may give very illusory results when there is much variation in the size of the drops. In addition, evaporation is a serious trouble in both parts of the experiment. The method finally adopted is convenient and accurate, and may be recommended for measurements of this character. Glass microscope slides were prepared by spreading out on each a layer of thick dark oil (density 0·9). When one of these slides was exposed to the spray, the small drops falling on it passed into the oil-layer and were there suspended, sinking very slowly. The rate of movement through the oil was so slow that the smallest drops did not reach the bottom of the layer until after forty-eight hours. While suspended in the oil the diameters of the drops could be easily measured with a low-power microscope. We were thus enabled, by taking a sufficient number of observations, to determine the sizes of the drops to any degree of accuracy required.

The total amount of water per second passing through the sprayer for any given air-pressure was easily measured by capturing all the water coming from

the nozzle before it spread out, and weighing. These observations were repeated from day to day in order to make sure that the sprayer was not varying in its action.

Purity of the Water.

In the previous work referred to, the ordinary distilled water of the laboratory was used. We had now access to water of a somewhat higher degree of purity. Experiments were therefore carried out with this water, and also with samples of different grades of purity prepared by mixing the distilled water in various proportions with tap-water (Vartry). In the course of each experiment samples were drawn off from the spraying bottle, and the conductivity determined. The purest water had a specific conductivity of $2{\cdot}4 \times 10^{-6}$ ohm^{-1}.

Results: Charge on water from sprayer driven at different pressures.

The continuous curves (fig. 1) show the total quantity of positive electricity

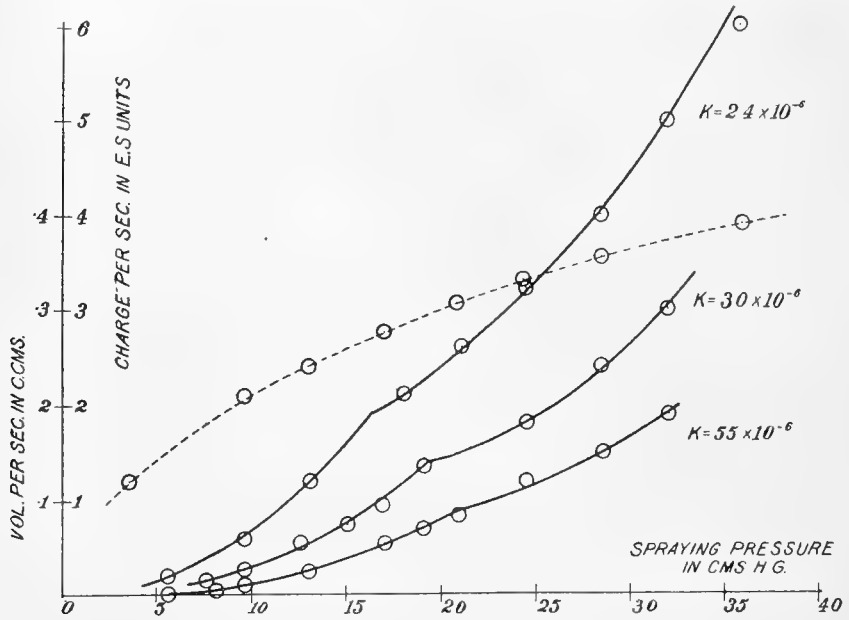

Fig. 1.

per second communicated to the water when the sprayer is driven at various air-pressures. Three curves are given corresponding to three samples of water of different degrees of purity. The conductivity of each sample of water is indicated on the curves. The dotted curve in the same figure shows the total quantity of water per second issuing from the sprayer at different pressures. Combining this curve with each of the other three in turn, we get the curves shown in fig. 2, which give the charge per c.c. in electrostatic units corresponding to different pressures on the sprayer. Some points may be at once noted. The charge per c.c. depends on the purity of the water, increasing as the conductivity decreases. The values are much higher than any previously reported, especially in the case of the purer sample. Each curve shows a discontinuity; the pressure at which the

discontinuity occurs being lower, the purer the sample of water. The pressure values are 16, 19, and 21 cms. Hg.

Measurement of Drops.

The next step is the measurement of the degree of pulverization produced in the water at different pressures of the sprayer. The method finally adopted has been indicated already; but before dealing with it in detail a few results of a general character may be mentioned. Observations made by different methods showed that the drops falling on any part of the receiving vessel varied considerably in size. The average size, however, did not vary much from place to place.

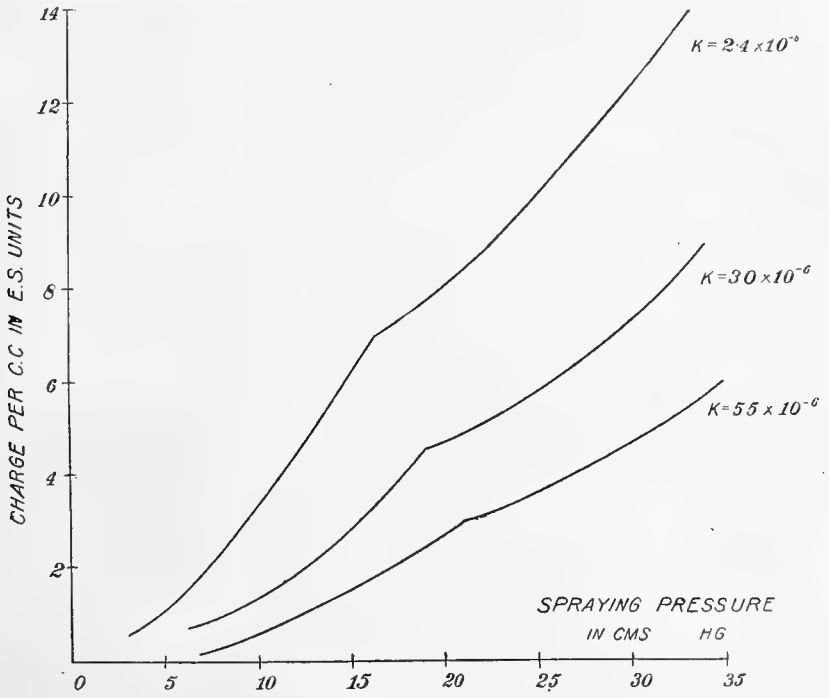

Fig. 2.

About the middle regions of the vessel, where the bulk of the spray fell, the average size was fairly constant. Out from this in each direction, that is back towards the sprayer and out towards the edges of the plume of spray, the drops tended to be smaller, but the difference was not very great. Electrical tests showed a corresponding slight variation in the magnitude of the charge per c.c. This was examined by putting a small vessel, suitably protected, insulated, and connected to the electrometer, at various positions on the surface of the receiving vessel. A difficulty arises here as to the charges due to the frictional effects at the nozzle. When capturing only a fraction of the water, it is no longer possible to secure automatic compensation by connecting the sprayer and the receiving vessel together. It is possible, however, to work at such an air-pressure that purely nozzle effects are inappreciable. Tests made in this way showed that the

charge per c.c. was fairly constant on different parts of the spray, tending to be somewhat higher towards the edges, where the small drops were more numerous. On the whole, the result of these tests showed that the character of the spray did not vary much from point to point, and that it was therefore justifiable to deal with it as a whole for purposes of measurement.

In order to get an estimate of the degree of pulverization for the whole spray, the following method was adopted: microscope slides, each having a thin layer of oil spread on its surface as already described, were arranged on a long glass plate at intervals of 10 cms. The plate was placed parallel to the long edge of the rectangular receiving vessel, and then drawn across quickly, traversing the full width of the vessel. Each of the microscope slides thus received a number of drops corresponding to the vertical section of the cone of spray directly above it. The drops captured on the slides were then measured and counted. In measuring the drops a low-power microscope was used, furnished with a scale in the eye-piece on which sixteen divisions corresponded to 1 mm. The drops were classified as of diameter $\frac{1}{2}$, 1, $1\frac{1}{2}$, . . . divisions; those of diameter between say $\frac{3}{4}$ and $1\frac{1}{4}$ division being returned as of diameter = 1 division. Very few drops of diameter less than $\frac{1}{4}$ division were observed. The method of working will be understood from the following table, which gives the results of a "census" of the drops produced from a sample of water at a certain spraying pressure. This table gives the number of drops of each of the standard sizes captured on the microscope slides at different parts of the spray. The results of a number of observations made under the same conditions are here combined:—

Diameter of drop in Scale divisions.	Horizontal distance of slide from nozzle in cms.									Total n	nd^2	nd^3
	5	15	25	35	45	55	65	75	85			
$\frac{1}{2}$	9	13	19	27	22	20	10	7	—	127	31·7	15·8
1	11	14	13	15	14	16	8	6	2	99	99·0	99·0
$1\frac{1}{2}$	5	6	4	3	2	1	—	1	—	22	49·5	74·2
2	3	7	3	2	2	1	1	1	—	20	80·0	160·0
$2\frac{1}{2}$	—	2	2	1	1	—	—	—	—	6	37·5	93·8
3	1	1	1	—	1	—	—	—	—	4	36·0	108·0
$3\frac{1}{2}$	—	—	—	1	—	—	—	—	—	1	12·3	43·0
4	—	—	—	—	—	—	—	—	—	—	—	—
$4\frac{1}{2}$	—	—	—	—	—	—	—	—	—	—	—	—
										$\Sigma nd^2 =$	346·0	
										Σnd^3	=	593·8

The question arises as to the proper method of treating these measurements of in order to express the degree of pulverization of the water. In the previous

work this was expressed in terms of the area of new surface created, and a connexion between this and the magnitude of the electrical charging was approximately established. We have adopted the same method in the present work.

If n_1 is the number of drops of diameter = d_1 scale divisions $\left(\frac{d_1}{160}\text{ cms.}\right)$, n_2 the number of diameter d_2, etc.,

then the volume of all the drops = $\Sigma\, n \frac{\pi}{6}\left(\frac{d}{160}\right)^3$ c.c.,

the total surface area = $\Sigma\, n\pi \left(\frac{d}{160}\right)^2$ sq. cm.,

and the surface area per c.c. = $960 . \frac{\Sigma nd^2}{\Sigma nd^3}$.

In the example given the surface area = $\frac{960 \times 346}{594} = 559$ sq. cm. per c.c.

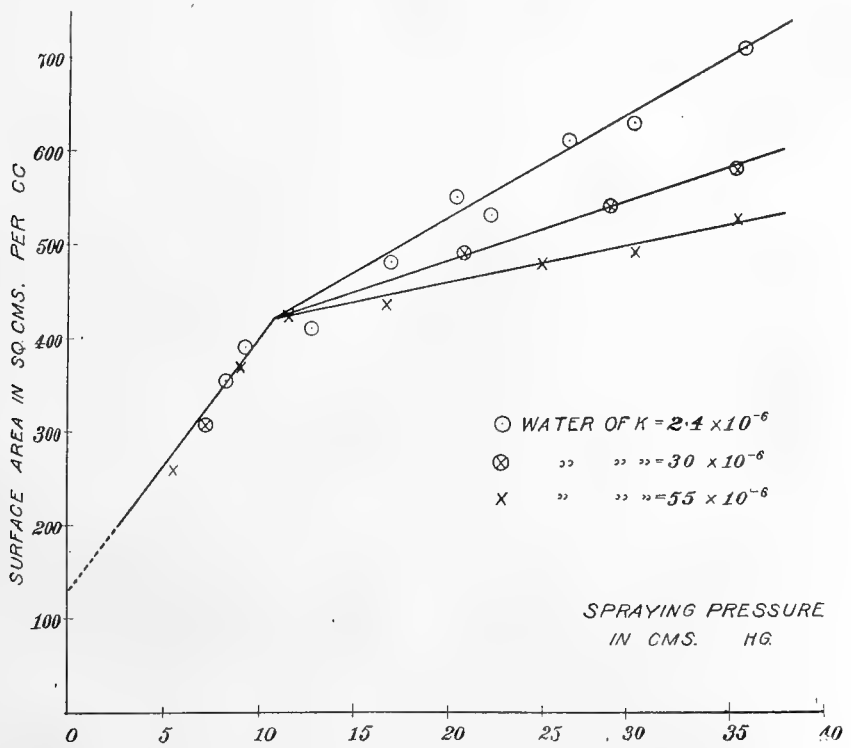

Fig. 3.

The results of observations made at different pressures of the sprayer are shown by the graphs of fig. 3. The area of water-surface per c.c. of water sprayed increases uniformly with the pressure up to a spraying pressure of about 11 cms. Hg., and the three samples of water behave in the same way. But at higher pressures the rate of increase of surface with rising pressure is much slower and different for the three samples. Thus we have the unexpected result

that the sprayer working at the same pressure produces a degree of pulverization depending on the purity of the water, the purer water being broken into smaller drops. It may be remarked that the output of the sprayer (as expressed in the dotted curve of fig. 1) is the same for all samples of water. The graphs of fig. 3 have been drawn as intersecting straight lines. It is possible that more accurate observation might reveal them as rounded in the region of inflexion. The bending over of the curve may really occur at slightly different pressures for the different samples of water. The pressure at which the change occurs is certainly well removed from the pressures 16, 19, and 21 cm. Hg. at which discontinuities occurred in the curves showing the charge per c.c. plotted against pressure (fig. 2).

The first part of the graph, which is common to the three samples of water, does not pass through the origin. It intercepts the vertical axis at a point corresponding to a surface of 130 sq. cm. per c.c. This is easily understood, for we have been plotting the total surface per c.c. of water, whereas what we are really interested in is the area of *new* water-surface. It would seem that this value of 130 represents the surface area per c.c. of the water issuing from the sprayer before it is broken up. If the issuing jet filled the whole orifice, it could be regarded as a cylinder, and its original surface per c.c. would be $2/r$, r being the radius of the orifice. The value of $2/r$ in this case is about 70. Now, the true value of the radius of the water-jet is certainly less than that of the orifice, the water being surrounded by an annular air-blast. To assume that the true radius of the water-jet is half that of the orifice would bring the numbers into approximate agreement. It is not unreasonable, then, to suppose that this intercept gives us the original water-surface per c.c.

Charge considered as a function of the new area produced.

We can now combine the results of these curves with those in fig. 2 (charge per c.c. against pressure), and, following the practice of the previous paper, plot the charge per c.c. against the area of new surface per c.c. These curves are given in fig. 4. In the previous work the points were found to lie, roughly, round a straight line passing through the origin, showing that the amount of charging was proportional to the area of new surface. In the present more accurate curves there is a rude suggestion of this relationship, especially if the proper origin of co-ordinates is taken (allowance being made for the original surface area of the water). The simple idea of direct proportionality, however, has to be abandoned.

Three notable points appear from a consideration of these curves. First, the effect of purity of the water on the charge developed is most important for the smaller degrees of pulverization. For example, the purer sample, when broken into drops of a certain comparatively large size, gives a charge of 2 e.s. units per c.c. The other samples, when broken into drops of the same size, give charges of 0·8 and 0·2 e.s. units per c.c. respectively. In the second place, for high degrees of pulverization it would seem as if the three curves were going to fuse into one, which means that if the water is broken into small enough drops the charge per c.c. will be the same whatever the purity of the water. The only difference is that (as the curves in fig. 3 show) it is apparently more difficult to break up the impure water. Finally, the discontinuities on the curves for electric charge, which could not be associated with any value of the electric charge, or any value of the spraying pressure, appear now on all three curves for the same value of surface-area per c.c., that is, for the same average size of drop. It would appear from these curves

that, as the size of the drops in the spray becomes smaller, the electrical separation occurs with greater and greater facility, especially in the case of pure water, until the average size of the drop reaches a certain value (radius = $6{\cdot}5 \times 10^{-3}$ cms.). At this stage there is a definite check. The further stages of the curve show, in the case of the two less pure samples, a tilt up, suggesting, as we have said, that they will fuse with the curve for the purer water.

In examining these curves we must consider to what extent they may have been effected by certain actions going on in the spray, which we have neglected up to the present, viz., electrical recombination and recombination of drops. The ions carrying the negative charge are not immediately separated from the spray. Some amount of recombination will take place, and the measured electric charge on the water will be correspondingly reduced. There may be two types of recombination,

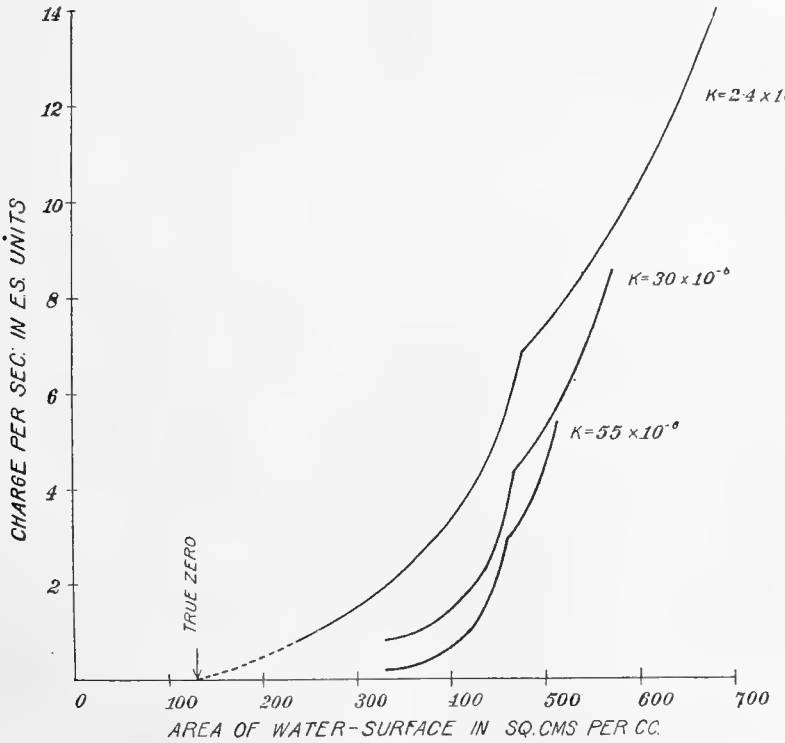

Fig. 4.

an initial type resembling (but on a different scale) the initial recombination, postulated in connexion with α-particle ionisation, and a type corresponding to later stages. If anything of the nature of the first type exists, it is probably beyond the range of detection in any of our experiments, and consequently does not influence the shape of our curves. The second type of recombination is certainly operative, but its effect may not be very great in this case, where the ions are carried away rapidly by the air-blast, while the drops sink steadily towards the receiver. It has been found[1] that the ions from a water-spray, examined as

[1] J. J. Nolan, Royal Irish Acad. Proc., A, 33 (1916).

soon as possible after their formation, consist, to a large extent, of the slowly moving type. They are probably, as a whole, not very effective for purposes of recombination. The extent to which their activity affects our values of charge is difficult to estimate. It should not vary much for different pressures of the sprayer, but its effect is possibly less at higher pressures, owing to the more rapid removal of the air. On the whole, we conclude that the electrical recombination should not affect the shape of our curves seriously beyond a possible apparent tilting up of the electrical values at higher pressures (smaller drops).

We have next to consider the possibility that coalescence takes place to some extent among the drops. This would result in our underestimating the extent of the pulverization undergone by the water. As is well known from the experiments of Lord Rayleigh, drops of pure water colliding will rebound if uncharged. A moderate degree of charging will promote coalescence, while a higher degree of charging will prevent it. It might be said, then, that all our measurements are affected by coalescence of the drops; that the effect is less with the purer water, owing to the higher charges developed; that the purer water being sprayed into smaller drops (fig. 3) merely means that in that case there has been less coalescence between drops. We are not in the possession of any quantitative data by which this view could be tested, but we are unwilling to believe that coalescence takes place between the drops to any serious extent, for a variety of reasons: (1) Except in the immediate vicinity of the nozzle of the sprayer, there is nothing to cause collision between drops. For most of their time, between production and capture, they are moving steadily through air that is practically at rest; and even when they are being driven forward by the air-current from the sprayer there is no appearance of whirling or violent motion which would throw them against one another. (2) When the vertical distance traversed by the drops before capture was varied from 20 to 80 cms. in steps of 10 cms., there was no appreciable change in the size of the drops examined. This experiment shows that the drops are certainly not coalescing in the later stages of their existence. (3) In fig. 3 the first part of the graph (pulverization against pressure) is common to the three samples. But this is just the stage at which the first part of the Rayleigh effect (moderate charges promoting coalescence) should show up distinctly. The charges on the three samples here differ most widely; for example, on the more impure sample the charge at 5 cm. pressure is practically zero. We should therefore be able to trace the effect of charges increasing from zero and promoting increasing degrees of coalescence, the effect developing more rapidly in the purer samples. But we have no evidence of any action of this kind. (4) If the separation of the later part of the graph (fig. 3) into three parts be taken to indicate the effect of the second part of the Rayleigh phenomenon (increasing charge preventing coalescence), it is difficult to see why the transition should occur at a certain value of the spraying pressure and size of drop, the charges at that stage being in the ratio roughly 1 : 3 : 8.

We believe therefore that recombination of drops, if it occurs at all, does not affect our measurements seriously. The tendency of the three curves in fig. 4 to join into one, which would seem to be very approximately a straight line passing through the true origin, we interpret as indicating that the charging of the water is a genuine surface effect. The charge produced is proportional to the area of new surface for any sample of water if the water is broken up to a sufficient extent. The full electrical separation is inhibited by impurities present if the degree of pulverization is small. There is apparently a critical size of drop at which the inhibiting effect of impurities becomes ineffective. The effect of the

impurities in modifying the electrical conditions at the surface-layer is probably connected with their effect in reducing the degree of pulverization produced at a given spraying pressure. The impure water can, to a certain extent, readjust its surface-tension under applied stresses; the pure water is, as it were, more "brittle."

Our measurements show that the quantity of electricity associated with the formation of 1 sq. cm. of new water surface is about 0·02 e.s. units.

Results considered in connexion with Simpson's "breaking-drop" theory.

In this connexion the outstanding feature of these experiments is the importance of purity of the water when the degree of breaking up is small. Our purest sample of water had a specific conductivity of $2{\cdot}4 \times 10^{-6}$ ohm^{-1}, which does not indicate a very high degree of purity. This water stood for days in an earthenware vessel in contact with air, and, of course, was also in contact with glass vessels. The nature of the contamination that affects the activity of the water from the electrical point of view we do not know. This is a point we hope to investigate subsequently. But it is not unreasonable to suppose that a rain-drop, formed in the well-filtered air of a thundercloud, is much purer than our purest sample. Hence, for moderate degrees of breaking up, we would expect the thunder-rain to acquire bigger charges than those observed by us. But even for the same degree of purity it can be seen that the charges observed on thunderstorm-rain can easily be reached. If a drop of radius r breaks up into 27 equal drops,[1] the change in surface per c.c. is $6/r$. Taking a drop of diameter 4 mm., the change in surface per c.c. will be 30 sq. cm. Reference to fig. 4 shows that this would produce, in our purest sample, a charge somewhat greater than 0·2 e.s. units per c.c. We see, therefore, that for the purer water of the thunderstorm we need not demand any high degree of breaking up or any very sustained repetition of the process in order to produce the charges observed on thunder-rain. There is another process at work which tends to concentrate the charge. It is recognized that evaporation from the falling rain-drops in a thunderstorm is very rapid. Evaporation no doubt also plays a part in increasing the magnitude of the charge per c.c. on ordinary rain. We consider, therefore, that if a moderate degree of purity be assumed for rain-drops in the upper atmosphere, the theory put forward by Simpson is fully competent to account for the observed electrical phenomena of thunderstorms. It also explains the sign and magnitude of the charge on the greater part of ordinary rain.

[1] See Hochschwender's photograph, fig. 6. Lenard, *loc. cit.*

No. 2.

CATAPHORESIS OF AIR-BUBBLES IN VARIOUS LIQUIDS.

By THOMAS A. McLAUGHLIN, M.Sc., A.Inst.P.,
University College, Galway.

[Read April 25. Printed June 1, 1922.]

On the subject of the electrification in colloids, that is, of fine particles in suspension in a liquid medium, much research has been done and a fair understanding obtained. Likewise, something has been done on the electrification in emulsions, but a broad field for research remains in this case of liquid suspensions in liquid. In the remaining case, of what, for the sake of continuity, may be termed gas suspensions in liquids, still less has been done, and, in view of the advanced state of knowledge on electric phenomena in gases, this seemed a promising field for research on cataphoresis phenomena.

Mention is made by Quincke[1] of the motion of air-bubbles under an electric field. He reports that air-bubbles in water, carbon disulphide, and turpentine move under an electric field, in water towards the positive pole, in carbon disulphide and turpentine towards the negative pole. McTaggart[2] verifies Quincke on air-bubbles in distilled water, and finds no motion of air-bubbles under an electric field in the pure alcohols, methyl, ethyl, propyl, and isobutyl. Otherwise, there seems to be no record of the motion of air-bubbles in pure liquids under an electric field. In the present research the plan was to make preliminary investigations into the charge on air-bubbles in pure liquids, with special reference to badly conducting liquids. It was thought possible, at first, that the investigation might be approached after the manner of Millikan's oil-drop experiments, that is, that the upward tendency of an air-bubble in a liquid might be balanced, owing to the charge on the bubble, by an electric field, and from such balancing experiments the charge calculated. It was decided, however, to proceed with preliminary investigations with apparatus of McTaggart's type, and, with a fuller knowledge of the phenomena, to return to the problem by other methods of investigation.

The apparatus used was a glass tube 18 mm. diameter and 5 cm. long, fitted with ground brass plugs, which were air-tight. On one plug a pulley was cut, and both were supported between pivotal ends on a heavy base, fitted with levelling screws. The tube was rotated, as in McTaggart's experiments, by a Rayleigh motor. The motor and the apparatus stand were clamped in position, so that everything was steady, and regular motion of the tube was obtained. The required air-bubble was simply introduced by removing one plug of the tube, pouring in liquid, and reintroducing the plug in time to catch a bubble which had been formed by the pouring of the liquid. The tube being in position and rotating, the bubble took up a position on the axis of the tube, and could be kept thus at rest during the period of observation by proper adjustment of the levelling screws and speed of the motor. The bubbles were illuminated by an electric lamp through a narrow

[1] "Pogg Ann." 113, 1861. [2] Phil. Mag., 27, p. 297, and 28, p. 367, 1914.

horizontal adjustable slit, and observed through a low-power microscope. The image of the bubble was clear and distinct, and its size could be determined on the scale-piece of the microscope, which read 25 divisions to 1 mm.

The first observations taken were on air-bubbles in distilled water. The results of Quincke and McTaggart, that they moved to the positive pole, were verified. McTaggart's results in the case of the alcohols were likewise verified. In *ethyl alcohol* bubbles of diameters 0·08, 0·10, 0·12, 0·16, 0·20, 0·24, and 0·28 mm. were observed under a field of 160 volts per cm., and showed no motion. In *butyl alcohol* bubbles of diameters 0·06, 0·12, 0·16, and 0·24 mm. showed no motion under a field of 95 volts per cm., being under observation in each case for at least sixty seconds. In *methyl alcohol* bubbles of 0·20 and 0·32 mm. diameter, respectively, showed no motion under 160 volts per cm. These bubbles were the only two which were successfully observed, the time of observation being only fifteen seconds. The difficulty arose from the fact that the field had the peculiar effect of causing air-bubbles to dissolve quickly. This phenomenon is mentioned by McTaggart. The following observations may be recorded in this connexion :—With no field in action a bubble of diameter 0·08 mm. remained unchanged while observed for forty-five seconds, but dissolved completely within three seconds under a field of 160 volts per cm. Again, bubbles of 0·12, 0·16, and 0·20 mm. diameters disappeared under field within ten seconds. A bubble of 0·20 mm. diameter grew to 0·28 mm. in thirty seconds without a field, and decreased to 0·12 mm. in fifteen seconds under field. Another of 0·28 mm. diameter gradually dissolved under field in forty seconds. At the same time, none of these bubbles showed any tendency to definite motion under the field. A similar effect of an electric field on the solution of the bubbles was looked for in the other alcohols and other liquids examined, but found to be absent in all cases. Observations were now proceeded with on air bubbles in a number of liquids not previously examined.

In *xylol* several bubbles, each of the following diameters, 0·08, 0·10, 0·12, 0·20, 0·24, 0·28 mm., were examined, and showed no motion under a field of 160 volts per cm. Each bubble was kept under observation with reverse fields for at least thirty seconds, except bubbles of 0·12 mm. diameter and under. These latter always dissolved quickly, while some of the larger bubbles showed a tendency to grow in the liquid, but in no case did the change in size affect the neutral attitude of the bubbles to the field. Air-bubbles in *benzene* were next examined. In the first sample they all moved to the negative pole under field. The benzene was probably impure, as the bottle contained some traces of red rubber cork, portion of which had probably gone into solution. In fresh pure benzene, under a field of 160 volts per cm. for periods of about thirty seconds, various bubbles of diameters 0·06, 0·12, 0·16, 0·20, 0·24, 0·28, 0·32, 0·40, and 0·48 mm. showed no motion. There was not the same difficulty with benzene as with xylol in investigating the smaller bubbles, but, as before, some bubbles grew in size while under examination, as, for example, from 0·32 to 0·48 mm. diameter, but the change did not affect the neutral attitude to the field.

In *toluene* air-bubbles 0·32 mm. and 0·48 mm. did not move under reversed fields of 95 volts per cm. for thirty seconds each way. Another bubble, 0·32 mm. diameter, was under observation for two minutes in the same field, and did not move. A small bubble, 0·12 mm. diameter, gradually dissolved, but was uninfluenced by the field.

It was now decided to proceed with investigations with benzene derivatives, some of which are chemically neutral, others acidic, others basic, in the hope that the presence of free ions might be the clue to the presence or absence of charge.

In bromobenzene, benzaldehyde, aniline, cinnamic aldehyde, ethyl malonate, and oleic acid, bubbles of diameters from 0·08 to 0·28 mm. did not move under a field of 95 volts per cm.

Benzyl alcohol disintegrated under 95 volts per cm., but bubbles 0·08 mm. and 0·12 mm. diameters showed no motion under a field of 14 volts per cm.

Lactic acid likewise disintegrated, but a bubble 0·12 mm. diameter did not move under field for thirty seconds prior to disintegration.

In *ethyl acetate* bubbles grew, but this growth was uninfluenced by field. A bubble 0·20 mm. diameter grew to 0·28 mm. while under observation for thirty seconds each way with a field of 95 volts per cm., but showed no sign of motion. Another bubble 0·16 mm. diameter was likewise uninfluenced by field.

It was not found possible to trap an air-bubble in acetone and such volatile liquids as carbon disulphide, but it was noticed that foreign matter moved to the positive pole in impure acetone.

Thus, in all pure organic liquids tested so far air-bubbles showed no cataphoresis. In one organic liquid, *nitrobenzene*, air-bubbles never failed to move under field. The first sample used was from ordinary laboratory supply, which was a dark yellow in colour, due to oxidation from exposure to light. It had been in store for some months.

Bubbles 0·08 and 0·16 mm. diameters undoubtedly moved towards the negative pole. Likewise, other samples from same source gave to air-bubbles a motion in the same direction, the bubbles being of diameters 0·08, 0·16, 0·24, and 0·32 mm. The latter moved 20 divisions per minute and 19 divisions per minute in opposite directions under reversed fields of 95 volts per cm. A fresh pure distilled sample of nitrobenzene was obtained. It was light yellow in colour, and was standing for about ten days in a dark place prior to use. A potential of 95 volts per cm. gave a motion of 4 divisions per minute in either direction, showing apparent positive charge. A later observation showed a motion of 11 divisions per minute; the charge was still positive, but seemed to have increased. The bubbles were 0·20 and 0·12 mm. diameter, respectively. A second sample from the same source gave to an air-bubble a negative charge, the motion being 3 divisions per minute under the usual field. The same sample, after standing for three days in light, again gave a negative charge to two bubbles of diameters 0·04 and 0·08 mm.; the charge had increased, the rate of motion now being 38 divisions per minute. Close examination of the liquid showed that some traces of red rubber from the cork of the bottle had got into it. A further sample of nitrobenzene was freshly distilled and carefully dried over phosphorus pentoxide. In all cases bubbles showed an apparent positive charge. In the first sample bubbles of diameters 0·08, 0·12, and 0·28 mm. all moved to the negative pole with velocity of 200 divisions per minute under 95 volts per cm. In a second sample from the same source bubbles of diameters 0·04 and 0·20 mm. moved towards the negative pole with a velocity of 140 divisions per minute under the same field. A third sample gave exactly the same motion to bubbles of 0·08 and 0·16 mm. diameters. In a fourth sample two bubbles of diameters 0·08 and 0·12 mm. acted likewise. Thus, bubbles in "pure" nitrobenzene move to the negative pole, but in impure nitrobenzene the charge seems to depend, both in sign and magnitude, on the purity of the nitrobenzene—a factor which is difficult to determine.

Owing to the general absence of cataphoresis in the great number of liquids tested, Quincke's statement that air-bubbles were positively charged in *turpentine* is of interest. On test, however, it could not be verified. Two distinct samples of turpentine from two different sources were tested. In one an air-bubble of

diameter 0·4 mm. gradually dissolved, and was observed for two minutes under a field of 90 volts per cm., but showed no motion. Another bubble 0·08 mm. gave the same result. In the second sample a bubble of 0·16 mm. diameter was watched gradually dissolving for two minutes under the same field, and likewise did not move. In view of these contradictory results, it is of interest to compare results on electric endosmose, reported in the same paper by Quincke, with results obtained by Perrin, and to recall Freundlich's comment on the apparent contradiction :—

> "Perrin fand keine Elektroendosmose bei . . . Terpentinöl . . . . . dagegen fand Quincke bei Terpentinöl eine Verschiebung der Flüssigkeit zum positivem Pol . . . Da schon beim Wasser kleine Zusätze grosse Änderungen der Elektroendosmose bedingen, ist es fraglos, dass Stoffe verschiedener Reinheit ein sehr verschiedenes Ergebnis zeitigen können" (Kapillarchemie von H. Freundlich, page 241).

It is of interest also to note that reference[1] is made by Coehn and Mozer to experiments showing that pure turpentine does not acquire a charge in bubbling, while they point out that Lenard found that ordinary unpurified turpentine acquired a charge.

Discussion.

The experiments on cataphoresis are meant to be purely of a preliminary nature, and the phenomenon is approached from the qualitative view-point rather than the quantitative. Adopting Lamb's[2] equation as governing the motion of an air-bubble, then the velocity U under field X volts per cm. is given by

$$U = \frac{qb}{u} X,$$

where q = surface density of charge in double layer
and b = thickness of double layer,
u = viscosity of liquid.

Thus the velocity of the air-bubble under a field in any given liquid is a measure of the charge in the inner double layer, i.e., the free charge which would accompany the bubble in motion in the liquid. This formula is laid down as applicable to all liquids which are not perfect insulators. Thus, absence of cataphoresis, under the conditions of the experiments, means either an entire absence of any double layer, or else that the charge is of a negligible amount as compared with that bound up in the double layer surrounding an air-bubble in distilled water. An air-bubble in nitrobenzene, or in impure liquids, is an exception. Thus, in benzene the presence of impurity brought about the cataphoresis phenomenon. The question arises, therefore, is the phenomenon in nitrobenzene due to the same cause? The unstable nature of nitrobenzene and the doubtful nature of its purity in all cases would seem to uphold such belief. In particular the variation in the motion of an air-bubble in different samples from the same source might be explained as due to the instability of the nitrobenzene, which darkens if exposed to daylight. The phenomenon in water might be explained similarly, as distilled water cannot be regarded as perfectly pure. It is

[1] Coehn u Mozer. Ann. der Physik, vol. xliii, p. 1045. 1914.
[2] Lamb, Brit. Ass. Rep., 1887.

of interest also to note that the two liquids which showed the phenomenon have a much higher dielectric constant than those liquids in which it was absent, and, accordingly, are much better ionisers. This might be expected from a law laid down by Perrin as a result of experiments on electric osmose—an allied phenomenon :—"Electric osmose is only appreciable with ionising liquids; or, in other words, ionising liquids are the only ones which give strong electrification by contact." Thus, the presence of impurities would be more likely to bring about cataphoresis in liquids of high dielectric constant, e.g., water and nitrobenzene. The results recorded in this paper are also in keeping with Coehn and Mozer's[1] results on contact electrification between gas-liquid surfaces investigated by bubbling experiments. They found that the greater the dielectric constant of the liquid, the greater was the charge recorded by bubbling a particular gas through it. It is remarkable that the charge produced by bubbling hydrogen through various liquids was much greater in the case of water and nitrobenzene than in any other tested. Benzaldehyde and aniline are recorded as giving charges in the bubbling experiments, while in these experiments they gave no charge to an air-bubble.

Summary.

Under the conditions described in the paper air-bubbles were found to show no cataphoresis in the following liquids :—Methyl, ethyl and butyl alcohols, xylol, benzene, toluene, bromobenzene, benzyl alcohol, benzaldehyde, aniline, cinnamic aldehyde, ethyl malonate, lactic acid, oleic acid, ethyl acetate, and turpentine. The results on turpentine are not in accordance with those obtained by Quincke.

It was not found possible to trap an air-bubble in acetone and such volatile liquids as carbon disulphide. In impure acetone foreign matter moved towards the positive pole.

In distilled water, air-bubbles moved towards the positive pole; in impure benzene, towards the negative pole. In "pure" nitrobenzene air-bubbles moved to the negative pole. In impure nitrobenzene the motion may be to either pole.

The solution of an air-bubble in methyl alcohol under an electric field previously noted by McTaggart was confirmed.

[1] Coehn u Mozer, Ann. der Physik, vol. xliii, p. 1048. 1914.

No. 3.

ON THE AERATION OF QUIESCENT COLUMNS OF DISTILLED WATER AND OF SOLUTIONS OF SODIUM CHLORIDE.

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Introductory.

In the course of his investigations on the downward transmission of atmospheric gases through quiescent columns of water five feet in depth, one of the authors showed that, as the oxygen and nitrogen are dissolved at the exposed surface of the water, they do not remain concentrated in the surface layer, but are distributed through the lower layers with comparative rapidity.

From the fact that the distribution of the dissolved gases, at various depths, after a comparatively short time was almost uniform, it appeared impossible to account for it on the assumption that it was entirely due to such an extremely slow process as that of the diffusion of the dissolved gases from the saturated surface layer.

In addition to diffusion, it was suggested that a process of downward "streaming" of the exposed layer of water occurs; and that it results largely, though possibly not wholly, from an increase in its density, which, in the case of distilled water, is caused by the lowering of temperature attending evaporation, and, in the case of salt solutions, by this factor and that of concentration.

The downward streaming sets up a process of mixing of the constantly changing air-saturated surface layer with the lower layers of the water. When a slow stream of dry air is continuously passed over the exposed layer of a column of water, surface density changes are constantly occurring, and comparatively rapid mixing ensues, with the result that, if the water be de-aerated at the commencement of the experiment, it becomes re-aerated with comparative rapidity.[1]

The experiments described in this investigation have been carried out with the object of investigating the process of the aeration of de-aerated columns of water to a depth of ten feet. Columns of de-aerated distilled water and of solutions of sodium chloride were exposed to a slow stream of dry air for periods of from two to eight weeks, when samples at different depths were drawn off, and the nitrogen content of each determined.

In general, it was found that re-aeration proceeded more rapidly in salt solutions than in pure water,[2] and a further series of experiments was carried out

[1] Unrecognised Factors in the Transmission of Gases through Water." By W. E. Adeney, Trans. R.D.S., p. 161, 1905; and Phil. Mag., 1905.

[2] See above references.

with solutions of sodium chloride, having a wide range of concentration, to ascertain the effect of concentration of the sodium chloride on the rate of aeration.

Experimental.

The de-aeration of the water employed in these experiments was effected by distillation in vacuo. In order to obtain water quite free from atmospheric gases, the distillation was at first carried on in a very slow current of hydrogen, or of carbon dioxide; but, although the resulting water was air-free, it was always found to contain undesirable traces of hydrogen or of carbon dioxide, according to which gas was employed during the distillation. It was consequently decided to rely upon distillation, under the reduced pressure obtained with the aid of a good water vacuum pump, employing a slow current of air, filtered through glass wool, to overcome difficulties from "bumping." The nitrogen content of the de-aerated water obtained did not exceed 1 c.c. per litre, which, in the case of pure water, amounts to about 7 per cent. of saturation at 15° C.

It was found unnecessary to determine the nitrogen content of each sample of water at the commencement of each aeration experiment, as it was found to be practically constant immediately after the de-aeration.

The glass tubes used in the experiments were about 3 cms. in diameter, and varied from 9 to 12 feet in length. T-pieces were fused on, at regular intervals, along the length of the tube, for drawing off samples of the water columns at different levels, and the lower ends of the tubes were sealed. The upper end of the tube was closed by a rubber stopper, fitted with an inlet and outlet tube, by means of which connexions were made to the pump and distilling apparatus; so that the water could be distilled directly into the experimental tube when desired.

In the case of salt solutions, the required quantity of sodium chloride was placed in a five-litre flask, connected with the vacuum distilling apparatus by means of a thick-wall rubber tube, and the required volume of pure water was allowed to distil into the flask and dissolve the salt, the exact concentration of the solution being subsequently determined by analysis. A screw clip was then used to close the connexion with the pump, the flask detached with its rubber connecting tube, and attached, with the aid of the same connecting tube, to the experimental tube. When the free end of this connecting tube was completely filled with freshly distilled water, it could be attached air-free to the previously exhausted experimental tube. After attaching the flask to the tube, the latter could be filled by inverting the flask and unscrewing the clip, when the solution flowed into the tube.

Experimental tubes were also employed, which were furnished with quill capillary tubes, instead of side tubes, for drawing off samples. These tubes passed upwards through a rubber stopper at the bottom of the experimental tube to different levels inside, the external ends being attached each to a piece of thick-walled rubber tubing closed by a screw clip.

Each experimental tube was attached to a stout lath of wood for facility of removal from one laboratory to another. The room in which the aeration was carried out was one which received no direct sunshine nor heat from artificial sources, so that the temperature prevailing in it was not subject to sudden fluctuations. In the earlier experiments, a zinc cylinder, 14 inches in diameter, and filled with water, was used as a jacket for the tubes. But it was subsequently found that a good wrapping of asbestos cloth was a sufficient guard against sudden variation in

temperature. Manipulative difficulties were thereby considerably reduced, and it was possible to work with a greater number of tubes simultaneously.

A recording thermometer was kept in the room, and the greatest variation in temperature in the room, during the exposure of any one tube, did not exceed 4·5 degrees centigrade. This variation, however, was exceedingly slow—too slow to give rise to convection currents.

The air passing over the surface of the water in the tubes was first dried by calcium chloride; and the inlet tube was so arranged that the air did not play directly on to the surface. The inlet and outlet tubes were fixed at a distance of 1·5 to 2 inches above the water. The moist air issuing from the tube was passed through weighed $CaCl_2$ tubes, so that the amount of water evaporated could be determined for each tube. In most of the experiments three tubes were connected in series, drying tubes being placed so that the moist air from one was dried before entering the succeeding tube.

A filter pump, worked by a constant head of water, seven feet in height, was at first employed to aspirate the current of air through the tubes, but subsequently, owing to shortage of the town water supply, caused by the drought last summer, a different arrangement had to be substituted. An electrically driven small air pump was utilized to force air into a large glass vessel, which acted as an equalizer, and thence through the drying vessels and experimental tubes. This arrangement worked very satisfactorily.

The experimental tubes, having been filled with de-aerated water, were fixed in a vertical position; the stoppers, which had been used during filling, were removed, and were replaced by others carrying inlet and outlet tubes for the air current. The air current was continued for two or three weeks in the case of salt solutions, and three to eight weeks for pure water. The apparatus employed for the determination of the dissolved gases was of the form devised by one of the authors.[1]

Method of withdrawing Samples from the Experimental Tubes.

Samples of water were withdrawn from an experimental tube, without at any time allowing them to come into contact with the air, with the aid of a modification of the gas burette used for the analysis of the dissolved gases.

By lowering the mercury reservoir, attached to the burette, a known volume of water was drawn from the tube into the latter, and thence transferred to a Plimpton gas holder to await examination. The depth from which the sample was drawn and the temperature of the water were at the same time noted. 50 c.c. of water were usually taken for the extraction and analysis of the dissolved gases in the case of the surface layer; and 100 c.c. for samples drawn from lower levels.

Determination of Saturation Values for Nitrogen of Sodium Chloride Solutions.

In order to calculate in percentages of saturation the observed rates of solution of atmospheric nitrogen by solutions of sodium chloride of the various concentrations employed in this investigation, it was necessary to determine the saturation values for atmospheric nitrogen of each solution. This was done by filling large tubes, about 5 cms. in diameter and 30 cms. in length, about two-thirds full of

[1] Sci. Trans. R.D.S., vol. v, Series 11, p. 548; also Supplemental vol. vi, Fifth Report of the Royal Commission on Sewage Disposal, p. 99.

the salt solution. A current of air, previously passed through a glass wool filter and through distilled water to saturate it with aqueous vapour, was drawn through the solution under examination. The inlet tube reached to the bottom of the solution; and the apparatus was immersed in a thermostat. The current of air was continued for a sufficient time to ensure equilibrium being reached throughout the tube at the observed temperature. The dissolved nitrogen and the sodium chloride in the solution were then estimated.

The experimental results are shown by the accompanying curve (fig. 1).

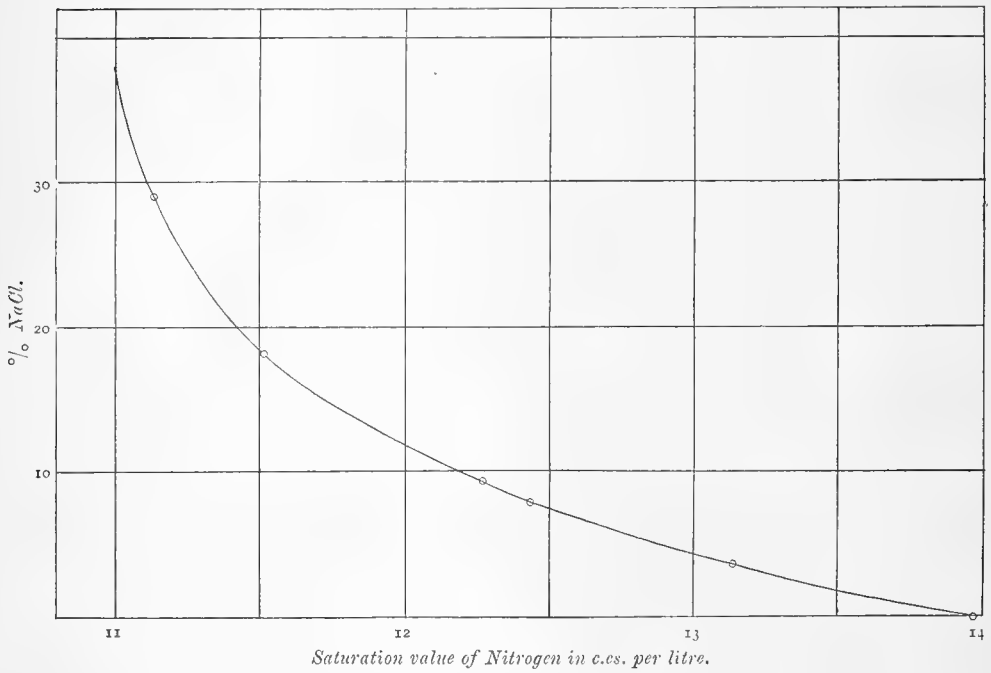

Fig. 1.—Saturation values of Nitrogen in solutions of varying NaCl concentration at 15° C.

Method of expressing Experimental Results for the purpose of Comparison.

The columns of water employed in these experiments were so deep—about ten feet—and the exposure of them to the air had to be continued over such extended periods of time—two to eight weeks—that it was decided not to attempt to control the temperature obtaining during the conduct of the experiments beyond preserving the room in which they were carried out, and which was exceptionally well circumstanced for such purpose, at as closely uniform temperature as possible during a set of experiments.

To have provided the means for controlling, at will, the temperature at which these experiments could be started and continued would have added very considerably to the practical difficulties of an already sufficiently laborious problem; and it was not thought necessary to attempt to do so, since the authors had good reasons, which will be explained later on, for assuming that when the gas concentrations of a de-aerated column of distilled water, or of a solution of

salt, at any time during re-aeration were expressed in percentages of saturation, they would be found to increase practically in the same proportions at different temperatures, provided that the variation in temperature did not exceed three or four degrees centigrade.

This view was based upon the following considerations :—

(1) Dittmar has determined the saturation values of distilled and of sea-water for atmospheric nitrogen and oxygen between 4° C. and 35° C.; and when his results are plotted against temperatures, the curves obtained approximate to parallel straight lines between the limits 8° C. and 35° C.[1]

(2) Adeney and Becker, in their work on the rate of solution of these atmospheric gases by distilled and by sea-water, found that, when the experimental observations from zero to saturation were expressed as percentages of saturation and plotted against time, the curves obtained were coincident when uniform conditions of exposure of water to the gas, of the mixing of the exposed with the unexposed portions of the water, and of temperature (within 1° C.) obtained.[2]

It may be assumed from this that the curves showing the rate of solution of atmospheric nitrogen and oxygen by dilutions of sea-water with varying proportions of distilled water would also be coincident with the curves showing the same for distilled and sea-water, separately, under like conditions of exposure to air, of mixing, and of temperature varying within 1° C.

(3) Adeney and Becker further found that the curves showing the rate of solution in water of nitrogen and of oxygen, when stated in percentages of saturation, lie very closely together for differences of 5° C. within the range of temperature from 0° C. to 30° C.

Consequently it is possible to convert approximately, by simple calculation, observations made at slightly different temperatures, to those that would obtain at a selected common temperature, varying even to as much as 2 or 3 degrees from them, and still obtain sufficiently accurate results for purposes of comparison.

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[1] Supplementary vol. vi to the Fifth Report, Royal Commission on Sewage Disposal, p. 59.

[2] "The Determination of the Rate of Solution of Atmospheric Nitrogen and Oxygen by Water." By W. E. Adeney and H. G. Becker, Part 1, Sci. Proc. R.D.S., 1918, and Phil. Mag., 1920, p. 385.

[Table.

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	D_1	D_2
Initial concn. of N. in c.cs. per litre, ...	0·9	0·662	0·662	0·662	0·618	0·618	0·618	0·618	0·618	1·06	0·618
Initial concn. of N. as percentage of saturation, ..	8·85	6·22	5·17	5·08	4·74	4·66	4·36	4·76	4·74	6·92	4·65
Percentage of NaCl, ..	3·303	1·97	0·94	0·41	0·25	0·116	0·068	0·029	0·0116	0	0
Temp. of room when set up,	11·8° C.	11·8° C.	13° C.	—	13·4° C.	14·0° C.	17·2° C.	17·8° C.	17·4 °C.	9·5° C.	14·1° C.
Temp. when taking samples,	10° C.	11·2° C.	12·7° C.	15·2° C.	16·7° C.	16·9° C.	20·4° C.	20° C.	18·8° C.	10·2° C.	17·8 °C.
Time in days, ...	14	17	18	18	18	18	19	21	23	27	56
Water evap. in gms., ..	—	—	12·07	10·815	6·212	7·632	6·915	7·98	8·3536	—	25·18
Saturation value of N. from curve at 15° C. in c.cs. per litre, ...	11·17	11·44	12·27	13·09	13·41	13·72	13·81	13·91	13·94	—	—
Saturation value of N. at temperature of experiment in c.cs. per litre, ..	12·27	12·28	12·79	13·037	13·02	13·26	12·59	12·97	13·047	15·31	13·29
Length of column in cms.,	317	269	269	317	317	269	269	317	347	360	347
Litres of air drawn over, ..	—	—	1024	843	452	542	398	456	481	—	1734
Litres of air per 24 hrs., ..	—	—	57	47	25	30	21	22	21	—	31

Experimental Results.

The conditions obtaining in some experiments with columns of salt solutions and of distilled water are given in the table on opposite page.

Curves showing the nitrogen content of columns of distilled water, and of salt solutions, at different depths, expressed as percentages of saturation, are given in fig. 2.

On comparing the curves D_1 for distilled water (exposed to the air for twenty-seven days) and S_1, for a 3·3 per cent. salt solution (fourteen days), the effect of the salt on the progress of aeration is well illustrated. The curve for the distilled water column shows a fall in nitrogen content from 40 per cent. at the surface to 13 per cent. at a depth of 311 cms., whereas the corresponding values for the 3·3 per cent. salt solution ranged from 42·7 to 38·3 per cent. respectively in about half the time.

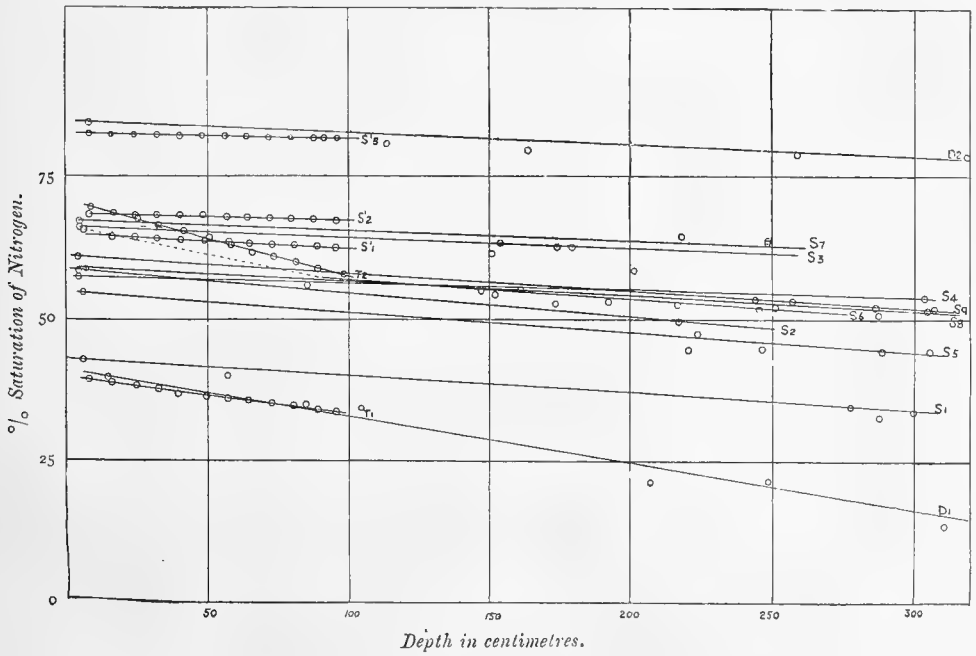

FIG. 2.—Nitrogen content expressed in percentages of saturation at different depths below the surface.

Even with a solution containing only 0·0116 per cent. sodium chloride (curve S_9), the effect of the salt in solution is well marked.

The curves S_1', S_2', S_3', T_1 and T_2 are plotted from results obtained from observations with columns of sea and tap water, 3 feet deep and 4 cms. diameter. The columns were exposed to the air at temperatures 14°, 12°, 13·5°, 13° and 13·5° C. for 7, 6·75, 11, 4, and 7 days respectively.

Samples of the salt solutions from the top and bottom layers of the columns, after aeration, were carefully examined by means of a Pulfrich refractometer to ascertain whether any difference in concentration of the sodium chloride existed between the two layers, but no difference could be detected.

In order to compare more clearly the experimental results obtained for the salt solution, an approximate correction for slight variations in the length of time of their exposure to the air has been made. Eighteen days was taken as the standard time, since four out of the nine tubes were exposed for that time.

Adeney and Becker's formula for calculating the rate of re-aeration was employed, viz.:—

$$w = (100 - w_1)\left(1 - e^{-f \cdot \frac{a}{v} t}\right),$$

where w = amount of gas dissolved, expressed in percentage of saturation.
w_1 = initial concentration in percentage of saturation.
f = coefficient of escape of the gas from the liquid per unit area and volume.
v = volume of liquid.
a = area of surface.
t = time of exposure.

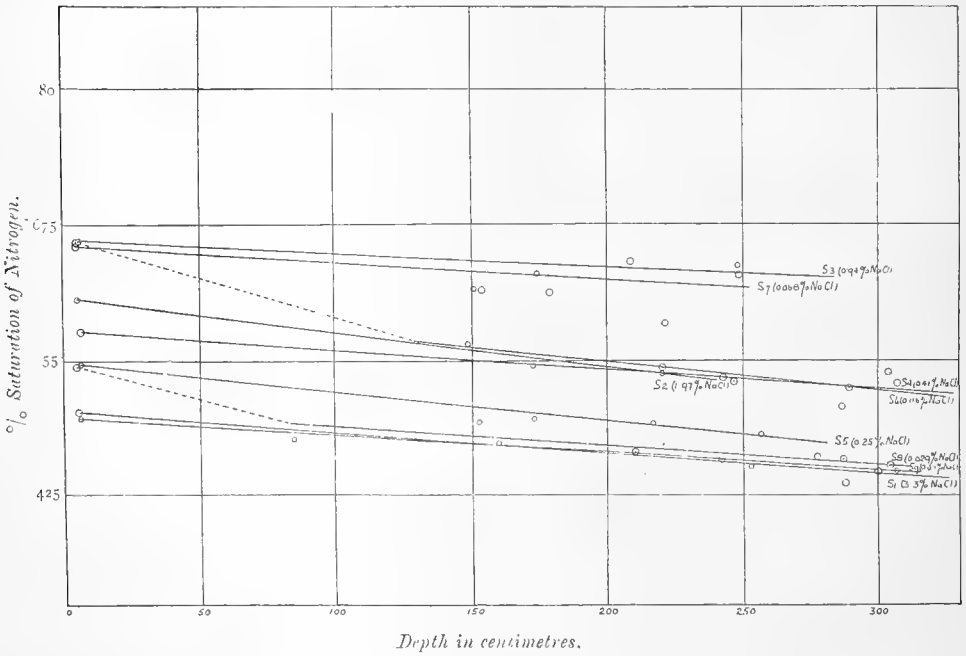

Fig. 3.—Concentration of Nitrogen at various depths after 18 days' exposure for different salt solutions.

By means of the above equation, if w be known for any time t, its value can be found for any other time t. The mean value of the concentration of the gases in solution in the liquid was taken to be w. The actual values at various depths were afterwards calculated. Curves were plotted for the columns of solutions, using observations made after eighteen days. They are given in fig. 3.

The values for the concentrations of nitrogen at a depth of 200 cms. were taken, and these values were plotted against the concentration of salt. The maximum concentration of nitrogen was found to obtain in a solution with a

concentration of about 1 per cent. of sodium chloride. The curves obtained are given in fig. 4.

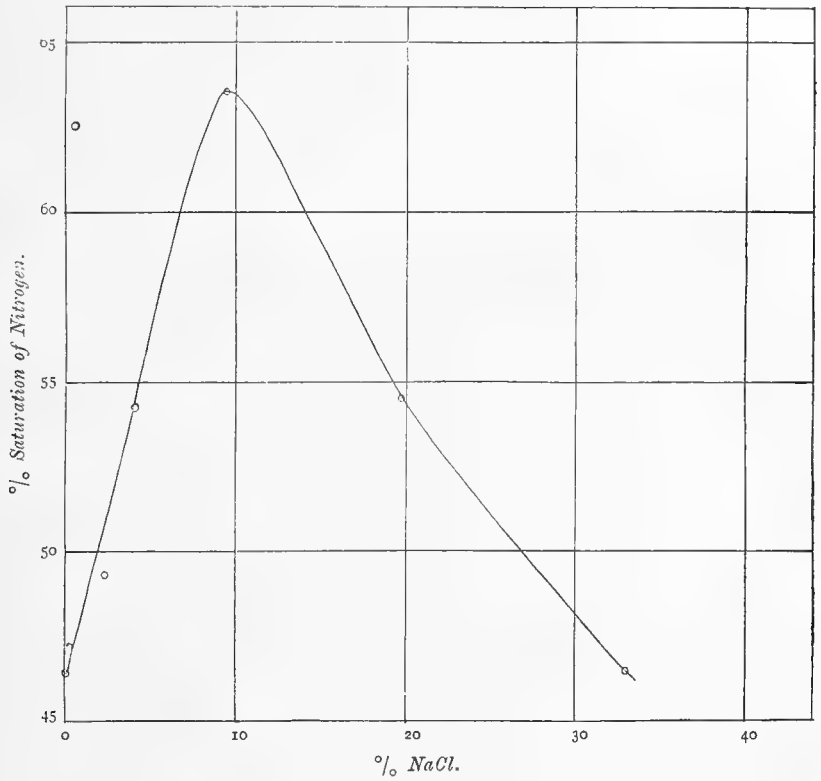

FIG. 4.—Relation between NaCl concentration and downward "streaming" of dissolved Nitrogen to depths of 200 cms. through solutions of that salt.

Conclusions.

The following conclusions may be drawn from the experimental results obtained in this investigation :—

1. The aeration of quiescent bodies of water, fresh and salt, under natural conditions is effected by a process of mixing of the exposed layer with the unexposed portions of the water to depths of at least 10 feet.

2. The process of mixing is caused by the downward "streaming" by the constantly changing layer of water exposed to the air. This downward "streaming" sets up a process of mixing certainly to depths of 10 feet, and in all probability to much greater depths.

3. The process of mixing set up by the downward "streaming" proceeds more rapidly, and more uniformly downwards, to depths of at least 10 feet, in salt water than in fresh water.

4. The rate at which the "streaming" proceeds depends largely, though not wholly, upon the rate at which the concentration and cooling of the surface layer of the water is brought about by evaporation from it.

5. The process of "streaming," and consequently of mixing, also proceeds more rapidly at temperatures at and above 10° C. than below it. It is distinctly less rapid and less uniform downwards to 10 feet deep, and probably to greater depths, at temperatures below 8° C., especially in fresh waters.

6. The rate at which the process of mixing from "streaming" proceeds also depends upon the concentration of salt in solution. The optimum concentration appears to be about 1 per cent. sodium chloride.

This last conclusion is based upon one series of experiments only, and should be confirmed by further experiments which the authors hope shortly to carry out.

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No. 4.

ON A PHYTOPHTHORA PARASITIC ON APPLES WHICH HAS BOTH AMPHIGYNOUS AND PARAGYNOUS ANTHERIDIA; AND ON ALLIED SPECIES WHICH SHOW THE SAME PHENOMENON.

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(Plates I and II.)

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The work described in the present paper originated from an examination of a couple of apples of the variety "Lane's Prince Albert," which were affected with an unusual form of rot, and were submitted for report in November, 1920, by Mr. E. Turner, one of the Department's Horticultural Inspectors, from Pilltown, County Kilkenny. The diseased fruits were apparently healthy when gathered on October 14th as portion of a lot specially selected for exhibition purposes, and they began to rot about ten days later. Whether infection occurred prior to gathering or during storage is not known.

I.—*Nature of the Rot.*

The decayed apples had dark brown skins, but they were more or less firm and elastic to the touch. They showed no signs of superficial wounds, nor were any external indications of fungus growth visible on them. After they had been kept under a bell-jar in the laboratory for a few days, however, small white tufts of hyphae were present at some of the lenticels. These proved to be composed of non-septate mycelium, which was almost entirely sterile; but prolonged search ultimately resulted in the discovery of two sporangia, suggestive of a Phytophthora.

Further examination, made at a later date, revealed the presence of a few sexual organs, borne on the mycelium in the basal portions of several of the tufts. The oogonia averaged 26·4μ and the oospores 24·4μ in diameter. The surprising thing about the sexual organs was that they were of two types. In the majority of cases the antheridia were lateral[1] (*paragynous*), but in a few instances they were observed to be of the type first discovered in *Phytophthora erythroseptica*, i.e., surrounding the base of the oogonium (*amphigynous*).[2]

The flesh of the diseased apples was brown in colour, fairly firm, and not pulpy.

[1] Not necessarily lateral in the strict sense of the word, because such antheridia are frequently situated more or less below or at the base of the oogonium near the stalk, but not surrounding or penetrated by the latter.

[2] The convenient terms, "amphigynous" and "paragynous," for expressing the position of the antheridium in relation to the oogonium, were suggested by P. A. Murphy, Ann. Bot., xxxii, 1918, p. 125.

In microscopical preparations it was found that rather coarse, non-septate mycelium permeated the tissues, being present both in and between the cells. White aerial mycelium was present in the cavities of the core, but it bore neither sporangia nor sexual organs. The latter, however, were found embedded in the soft tissues above and below these cavities; and antheridia of both the amphi- and paragynous types were present, the latter in much greater number than the former.

Eighteen small pieces of affected tissue were removed aseptically, and each was placed in the centre of a Petri dish, in which a sterile nutrient agar medium had previously been allowed to set. In all cases mycelium grew from the tissue into the medium; but, in the early stages at least, with one exception, the mycelium remained barren. This exception was Quaker Oat agar. On this medium the fungus developed freely, and sexual organs with both types of antheridia were produced on the mycelium in close proximity to the original piece of tissue, whilst they were also present in the tissue itself. Photographs of each type are reproduced on Pl. II, figs. 1 and 2, and the two types are figured on Pl. I, figs. 1 and 2.

Sub-cultures were prepared from the growth on Quaker Oat agar, and from them inoculations were made into healthy apples, the necessary controls being also provided. A rot identical with that found in the original material was produced in every case where the inoculum was introduced, whilst the controls remained sound.

It appeared, therefore, that the rot was caused either by a single species of fungus having sexual organs of two types or by one or both of two associated species, each producing its own particular type of antheridium. In any case, the matter was of more than usual interest, and demanded further study.

II.—*Isolation of the Fungus and Proof of Pathogenicity.*

An attempt was first made to discover by direct microscopical observation whether the two types of sexual organs were borne on one and the same portion of mycelium, but this attempt gave only uncertain results. It was therefore decided to raise pure cultures.

(*a*) *Isolation from portions of single hyphae.*—When radiating growth had proceeded for some time on the Quaker Oat agar Petri dish cultures, mentioned above, a narrow circular band of the medium was removed just beyond the limit of growth. When the tips of the growing hyphae reached the inner margin of this annular space, they proceeded to cross it; but it was found possible, by careful manipulation with sterile needles under the low power of the microscope, to prevent the crossing of this space by all hyphae in a given region save by a single selected one. When the latter had crossed and had become well established in a completely isolated condition in the medium on the other side of the annular space, it was removed, together with a small portion of the medium in which it was embedded, and placed on a suitable medium slanted in a test tube. In this way nine pure cultures were raised, each derived from an isolated portion of a single hypha.

As a means of isolating both members of a pair of fungi intimately associated with each other during growth, the above described technique is undoubtedly open to criticism. If, for example, the rate of growth of one of the pair were greater than that of the other, the former would invariably reach the annular space first, and the cultures derived, as described above, would almost certainly represent only one of the two organisms. Nevertheless, to obtain even one of them in pure culture was a step in advance, and, as the sequel will show, the method adopted served its purpose.

Sub-cultures of the nine pure stocks raised were made in parallel series in various media, and close study of them showed that they were identical in all respects. The production of sexual organs, however, on the media employed was extremely limited. Cases were found where the antheridium was undoubtedly paragynous, and certain others occurred in the same cultures in which this organ appeared to be amphigynous, but absolute certainty on the matter could not be arrived at.

In order to clear up this important point it was necessary to make cultures on a medium in which the sexual organs were produced in greater abundance. For this purpose cylinders of tissue, cut with a sterile cork-borer from a healthy raw apple under aseptic conditions, were placed in sterile plugged test tubes. One-half of the number of cylinders was inoculated from a sub-culture of one of the nine stocks alluded to above; the other cylinders were left uninoculated to serve as controls.

After the lapse of about a month the cylinders were examined. The controls had remained sound, and no trace of fungus or other growth could be discovered on or in them when examined microscopically. The inoculated cylinders had rotted and become brown, while traces of aerial mycelium were visible on them. Microscopical investigation revealed the presence of no sporangia, but sexual organs were present in abundance. The antheridia in the vast majority of cases were paragynous; but in two instances, at least, antheridia undoubtedly of the amphigynous type were present in the same culture along with them. A photograph of one of these is reproduced in fig. 6, Pl. II.

It was thus fairly clear that only a single fungus was responsible for the apple rot, and that this fungus produced both paragynous and amphigynous antheridia.

(*b*) *Isolation from a single sporangium.*—The fungus does not produce sporangia at all abundantly, but in an old growth on oat-extract agar, derived from a piece of tissue removed aseptically from one of the original decaying apples, a few were found. The culture was in a Petri dish, and at the time was not free from bacterial contamination. In the tissue from which the growth had emanated sexual organs of both types were present.

Under microscopical control six isolated sporangia were successfully removed, one at a time, by means of sterile capillary tubes, and each was washed in about twenty changes of sterile water to remove bacterial contamination as far as possible.

On a healthy apple, and with aseptic precautions, six small, well-isolated cut surfaces were prepared, and on each a single sporangium was placed. A similarly prepared healthy apple, on which no sporangia were placed, was used as a control, and both fruits were kept in a moist atmosphere under the same bell-jar. Within sixteen days a rot of the first apple had started at three out of the six places where sporangia had been introduced. No rot occurred at the other three places, the sporangia in these cases being apparently not viable. The uninoculated apple remained perfectly sound.

Before the rot had extended from the three centres sufficiently to become coalescent, several small portions of the affected tissues were removed from one of the centres to set media in Petri dishes, aseptic precautions being observed as before. After a few days mycelium grew into the media from the pieces of tissue, and in each case the growth was found to be free from bacterial contamination. From these growths—each the product of a single sporangium—sub-cultures in test tubes were prepared.

At a later stage the pieces of tissue on the Petri dishes were examined, and

sexual organs with both para- and amphigynous antheridia were found in them, the latter being decidedly in the minority. One of each type, isolated from this material, is illustrated in figs. 3 and 4, Plate II.

(*c*) *Isolation from a single oospore.*—Small portions of the affected tissues which had been removed from one of the original diseased apples to solidified media in Petri dishes were macerated in water, and sexual organs having antheridia of the amphigynous type, containing apparently ripe and viable oospores, were sought out under the microscope. Several were found and were removed by capillary tubes, one at a time, to films of oat-extract agar on cover-glasses, which were then inverted over excavated microscope slides in the usual way. Before removal, great care was taken to ensure that no living hyphae remained attached either mechanically or otherwise to the oogonia and antheridia. Each slide thus prepared contained a single oospore; but the films, although quite free from any other fungus, were not altogether free from bacteria.

The slides containing the oospores were kept under close microscopical observation. In the majority of instances the oospores either remained in a resting condition or germinated and produced short germ tubes which soon died. In one case, however, greater success was achieved. The inner portion of the thick wall of the oospore was observed to dissolve in a manner similar to that which occurs during the germination of the oospores of *Phytophthora erythroseptica*. A germ-tube then arose which passed through the wall of the oogonium, grew for a short distance, and then developed a sporangium at its distal end. This sporangium produced a number of zoospores which, after actively swimming about for some time, came to rest, developed thin walls, and proceeded to form germ-tubes.

At this stage the cover-slip containing the film culture was carefully inserted into a slit made in the flesh of a healthy apple, which was then kept for thirteen days in a moist atmosphere at laboratory temperature. At the end of this time a rot had developed in the apple, starting from the film side of the cover-glass, on which the germinating zoospores were originally present.

After seventeen days portions of the rotted tissues were removed (aseptic precautions being still maintained) and were placed on solidified oat-extract agar in Petri dishes, and from the growths which arose in the agar medium sub-cultures were prepared. No sexual organs or sporangia had developed in the growths at the time the sub-cultures were made; but microscopical examination of the portions of tissue themselves revealed the presence in them of sexual organs with both amphi- (Plate II, fig. 5) and paragynous antheridia.

From the foregoing it will be seen that pure cultures were raised by three different methods, and that in all cases a single fungus was derived which produced sexual organs, having both amphi- and paragynous antheridia. It might be maintained that the use of raw apple tissue in the course of the work left the question as to the absolute certainty of the results in some doubt; and, in order to obviate this, sterilised carrot tissue was subsequently employed. The results obtained were the same as before.

As regards pathogenicity, the fungus in pure culture has repeatedly been inoculated into healthy apples, and always with the same result, viz., the production of a characteristic form of rot, similar to that exhibited by the original naturally infected fruits. In each case, where the attempt was made, the fungus was re-isolated in pure culture from the artificially infected apples. Non-inoculated controls, kept under similar conditions, remained unaffected in every case.

In the diseased tissues of both naturally and artificially affected apples sexual organs occur, but not abundantly. When small portions of such tissue, however,

are removed to media, or even to sterilised moist filter paper in Petri dishes, sexual organs are produced within the tissues in much greater abundance. Possibly this may be the result of an increased supply of oxygen; but the matter was not further investigated.

The fungus is also pathogenic to pears. When inoculated into unripe fruits a firm brown rot quickly develops. On keeping them in a moist atmosphere tufts of mycelium, sometimes bearing sexual organs, but not, so far as was observed, sporangia, arise from some of the lenticels. Sexual organs are also developed within the rotting tissue.

As regards potato tubers, repeated inoculations invariably gave negative results; hence the fungus is not pathogenic to the potato.

III.—*Cultural Characteristics and Morphology of the Fungus.*

The fungus has been cultivated on a number of media, parallel cultures having been made with stocks raised by the various methods already described. On a given medium, as was to be expected, the behaviour of the fungus has always been the same, regardless of the particular method by which it was isolated.

On *Quaker Oat* agar the growth was submerged, creeping, or very slightly raised above the surface of the medium. Sporangia were few, and occurred in small localized tufts on long and rather ill-defined sporangiophores. Oospore production was very scanty, or even entirely suppressed. The oogonial wall was yellow where embedded in the substratum, but hyaline when formed on or above its surface. Yellow-walled oogonia generally imparted a faint tinge of yellow to the walls of the oospores within them.

On *oat-extract* agar the growth was sparse, creeping, or submerged. Sporangia were few, and sexual organs absent. On this medium the mycelium, when examined microscopically, had a characteristic appearance. Towards the centre of the growth the hyphae were distinctly tuberculate, with numerous short lateral branches; while at the margins each radiating hypha branched copiously, producing a dense fan-like growth.

Cooked prune-extract and *cooked apple-extract* agars proved to be unfavourable media for the fungus. The limited growth on each was submerged or raised slightly above the surface. Sporangia were absent, and sexual organs almost completely so.

On *cooked potato stalk* and *cooked potato plugs* the fungus was completely sterile and confined to the tissues, while on *cooked potato agar* a very sparse and sterile growth was produced, which resembled that on oat-extract agar.

Cooked or raw apple plugs and *cooked carrot plugs* were the media on which the best production of oospores was obtained; but on none of these media were sporangia observed.

The sporangia, which, as has already been stated, are produced only sparingly by this fungus, are borne on long hyphae which branch in a sympodial manner, typical of the genus Phytophthora. Each sporangium is spherical when young, but becomes inversely pyriform as it matures. The wall is thin, except at the narrower free end, where it is thickened, but distinctly hyaline. The free end is blunt, and not papillate; whilst at the basal end of the detached sporangium there remains a small portion of the parent hypha on which it was borne. In size the sporangia varied greatly, but were found to average 40μ long by 27μ broad. A single normal sporangium is illustrated on Plate I, fig. 15, and Plate II, fig. 16.

When mature sporangia were placed in tap-water, a certain number of them

liberated their zoospores after twenty minutes. The broad, apical, hyaline, thickened portion of the wall became suddenly stretched and expanded to form a sac-like structure, while the zoospores rushed out in mass, partially filling it. Almost simultaneously the thin wall of the sac became ruptured, allowing the zoospores to escape, whilst the wall itself quickly became unrecognizable. The zoospores, which are typical of the genus Phytophthora, are lemon-shaped, and each is provided with two cilia. After some time the zoospores came to rest, rounded themselves off, and each produced a single germ tube. Individual sporangia were seen to produce from four to twenty-six zoospores; and the openings at the ends of the empty sporangia averaged 9μ in breadth.

In water a few sporangia produced a single germ-tube each, which generally emanated from a point a little to one side of the hyaline apical region. In many cases the germ-tube, after growing for a distance equal to the length of the parent sporangium, developed at its extremity a second sporangium, which, in turn, germinated by producing a germ-tube, and, as before, a terminal sporangium. In this way short chains of sporangia, gradually diminishing in size, were very frequently formed; and all, with the exception of the terminal one, were devoid of contents.

The sexual organs.—Specimens with paragynous antheridia resemble in shape similar organs figured from time to time for species of the Cactorum group. The oogonia are pear-shaped, and arise as terminal swellings on rather short lateral hyphae. The wall of the oogonium is thin compared with that of the oospore, but considerably thicker than that of the hypha on which it is borne. The average diameter of oogonia of this type from pure cultures of the fungus on artificial media was found to be 28μ.

The antheridia are small, irregularly shaped terminal swellings on short hyphae, which spring from the parent oogonial hypha or from a neighbouring one. Antheridia were occasionally observed with one or more short, finger-like, hyphal outgrowths, which gave them a false appearance of being intercalary in origin.

In the early stages of sexual reproduction an antheridium and a developing oogonium meet; the former becomes firmly attached to the wall of the latter at any point on its surface, but perhaps most frequently somewhere in the region of the oogonial stalk. During fertilization only a portion of the contents of the antheridium passes into the oosphere.

The oospore, which partially or almost completely fills the oogonium, is generally hyaline, spherical, thick-walled, and filled with protoplasmic contents, in which are to be seen a large central oil-drop and a smaller highly refractive body. Oospores from sexual organs of this type were found to average $25{\cdot}4\mu$ in diameter, and their walls varied from $1{\cdot}5\mu$ to 2μ in thickness.

The sexual organs of the second type develop in a similar manner to that described for the first time in *P. erythroseptica*.[1] The oogonial incept grows through the antheridium, and on emergence swells rapidly. The protoplasmic contents gradually contract to form an oosphere, and, after fertilization, an oospore. The average diameters of oogonia and oospores from sexual organs of this type were found to be $27{\cdot}3\mu$ and 25μ respectively, and the walls of the oospores varied from $1{\cdot}5\mu$ to 2μ in thickness.

From the characters described, and as a result of the cultural studies which will be dealt with in the following section of this paper, the fungus was ultimately recognized as being identical with *Phytophthora Syringae* Klebahn.

[1] Sci. Proc. Roy. Dublin Soc., xiii (N.S.), No. 35, 1913, p. 529.

IV.—*Previous Work on similar Types of Rot.*

During recent years several cases of Phytophthora attack on apples as well as on pears have been recorded both in Europe and in America. Thus, Osterwalder[1] described one on apples in Switzerland in 1904; Marchal,[2] Bubák,[3] Unamuno,[4] and Schoevers[5] reported attacks on pears in Belgium, Bohemia, Asturia, and Holland respectively at various times from 1908 to 1915; Whetzel and Rosenbaum[6] recorded an attack on apples in America in 1916; Wormald[7] dealt with attacks on pears and apples in England in 1919; Güssow[8] noted an attack on pears in Nova Scotia in 1920, while in the same year Clinton[9] gave particulars of attacks on both apples and pears in Connecticut, U.S.A.

In all these instances the rot was attributed either to *Phytophthora Cactorum* (Leb. et Cohn) Schröt. or to its synonym *P. omnivora* de Bary,[10] but in no case was any intensive study of the sexual organs of the fungus made, nor was the existence of two kinds of antheridia suspected or discovered.

At the outset it appeared possible that the rot in the Irish apples was caused by a new species of Phytophthora, because neither in *P. Cactorum* nor in any other species of Phytophthora hitherto described had the production of two kinds of antheridia in one and the same species been noted. On the other hand, it was not impossible that antheridia of the two types really did occur in *P. Cactorum*, and perhaps in other species of the genus, but that the fact had merely been overlooked or not apprehended by previous workers, owing to the comparative scarcity of cases in which the antheridia are amphigynous as compared with those in which they are paragynous.

This latter surmise was strengthened as a result of a consideration of the relevant literature. Thus, Hartig,[11] in his description of the development of the oospore in *P. Fagi*, states that in exceptional cases the stalk of the oogonium swells out directly under the oogonium itself to form the antheridium, and his figure of this condition (Taf. III, fig. 24b) suggests very strongly that this was an instance in which the antheridium may have been amphigynous. Butler and Kulkarni,[12] after referring to Hartig's figure and to the statement of Himmelbaur,[13] that in *P. Fagi* the antheridium is attached to the underside of the oogonium near its base, says: "It appears probable that a penetration of the antheridium by the oogonial stalk sometimes occurs in this species."

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[1] Centralb. f. Bakt., ii, xv, 1906, p. 435.
[2] Bull. Soc. Roy. de Belgique, xlv, 1908, p. 343.
[3] Zeitschr. f. Pflanzenkrankheiten, xx, 1910, p. 257.
[4] Zeitschr. f. Pflanzenkrankheiten, xxi, 1911, p. 379 (abstract).
[5] Tijdschr. over Plantenziekten, xxi, 1915, p. 153.
[6] Phytopathology, vi, 1916, p. 89.
[7] Ann. App. Biology, vi, 1919, p. 89.
[8] Phytopathology, x, 1920, p. 50.
[9] Conn. Agric. Expt. Station, Bull. 222, 1920, p. 406 and p. 454.
[10] It will be remembered that de Bary in 1881 assembled Schenk's *Peronospora Sempervivi* and Hartig's *Peronospora Fagi* under the new name *Phytophthora omnivora*; and, later in the same year, also included Lebert and Cohn's *Peronospora Cactorum* under the same name. (Beitr. z. Morph. u. Phys. d. Pilze, iv, 1881, and Bot. Zeit., xxxix, 1881.) Himmelbaur, however, has shown that *P. Fagi* and *P. Cactorum* are dissimilar (Jahrb. Hamb. Wiss. Anst., xxviii, 1910), and for this reason as well as on priority grounds the combination *P. omnivora* should no longer be used.
[11] Untersuch. forstbot. Inst. München, i, 1880, p. 49.
[12] Mem. Dept. Agric., India, Bot. Series, v, 1913, p. 257.
[13] Jahrb. Hamburg Wissensch. Anstalten, xxviii, 1910, p. 450.

Again, some of the figures illustrating de Bary's[1] own account of *P. omnivora*, especially figs. 25, 26, and 27 on Taf. III (concerning which it is stated that the point of origin of the oogonium was hidden by the antheridium), are suggestive, at least, of cases where the antheridia may have been amphigynous.

Rosenbaum,[2] in dealing with *P. Cactorum* isolated from diseased Ginseng, figures the sexual organs with paragynous antheridia only; but he states that "in some cases the stalks bearing the antheridium and oogonium are on the same side, and the antheridium then falls on the oogonial stalk. Under the microscope, such a condition may present the appearance that the oogonium has grown through the antheridium." It seems quite likely that the "appearance" in the cases noted may, indeed, also have been the reality.

In view of the uncertainty which appeared to exist in the matter, it was considered desirable to make renewed studies of *P. Cactorum* and *P. Fagi*, and to compare the fungus isolated from the Irish rotted apples with these species, and also with others isolated from decayed apples and pears in other countries, so far as it was possible to obtain them. Later on it was found necessary to re-examine *P. Syringae*.

As soon as it had been established clearly that the two types of sexual organs found in the Irish apples were produced by one and the same fungus, the attention of mycologists was called to the matter in a letter to "Nature,"[3] and an appeal was made for cultures of species of Phytophthora from other workers.

From Dr. W. F. Bewley, of the Experimental and Research Station, Cheshunt, Herts, a culture was received provisionally regarded by him as one of *P. Cactorum*. The fungus had been isolated from a rotten apple purchased in Cheshunt. Professor H. H. Whetzel was good enough to send a culture of what he regarded as *P. Cactorum*, isolated from a decayed apple grown in his own garden in Cornell University, Ithaca, New York State, U.S.A. Dr. A. Osterwalder kindly sent from the Swiss Research Station for Fruit, Vine, and Garden Cultivation in Wädenswil a pear affected with a Phytophthora-rot, from which what appeared to be *P. Cactorum* was isolated without difficulty. Cultures of *P. Cactorum* and *P. Fagi* (stated to have been isolated by Peters from Cactus and *Fagus sylvatica* seedlings respectively) and one of *P. Syringae* (originally isolated in Hamburg by Klebahn from *Syringa vulgaris*) were obtained from the Centraalbureau voor Schimmelcultures in Baarn, Holland, through the kindness of Dr. J. Westerdijk.

Including the species isolated from the Irish apples, there were thus seven cultures of allied Phytophthoras, each from a different source; and these formed the basis of a series of sub-cultures on various media, which were studied in considerable detail. Limitations of space preclude the description *in extenso* of this comparative cultural work, and only the most important results can be dealt with here.

In the first place, it may be stated that each of these fungi, when inoculated through wounds into healthy apples and pears, was found to be capable of setting up in them a characteristic form of brown rot, similar to that described in the present communication. Apart from minor variations, such as the number and size of the tufts of hyphae protruding through the lenticels, and so on, these forms of rot were indistinguishable from one another by the naked eye.

Secondly, it was found that each of the fungi produced sexual organs of the

[1] Beitr. z. Morph. u. Phys. der Pilze, iv, Frankfurt a. M., 1881, p. 22.
[2] N.Y. State Coll. Agric. Cornell Univ., Bull. 363, 1915, p. 100.
[3] Vol. cvii, No. 2685, April 14th, 1921, p. 204.

two types already described; and in all cases those with amphigynous antheridia were very much rarer than those in which the antheridia were paragynous. Illustrations of both types of these organs for the Phytophthoras under consideration are provided in Pl. I, figs. 1–14, and Pl. II, figs. 1–12.

Further, in all cases except *P. Syringae* and the Irish fungus, a second form of asexual reproductive body was found in varying abundance, which had not hitherto been described for *P. Cactorum* or *P. Fagi*. With regard to the latter fungus, these bodies were not found in cultures on sterilised media, but only on cultures on living apples.

These bodies, which may provisionally be called *sphaero-conidia*, are usually intercalary in origin, although they may sometimes be terminal. The hyphae on which they are borne are generally somewhat stouter than those which bear sporangia. They are, as a rule, uniformly spherical in shape; but, in rare instances, they develop a papilla resembling that found in a sporangium. Their thin walls give the same micro-chemical reactions as those given by the sporangial walls. In diameter they vary from 33μ to 40μ, and there is a tendency for them to be slightly larger when produced on living apple than on sterilised media, such as Quaker Oat agar, &c. Not infrequently short, slender hyphal outgrowths proceed from them, which, however, must not be confounded with germ tubes. Illustrations of sphaero-conidia are reproduced in fig. 13, Pl. II.

In hanging drops of water these sphaero-conidia have been found to produce germ tubes, which may arise at any point on their surfaces. After growing a short distance a germ tube may cease to grow, or may give rise to a terminal second sphaero-conidium or to a normal sporangium. It is possible that the sphaero-conidia possessing papillae may germinate by means of zoospores; but up to the present only the germination of the much more abundant non-papillate forms has been observed.

"Resting" conidia and chlamydospores have been described as occurring in *Phytophthora Arecae* (Colem.), *P. Colocasiae* But. & Kul., *P. Faberi* Maubl., *P. jatrophae* Jens., *P. Meadii* M'Rae, *P. Nicotianae* de H., *P. parasitica* Dast., *P. terrestria* Sherb., and *P. Theobromae* Colem. In some cases they are thick-walled, and are perhaps true chlamydospores, or possibly parthenogenetic oospores. In others the walls are not so thick, and germination is sometimes by zoospores. The relation of these bodies to one another and to those described here for *P. Cactorum* and *P. Fagi* require further investigation.

The sporangia of the English, American, and Swiss Phytophthoras, as well as those of *P. Cactorum* and *P. Fagi*, resembled one another in being always papillate, the papilla being, in reality, a hyaline thickening of the sporangial wall, and forming a distinct projection or protuberance. (See Pl. I, fig. 16, and Pl. II, figs. 14 and 15.) In the Irish fungus and in *P. Syringae*, on the other hand, although the wall at the distal end of the sporangium is thickened and hyaline, yet there is no distinct papilla, the end being flattened or rounded.

There was considerable variation in the size of the sporangia. In the case of the four first-mentioned fungi they averaged 37μ × 27μ on artificial media, such as Quaker oat agar, &c.; but they were considerably larger, viz. 52μ × 30μ, on raw apple tissue. In the case of *P. Fagi* they averaged 45μ × 31μ on artificial media and 56μ × 31μ on raw apple tissue. In *P. Syringae* and in the Irish fungus they measured 38μ × 26μ and 40μ × 27μ respectively on artificial media, but they were not abundant enough on raw apple tissue to permit average measurements being made.

Too much stress, of course, must not be laid on the sizes of sporangia as a

diagnostic character; but it was clear that, on the average, the sporangia of the English, American, and Swiss fungi and of *P. Cactorum* were considerably smaller than those of *P. Fagi*, whilst those of the Irish fungus and *P. Syringae* were intermediate in size.

There was also considerable variation in the abundance of sporangium formation; but it was clear throughout the work that the Irish fungus and *P. Syringae* formed sporangia on the various media tried with considerably more reluctance than the five others did.

Having regard to all the characters shown in cultural and inoculation trials, it was found impossible to distinguish between the English, American, and Swiss Phytophthoras and the *P. Cactorum* stated to have been isolated by Peters from Cactus seedlings; and it is believed that the authors mentioned, who described the rotting of apples and pears in the instances coming under their notice as being due to *P. Cactorum*, were perfectly correct in so doing.

That *P. Fagi* and *P. Cactorum* should not be grouped together under the name *P. omnivora* has already been pointed out by Himmelbaur.[1] The present investigations confirm this view. The sporangia of *P. Fagi* were found on the average to be distinctly longer than those of *P. Cactorum*. Moreover, *P. Fagi* was found incapable of infecting living specimens of *Sempervivum arboreum-rubrum*, *S. Berthelotianum*, and a species of Mammilaria, whilst *P. Cactorum* was pathogenetic to, and caused a rot in these plants.

As the cultural work progressed it became clear that the Irish fungus was neither *P. Cactorum* nor *P. Fagi*. On the other hand, it seemed to be closely allied to, if not identical with, *P. Syringae*. Hence, careful parallel cultures and inoculation experiments with the latter species and the Irish fungus were made, with the result that no essential differences could be found between them.

The rot produced by inoculating *P. Syringae* into healthy apples was similar to that originally found in the Irish apples. Further, the Irish fungus and *P. Syringae* when inoculated into buds of *Syringa vulgaris* caused identical forms of rot; and oospores of identical character were developed in the dead tissues in both cases. Hence it is concluded that the Phytophthora which caused the rot in the Irish apples is *P. Syringae*; and it is believed that this is the first record of attack by this species on apples, at any rate in Ireland.[2]

V.—*Classification of Phytophthoras.*

The penetration of the antheridium by the oogonial incept and the development of the oogonium proper on the summit of the antheridium in Phytophthoras were first established with certainty in the case of *P. erythroseptica*.[3] At the same time, this method of development of the sexual organs was found to occur in *P. infestans* and *P. Phascoli*, while it was surmised (correctly, as has since been shown by Rosenbaum) to occur also in *P. Arecae*. In the paper referred to, and in

[1] See foot-note, p. 35.

[2] There is some reason to believe that *P. Syringae* occurs in Ireland on *Syringa vulgaris*. On two occasions within the past two years diseased Lilac leaves have been forwarded for examination on which a Phytophthora was present which, there was every reason to suspect, was *P. Syringae*; but the material submitted was not sufficient or suitable for raising cultures and thus determining the species with certainty. Rosenbaum's statement (Journ. Agric. Research, viii, 1917, p. 235), that he worked with *P. Syringae* isolated from Lilac in Ireland, is an error. Nor was his culture of *P. Fagi* derived from Fagus seedlings in Ireland.

[3] Sci. Proc. Roy. Dublin Soc., xiii (N. S.), No. 35, 1913, p. 529.

one[1] published in the following year, the various species of Phytophthora which had been described up to that time (numbering fourteen, or possibly fifteen) were enumerated. Since then the following seven species have been recorded :—

P. fici Rau[2]
P. citri Rau[2]
P. Allii Saw.[3]
P. Melongenae Saw.[3]
P. terrestria Sherb.[4]
P. Meadii McRae[5]
P. cryptogea Pethyb. and Laff.[6]

—whilst what may possibly turn out to be three new species have been reported as attacking Paeony, Rhubarb, and Oats respectively in the United States of America,[7] and another unidentified species has been found by Brittlebank, causing a disease of *Papaver nudicaule*, in Australia.[8] Thus, there would now appear to be some twenty-two species; but further study of them will probably result in a slight reduction of this number, since some of them, like *P. Theobromae* and *P. Faberi*, *P. parasitica* and *P. terrestria*, *P. Nicotianae* and *P. jatrophae*, are probably identical.[9]

When amphigyny was first discovered, it was proposed that the genus Phytophthora should be divided into two. The generic name Phytophthora was to be retained only for those species with amphigynous antheridia, with *P. infestans* as type; while those in which the antheridia are lateral were to be placed in a genus for which the name Nozemia was suggested, with *N. Cactorum* as type, it being assumed, of course, that in a given species only one and the same type of antheridium would be present. The Cactorum group then included *Nozemia Cactorum*, *N. Fagi*, *N. Syringae*, and *N. Nicotianae*. But it is clear from the work described in the present paper that the first three of these species cannot well remain in it. Having both amphigynous and paragynous antheridia, they constitute an intermediate group linking Phytophthora with Nozemia.

On the removal of these three species the sole representative of the latter genus would now be *N. Nicotianae*. It is true that de Haan's description and illustrations of the morphology of the sexual organs in this species clearly show that the antheridia are paragynous; nevertheless, in view of the discovery of cases of amphigyny in the other three species, it seems not at all improbable that examples of this condition might be found to occur also in *N. Nicotianae*; and further investigations on this species are highly desirable. For pure culture work, however, no medium has yet been found on which this species produces its sexual organs with certainty or in abundance; and the point cannot therefore at the moment be cleared up. Furthermore, renewed investigations directed to this special end might eventually show, as regards those Phytophthoras in which the antheridia are, so far as is known at present, amphigynous, that these organs may occasionally be paragynous.

In view of these and other considerations arrived at as a result of more extended research, it is now thought better that the recently erected genus

[1] Journ. Econ. Biol., ix, No. 2, 1914, p. 53. [2] Journ. Bombay Nat. Hist. Soc., xxiv, p. 615.
[3] Mycologia, ix, No. 4, 1917, p. 249. [4] Phytopathology, vii, No. 2, 1917, p. 119.
[5] Mem. Dept. Agric. India Bot., Series ix, No. 5, 1918, p. 219.
[6] Sci. Proc. Roy. Dublin Soc., xv (N.S.), No. 35, 1919, p. 487. It is of interest to note that when this species was described its sexual organs were known only from pure cultures. Since that time, however, the authors have found them in the decayed tissues of the host plant.
[7] Science, N.S., liv, 1921, p. 170; Phytopathology, xi, 1921, p. 55; and Science xliii, 1916, p. 534.
[8] Journ. Dept. Agric. Victoria, xvii, Pt. 2, 1919, p. 700.
[9] While the present paper was in the press a reference to Ashby's *P. palmivora* was noted. We have not yet seen the paper in which it is described. (West Indian Bull., xviii, No. 1 1920, p. 61.)

Nozemia should be abandoned. The name has as yet scarcely had time to become definitely established in the literature; and there need be therefore the less reluctance to revert to former usage, and to unite all the species in the one genus Phytophthora.

Based on the mode of development of their sexual organs, the species contained in the genus may now be grouped as follows :—

A.—*Species in which, so far as is known at present, the antheridia when present are always amphigynous*:—

1. *P. infestans* (Mont.) de Bary
2. *P. Phaseoli* Thaxt.
3. *P. Colocasiae* Racib.
4. *P. Arecae* (Colem.).
5. *P. erythroseptica* Pethyb.
6. *P. parasitica* Dastur
7. *P. terrestria* Sherb.
8. *P. Allii* Saw.
9. *P. Melongenae* Saw.
10. *P. Meadii* McRae
11. *P. cryptogea* Pethyb. and Laff.

B.—*Species in which the antheridia are preponderatingly paragynous, but are sometimes amphigynous*:—

12. *P. Cactorum* (L. and C.) Schroet.
13. *P. Fagi* Hartig
14. *P. Syringae* Klebahn

C.—*Species in which, so far as is known at present, the antheridia are always paragynous*:—

15. *P. Nicotianae* de Haan

D.—*Species in which the mode of development of the sexual organs is not fully known, or in which these organs have not yet been found*:—

16. *P. Thalictri* Wilson and Davis
17. *P. agaves* Vill. (?)
18. *P. Faberi* Maubl.
19. *P. Theobromae* Colem.
20. *P. jatrophae* Jens.
21. *P. fici* Rau
22. *P. citri* Rau

P. Thalictri is probably closely allied to *P. Phaseoli*, and may ultimately be found to belong to Group A. Gandara[1] states that a Phytophthora, specified by Villada as *P. agaves*, causes a disease of Agave in certain parts of Mexico; but we have not succeeded in finding any description of this species. *P. Faberi* and *P. Theobromae* are probably synonymous. According to Coleman, the latter species is closely allied to *P. Arecae*; if this be really so, it should be placed in Group A; but antheridia are absent, or are only rarely formed; and it is not certain whether they are amphigynous or paragynous. *P. jatrophae* has been issued in culture form, but apparently has not been described; it may be identical with *P. Nicotianae*. *P. fici* and *P. citri* were named provisionally in 1915; but we are officially informed that nothing further has yet been published on them.

[1] Mem. y. Rev. Soc. Cient. Antonio Alzale, xxv, 1908-9, p. 293.

VI.—*Practical Considerations.*

There is no reason to suppose that this particular form of apple-rot is likely to become a serious menace to fruit-growers in this country. It is not known how or from what source the fruits became infected. In other cases of Phytophthora-rot of apples, however, it has been observed that the affected fruit was confined to the lower branches of the trees near the ground; and it is believed that infection came from the soil as a result of rain splashes, &c. In such cases judicious propping up of the hanging branches would probably suffice to prevent infection. In some cases, too, the rot has been recorded as being particularly prevalent in fallen apples; but whether infection took place before or after falling is not clear.

Seeing that Osterwalder found *P. Cactorum* (*omnivora*) causing a die-back of apple-shoots in Switzerland, a careful search was made amongst the trees in the orchard at Pilltown, Co. Kilkenny, from which the Irish apples came; but no trace of any such injury was to be found there. Further, an attempt made to infect apple twigs through wounds in the bark with a pure culture of the Irish fungus was not successful.

Since oospores of the fungus are formed in the tissues of the rotted apples, it is clear that such sources of re-infection for the following season should not be allowed to remain in the orchard. From a general hygienic point of view, as well as in regard to this particular fungus pest, all rotted fruit should be collected and suitably destroyed.

We desire to express our indebtedness to the persons named in the text of this paper who were good enough to furnish us with material for comparative study, and also to Sir Frederick W. Moore, of the Royal Botanic Gardens, Glasnevin, who kindly placed at our disposal Cereus, Cactus, and other plants for inoculation purposes. We also desire to record our indebtedness to the Imperial Bureau of Mycology in Kew, and to Dr. H. M. Quanjer, Wageningen, for assistance in the matter of literature.

VII.—*Summary.*

The present paper deals with a rot occurring in apples in Ireland which was found to be caused by a species of Phytophthora which proved to be *P. Syringae* Klebahn, and not *P. Cactorum* Schroet., a species that has been recorded several times as the cause of a similar rot in apples and pears in other countries.

The causative fungus was isolated and studied in detail, in pure culture. It was found to produce both paragynous and amphigynous antheridia, but the latter are formed in relatively quite small numbers.

Renewed studies of *P. Cactorum* Schroet. and *P. Fagi* Hartig revealed the fact that in these species also amphigynous antheridia occasionally occur, though they are much rarer than paragynous antheridia.

Apart from those species in which the sexual organs are imperfectly known or have not been discovered, *P. Nicotianae* de Haan is the only species of this genus in which the antheridia, so far as is known at present, are exclusively paragynous.

It is suggested that renewed investigation of this species might lead to the discovery of some medium on which its sexual organs would be developed in abundance, and it is thought that amongst them examples with amphigynous antheridia would possibly be found. There is also a possibility that some of the species of Phytophthora which apparently have amphigynous antheridia only may yet be found to produce paragynous antheridia occasionally.

It is proposed, therefore, to discard the generic name Nozemia, suggested a few years ago for those species having paragynous antheridia only, and to re-unite in the one genus Phytophthora all the species hitherto described under that name, irrespective of the types of antheridium which prevail in them.

A classified list of the twenty-two species of Phytophthora recorded up to the present, with brief notes thereon, is furnished.

The economic significance of the rot does not appear to be great. Supporting heavily laden, drooping branches with props and attention to orchard hygiene are suggested as preventive measures.

EXPLANATION OF PLATES.

Plate I.

All drawings were made with the aid of a camera lucida at a time when the oospores were practically ripe, and all are magnified 645 diameters.

Fig.

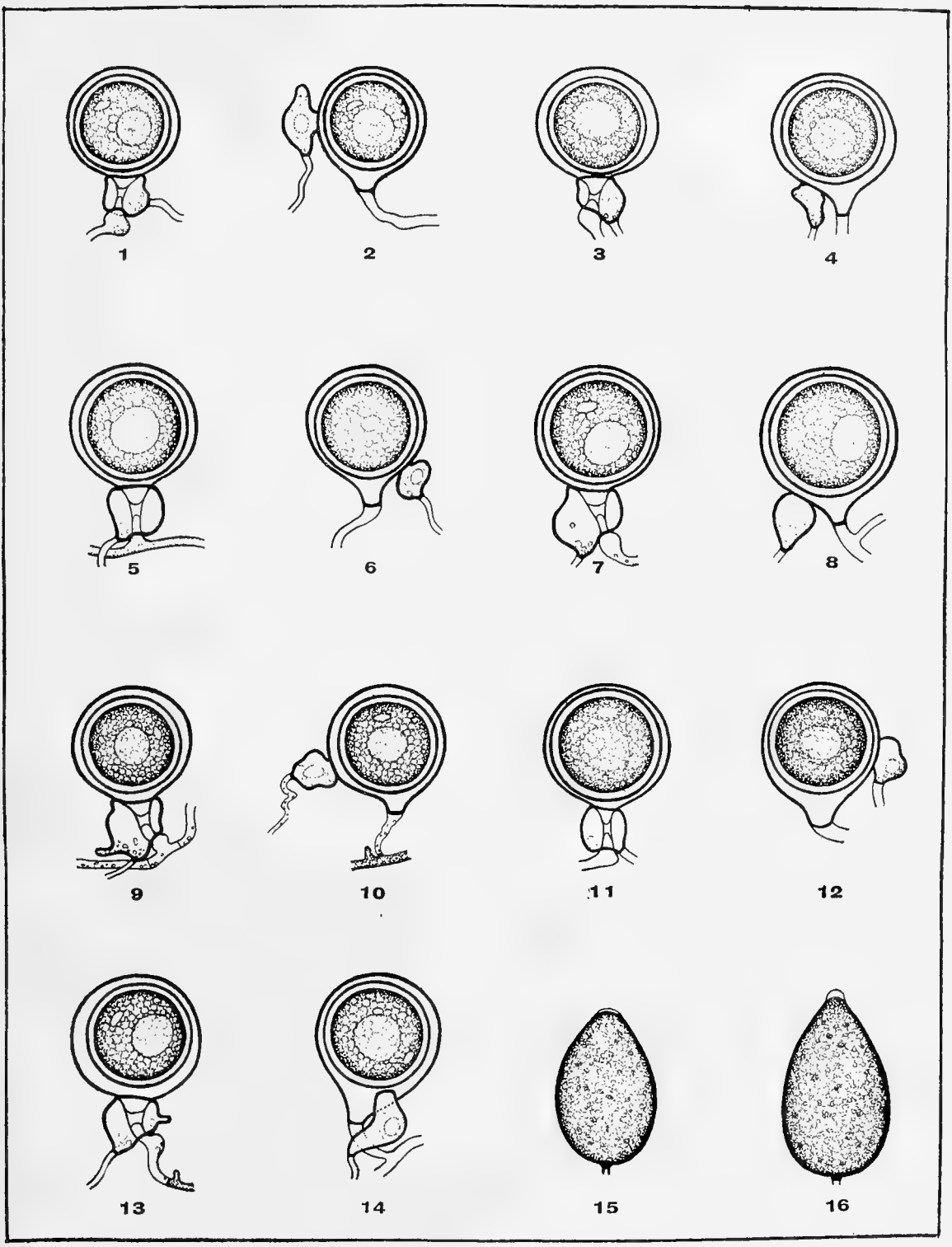
1. Sexual organs of *P. Syringae* in which the antheridium is amphigynous; from a naturally rotted apple.
2. The same as 1, from the same source, but with the much more common paragynous antheridium.
3. Sexual organs with amphigynous antheridium of *P. Syringae*, originally isolated from diseased Lilac in Germany.
4. The same as 3, from the same pure culture, but with paragynous antheridium.
5. Sexual organs of *P. Cactorum* with amphigynous antheridium, from culture of Whetzel's organism isolated from diseased apple in America.
6. The same as 5, from the same pure culture, but with paragynous antheridium.
7. Sexual organs of *P. Cactorum* with amphigynous antheridium, from culture of organism isolated by Bewley from diseased apple in England.
8. The same as 7, from the same pure culture, but with paragynous antheridium.
9. Sexual organs of *P. Cactorum* with amphigynous antheridium, from culture of organism isolated from diseased pear, received from Osterwalder (Switzerland).
10. The same as 9, from the same pure culture, but with paragynous antheridium.
11. Sexual organs of *P. Cactorum* with amphigynous antheridium, from culture stated to have been isolated by Peters from Cactus seedlings.
12. The same as 11, from the same pure culture, but with paragynous antheridium.
13. Sexual organs of *P. Fagi* with amphigynous antheridium, from culture stated to have been isolated by Peters from seedlings of *Fagus sylvatica*.
14. The same as 13, from the same pure culture, but with paragynous antheridium.
15. A sporangium of *P. Syringae* from culture from Irish apple, showing the flattened apex.
16. A sporangium of *P. Cactorum* from tuft of mycelium on apple, showing papillate apex.

Plate II.

All figures reproduced from untouched negatives. Fig. 13 magnified 250; fig. 14, 300; the remaining figures 720 diameters.

Fig.

1. Sexual organs of *P. Syringae* from naturally rotted apple tissue. The antheridium is paragynous and the oospore not yet ripe.
2. From the same source as 1. The antheridium is amphigynous and the oospore is ripe.
3. Sexual organs of *P. Syringae* from an apple previously inoculated with a single sporangium from a culture from the original rotted Irish apple. The antheridium is paragynous and the oospore nearly ripe.
4. Sexual organs of *P. Syringae* from the same source as in 3, but with amphigynous antheridium.
5. Sexual organs of *P. Syringae* from an apple rotted by product of germination of a single oospore derived from the original rotted apple. Antheridium amphigynous. Contents of oospore disorganized by clearing.
6. Oogonium and amphigynous antheridium of *P. Syringae* from single hypha culture from original rotted apple on raw apple cylinder.
7. Sexual organs of *P. Syringae* with amphigynous antheridium from pure culture on Quaker-oat agar originally isolated in Germany from *Syringa vulgaris*.
8. Sexual organs of *P. Cactorum* with amphigynous antheridium on prune agar, from pure culture stated to have been isolated from Cactus seedlings.
9. The same as 8, but fungus originally isolated from rotted apple in America (Whetzel).
10. The same as 8 and 9, but fungus originally isolated from rotted apple in England (Bewley).
11. The same as 8, 9, and 10, but fungus isolated from rotted pear from Switzerland (Osterwalder). Oogonial contents plasmolysed.
12. Sexual organs of *P. Fagi* with amphigynous antheridium, from culture on cooked carrot, original isolation from Cactus seedlings (Peters).
13. "Sphaero-conidia" of *P. Cactorum* isolated by Bewley from rotted apple in England. The lower one has a papilla over the empty portion of the hypha on the left; it also has a hyphal outgrowth above on which the upper intercalary sphaero-conidium is borne.
14. One unripe and two ripe papillate sporangia of *P. Cactorum* from rotted pear. Swellings similar to those seen in *P. infestans* are present on the sporangiophore, where the two lower sporangia are borne.
15. A single sporangium of *P. Cactorum* from rotted pear, showing papilla and swelling of sporangiophore at point of attachment.
16. A single sporangium of *P. Syringae* from Irish rotted apple, showing flattened apex and absence of papilla.



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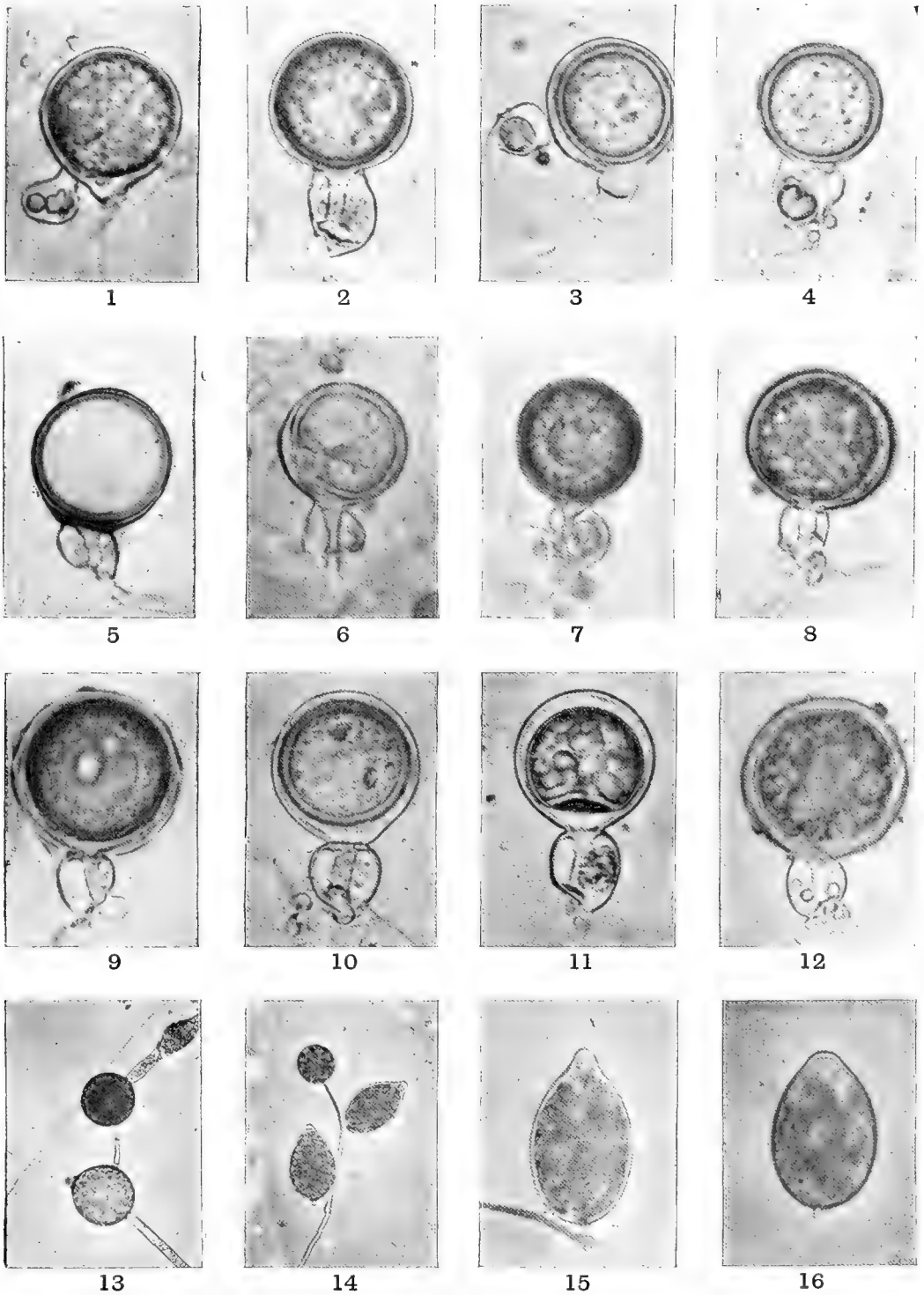

LAFFERTY AND PETHYBRIDGE.

No. 5.

SOME FURTHER NOTES ON THE DISTRIBUTION OF ACTIVITY IN RADIUM THERAPY.

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[Read May 23. Printed June 21, 1922.]

An account has recently been given [Sc. Proc. R. Dub. Soc. N.S. xvi, 35, 1922] of some determinations of the total activity due to the β and γ rays from an emanation tube with various screens. These results were combined with the geometrical law of distance, and in this way, on certain assumptions, the activity to be expected at various depths in the flesh was calculated for a tube enclosed in a serum needle, and also for several arrangements of surface applicators. At the discussion which followed the reading of the paper it was suggested that additional figures were desirable, so as to represent the activity at various points in the neighbourhood of one or more emanation tubes. Further calculations have accordingly been made, the method already given for a single tube being extended to points off the "equatorial plane" of the tube [*i.e.*, the plane perpendicular to the axis of the tube at its middle point], and the effect of the use of multiple tubes worked out for a couple of typical cases.

The results are shown in Tables 1 to 4, the corresponding conditions being given with the respective tables. In order to simplify the printing of the tables, the unit adopted has been changed. In every case the figure in the table may be taken to represent the action per millicurie hour of total dose, the action due to a dose of one millicurie hour concentrated at a point at a distance of one centimetre, without any screening except that due to the glass wall of the tube, being taken as 1000. The unit previously employed referred to a distance of one millimetre, and so was a hundred times as great as the new unit. On the other hand, the old figures referred to the activity of ten millicuries, or the total action produced by ten millicurie hours, so that figures in the new tables are ten times as great as the corresponding figures in the old. For example, the figure for the action on the surface of a serum needle is 6,200 in Table 1, and 620 in the table on p. 475 of the previous paper.

Objection might be taken to the unit adopted, inasmuch as the screening action of the wall of an emanation tube must vary greatly. The unit, however, really refers to one particular tube which was used as a standard. Taking the "bare" activity of this tube as 1,000, the activity at the same distance through 1·8 mm. of brass was 10·0. The action through this thickness of brass, which stops all the β rays, is sensibly independent of the thickness of the tube, and is

the real standard adopted in comparing the various figures. In the case of very thin screens the unavoidable variation in thickness of wall of different emanation tubes would cause appreciable errors, but for brass screens thicker than 0·2 mm. this variation would be unimportant.

In each of the four tables the action is shown at various points on a plane parallel to the tube or tubes. In Tables 1 and 3 this plane passes through the axis of the tube. In Tables 2 and 4 it bisects the rectangle formed by the tubes at right angles. The relations of the various planes and distances involved may, perhaps, be described as follows:—

Suppose that the emanation tube or tubes are placed horizontally in a north and south direction. Tables 1 and 3 refer to short and long single tubes respectively, while Tables 2 and 4 refer respectively to four parallel short tubes and six parallel long tubes, covering in each case a horizontal rectangular area. What has been called the "equatorial plane" is now a vertical east and west plane, passing through the centre of the tube or tubes. The figures in the tables refer to points in a vertical north and south plane, which, in the case of Tables 1 and 3, passes through the axis of the tube, and, in the case of Tables 2 and 4, passes midway between the two central tubes of the area: a in the tables is the vertical height of the given point above or below the tube or tubes; b is the horizontal distance of the given point north or south of the centre of the tube or tubes.

In the case of a single tube [Tables 1 and 3] the distribution is obviously cylindrical round the axis of the tube, so that the action at any point may be found from the tables. It is only necessary to choose as the plane of the table one which passes through the axis of the tube and the required point, and measure a and b in this plane. In Table 2 the effective area covered by the four tubes is approximately square, so that the distribution in the "east and west" or "equatorial" plane is almost identical with that in the "north and south" plane, at distances greater than 5 mm. from the tubes. In Table 4 the effective area covered by the tubes may be taken as 100 mm. × 60 mm., so that 30 mm. east or west of the centre brings us to the edge, whereas 50 mm. is required to do so in the plane of the table. It would appear then that, if we wish to find the distribution in the equatorial plane, no large error would be introduced by taking from the table a value for the action at a point corresponding to a value of b 20 mm. greater than the actual distance east or west of the centre. This would only apply to points at distances of 10 mm. or more from the plane of the tubes.

The figures in the second column of Table 1 correspond to those in column A of the table in the previous paper. The slight discrepancies observable in some cases are due to the use of a slightly different absorption coefficient for γ rays in flesh, as a small discrepancy had occurred between the values used for column A and those employed in columns B, C, and D of the previous table. Results are shown to two significant figures only.

Some examples on the reading of the tables may be of use. Suppose a small tumour is to be treated, and it is decided to use an arrangement of needles similar to that given for Table 2. If the total dose distributed over the four tubes is 50 millicurie hours, the action to be expected would be:—

On the surface of a serum needle						.	.	50 × 1600 =	80,000 units.
At a depth of 1 cm. opposite to centre of tubes								50 × 9·1 =	455 „
„	„	„	„	„	ends	„		50 × 6·9 =	345 „
„	„	5 cms.	„		centre	„		50 × 0·37 =	18·5 „
„	„	„	„	„	ends	„		50 × 0·37 =	18·5 „

If a larger area has to be treated, an arrangement similar to that of Table 4 might be used. Suppose the total dose increased to 300 mc. hrs., the actions would be:—

On the surface of a serum needle 300 × 160 = 48,000 units.
At a depth of 5 cms. opposite centre of area of application 300 × 0·26 = 78 units.
And so on.

For a single dose with a flat surface applicator of 6 sq. cm. area and 3 mm. thick (brass), in contact with the skin, the emanation tubes in the applicator being in contact with the base, and as uniformly distributed as possible, we can use the figures in column B of the table in the last paper, multiplying by 10 to allow for the change of units. Thus, for a dose of 300 mc. hrs. the actions would be:—

On skin at centre of applicator 300 × 12·4 = 3,720 units.
At a depth of 1 cm. opposite to centre of applicator 300 × 3·6 = 1,080 units.
„ 5 cms. „ „ „ 300 × 0·27 = 81 units.

In this way a comparison may be made between the actions to be expected at various depths with different arrangements and doses.

As in numerous cases the permissible dose is limited solely by the damage done to the skin or flesh in contact with the applicator, it is interesting to work out the skin action through various thicknesses of brass in direct contact with the tissues. This is especially important in the case of internal tubular applicators where no intervening material is employed. The figures are shown in Table 5. Here F represents the activity at a fixed distance through t mm. of brass, the bare tube being 1000. D is the external diameter of a tubular applicator of thickness t with a bore of 1 mm. to take a single central capillary. A_d is the skin activity on the surface of such an applicator, or of any applicator, in which only a thickness t of brass is interposed between the emanation tube [assumed 15 mm. long] and the flesh. A_d thus also applies to a single tube in a flat applicator, or a large bore tubular applicator in which the emanation tube rests against one side.

The figures in columns A_3 to A_7 represent approximately the skin activities on the surface of tubular applicators 3 to 7 mm. external diameter and t mm. thick. The interior of the applicator, which in all these cases is at least 2 mm. in diameter, is assumed to contain as many uniformly active capillaries as it will hold, so that the distribution may be regarded as symmetrical. These values are obtained by multiplying the value of A_d for the appropriate diameter by the ratio of the values of F for the thin- and thick-walled tubes respectively. Thus, a tube 6 mm. in diameter and 2·5 mm. thick would have a bore of 1 mm., and contain a single emanation capillary. Its surface action would be 50, the value of F being 9·7. If its wall was only 0·5 mm. thick, the internal bore would be 5 mm., and would contain a large number of capillaries. The value of F for 0·5 mm. being 30, the surface action, A_6, is assumed to be 30 × 50 ÷ 9·7, i.e., about 150, as shown in the table. This method is only approximate; but it seems unlikely that any errors due to it would be comparable with those due to the variations from the assumed conditions which are likely to occur in practice.

TABLE 1.

Single emanation tube, 15 mm. long in serum needle.

a = distance in millimetres from axis of tube.
b = „ „ „ „ "equatorial plane."

a mm.	0	2·5	5	7·5	10	15	20	25	30	40	50 mm. b
0·6	6200	6200	6200	3100	39	6·0	2·7	1·6	1·0	0·52	0·29
1	2300	2300	2300	1200	47	6·2	2·8	1·7	1·1	0·57	0·33
2	590	580	550	300	47	6·4	2·8	1·7	1·1	0·60	0·35
3	240	230	210	120	36	6·3	2·8	1·7	1·1	0·61	0·37
4	120	110	98	62	26	6·0	2·8	1·7	1·1	0·61	0·37
5	66	64	55	37	19	5·6	2·7	1·7	1·1	0·61	0·37
6	42	40	35	25	15	5·2	2·6	1·7	1·1	0·61	0·37
7	28	27	23	17	11	4·8	2·5	1·6	1·1	0·61	0·37
8	19	19	16	13	8·9	4·4	2·4	1·6	1·1	0·60	0·36
9	14	14	12	9·9	7·4	4·0	2·3	1·5	1·1	0·59	0·36
10	11	10	9·5	8·0	6·2	3·7	2·2	1·5	1·0	0·59	0·35
12	7·2	7·1	6·5	5·7	4·8	3·2	2·0	1·4	0·98	0·58	0·35
14	5·3	5·2	4·8	4·3	3·8	2·7	1·8	1·3	0·93	0·56	0·35
16	4·0	3·9	3·7	3·4	3·1	2·3	1·6	1·2	0·88	0·54	0·34
18	3·2	3·1	3·0	2·8	2·5	2·0	1·5	1·1	0·84	0·51	0·33
20	2·6	2·5	2·4	2·3	2·1	1·7	1·3	1·0	0·79	0·49	0·32
25	1·6	1·6	1·6	1·5	1·4	1·2	1·0	0·81	0·65	0·43	0·30
30	1·1	1·1	1·1	1·0	1·0	0·90	0·77	0·65	0·54	0·38	0·27
40	0·61	0·61	0·60	0·59	0·57	0·53	0·48	0·43	0·38	0·29	0·22
50	0·37	0·37	0·37	0·37	0·36	0·34	0·31	0·29	0·27	0·22	0·17
60	0·25	0·25	0·25	0·25	0·24	0·23	0·22	0·21	0·20	0·17	0·14
70	0·17	0·17	0·17	0·17	0·17	0·16	0·16	0·15	0·14	0·12	0·11
80	0·13	0·13	0·13	0·13	0·12	0·12	0·12	0·11	0·11	0·11	0·09
90	0·10	0·10	0·10	0·10	0·10	0·09	0·09	0·09	0·08	0·08	0·07
100	0·07	0·07	0·07	0·07	0·07	0·07	0·07	0·07	0·06	0·06	0·05

TABLE 2.

Four emanation tubes, each 15 mm. long, in coplanar, parallel serum needles equally spaced 4 mm. apart, forming a rectangle 15 mm. × 12 mm.

a = distance in millimetres from plane containing the four tubes.
b = " " " " "equatorial plane."

The plane of the table passes midway between two tubes, so that for $a = 0$ and $b < 7{\cdot}5$ the nearest tubes are at 2 mm. on either side. The maximum activity on the surface of one of the needles (1·2 mm. diameter) would be 1600 on the same scale.

For values of a greater than 20 the activities are the same as for the corresponding points in Table 1, from which they may be taken.

With the exception of a layer a few millimetres thick next to the plane of the tubes, the distribution in the "equatorial plane" would be nearly the same, b being now measured from the original plane of the table.

a mm.	0	2·5	5	7·5	10	15	20	25	30	40	50 mm. b
0	310	310	290	160	31	5·8	2·7	1·7	1·1	0·60	0·36
1	250	240	220	130	30	5·8	2·7	1·7	1·1	0·60	0·36
2	160	150	130	80	25	5·7	2·7	1·7	1·1	0·61	0·36
3	89	86	72	48	21	5·5	2·6	1·7	1·1	0·61	0·37
4	56	54	47	32	17	5·3	2·6	1·6	1·1	0·61	0·37
5	38	36	31	23	13	5·0	2·5	1·6	1·1	0·61	0·37
6	26	25	22	16	11	4·6	2·5	1·6	1·1	0·60	0·36
7	19	18	16	13	8·8	4·3	2·4	1·6	1·1	0·60	0·36
8	15	14	12	10	7·2	4·0	2·3	1·5	1·1	0·59	0·36
9	11	11	10	8·2	6·3	3·7	2·2	1·5	1·0	0·59	0·35
10	9·1	8·8	8·0	6·9	5·5	3·4	2·1	1·4	1·0	0·58	0·35
12	6·4	6·3	5·8	5·3	4·4	3·0	2·0	1·3	0·97	0·57	0·35
14	4·8	4·7	4·4	4·0	3·5	2·6	1·8	1·2	0·91	0·55	0·34
16	3·8	3·7	3·5	3·2	2·9	2·2	1·6	1·2	0·87	0·53	0·34
18	3·0	2·9	2·8	2·6	2·4	1·9	1·4	1·1	0·83	0·50	0·33
20	2·5	2·4	2·3	2·2	2·0	1·6	1·2	1·0	0·78	0·48	0·32

TABLE 3.

Single emanation tube in long serum needle, partially withdrawn at intervals so as to distribute the activity as uniformly as possible along a line 100 mm. long.

a = distance in millimetres from axis of tube.

b = " " " " "equatorial plane" (perpendicular to axis at centre point of this line).

a mm.	0	10	20	30	40	50	60	70 mm. b
0·6	930	930	930	930	930	470	0·80	0·30
1	350	350	350	350	350	180	0·84	0·33
2	90	90	90	90	89	45	0·88	0·35
3	38	38	38	37	37	19	0·87	0·37
4	20	20	20	19	19	9·9	0·86	0·38
5	12	12	12	12	11	6·1	0·84	0·38
6	8·3	8·2	8·2	8·0	7·6	4·2	0·81	0·37
7	6·1	6·1	6·0	5·8	5·4	3·1	0·78	0·36
8	4·7	4·7	4·6	4·4	4·1	2·4	0·75	0·36
9	3·8	3·8	3·7	3·6	3·2	2·0	0·72	0·36
10	3·2	3·2	3·1	3·0	2·6	1·7	0·69	0·35
12	2·5	2·4	2·4	2·3	2·0	1·3	0·64	0·34
14	2·0	2·0	1·9	1·8	1·6	1·1	0·58	0·32
16	1·7	1·6	1·6	1·5	1·3	0·91	0·53	0·31
18	1·4	1·4	1·4	1·3	1·1	0·79	0·49	0·30
20	1·2	1·2	1·2	1·1	0·93	0·69	0·45	0·28
25	0·90	0·89	0·85	0·79	0·68	0·52	0·37	0·25
30	0·69	0·68	0·65	0·59	0·51	0·41	0·30	0·22
40	0·43	0·42	0·40	0·37	0·33	0·27	0·22	0·17
50	0·29	0·28	0·27	0·25	0·22	0·19	0·16	0·13
60	0·20	0·20	0·19	0·18	0·16	0·14	0·12	0·10
70	0·15	0·14	0·14	0·13	0·12	0·11	0·09	0·08
80	0·11	0·11	0·10	0·10	0·09	0·08	0·07	0·06
90	0·08	0·08	0·08	0·08	0·07	0·07	0·06	0·05
100	0·07	0·07	0·06	0·06	0·06	0·05	0·05	0·04

TABLE 4.

Six emanation tubes in long, coplanar, parallel serum needles, equally spaced 10 mm. apart, and partially withdrawn at intervals so as to distribute the activity of each needle as uniformly as possible along a line 100 mm. long, thus forming a rectangle 100 mm. × 50 mm.

a = distance in millimetres from plane containing the four tubes.
b = „ „ „ "equatorial plane."

The plane of the table passes midway between two tubes, so that for $a = 0$ and $b < 50$ the nearest tubes are at 5 mm. on either side. The maximum activity on the surface of one of the needles (1·2 mm. diameter) would be 160 on the same scale.

a mm.	0	10	20	30	40	50	60	70 mm. b
0	4·9	4·9	4·8	4·7	4·5	2·5	0·59	0·31
1	4·7	4·7	4·7	4·6	4·3	2·4	0·59	0·31
2	4·3	4·3	4·2	4·1	3·9	2·2	0·58	0·31
3	3·8	3·8	3·7	3·6	3·4	2·0	0·57	0·31
4	3·3	3·3	3·2	3·1	2·9	1·7	0·57	0·31
5	2·8	2·8	2·8	2·7	2·4	1·5	0·56	0·31
6	2·5	2·4	2·4	2·3	2·1	1·3	0·55	0·30
7	2·2	2·2	2·1	2·0	1·8	1·2	0·53	0·30
8	2·0	1·9	1·9	1·8	1·6	1·1	0·52	0·30
9	1·8	1·8	1·7	1·6	1·4	0·97	0·51	0·30
10	1·7	1·6	1·6	1·5	1·3	0·90	0·50	0·29
12	1·5	1·4	1·4	1·3	1·1	0·79	0·47	0·28
14	1·3	1·2	1·2	1·1	0·97	0·71	0·44	0·27
16	1·1	1·1	1·1	1·0	0·86	0·64	0·41	0·27
18	1·0	1·0	0·96	0·89	0·76	0·58	0·39	0·26
20	0·91	0·90	0·87	0·80	0·68	0·53	0·36	0·25
25	0·72	0·71	0·68	0·63	0·54	0·42	0·31	0·22
30	0·57	0·56	0·53	0·49	0·43	0·35	0·27	0·20
40	0·38	0·37	0·36	0·33	0·29	0·25	0·20	0·16
50	0·26	0·26	0·25	0·23	0·21	0·18	0·15	0·12
60	0·19	0·19	0·18	0·17	0·15	0·13	0·12	0·10
70	0·14	0·14	0·13	0·12	0·11	0·10	0·09	0·08
80	0·10	0·10	0·10	0·10	0·09	0·08	0·07	0·06
90	0·08	0·08	0·08	0·07	0·07	0·06	0·06	0·05
100	0·06	0·06	0·06	0·06	0·05	0·05	0·05	0·04

TABLE 5.

Skin action with various flat and tubular brass applicators.

t = Thickness in mm.

D = External diameter of tubular applicator of wall-thickness t, allowing an internal diameter of 1 mm. to hold a single central emanation tube.

F = Relative activity through t mm. at any fixed distance.

A_d = Skin action per mc. hr. for single emanation tube in tubular applicator of thickness t and diameter D, or in contact with a flat applicator of thickness t. This also represents the *maximum* skin action for a single tube in a larger tubular applicator, in which the tube rests against one side.

A_3 = Skin action for applicator thickness t, external diam. 3 mm., completely filled with emanation tubes.

A_4 = Ditto ditto ditto 4 ditto ditto.

A_5 = Ditto ditto ditto 5 ditto ditto.

A_6 = Ditto ditto ditto 6 ditto ditto.

A_7 = Ditto ditto ditto 7 ditto ditto.

t mm.	D mm.	F	A_d	A_3	A_4	A_5	A_6	A_7
0·1	1·2	208	5100	2200	1800	1400	1100	890
0·2	1·4	107	2000	1100	900	690	550	460
0·3	1·6	60	970	630	510	390	310	260
0·4	1·8	40	570	420	340	260	200	170
0·5	2·0	30	390	320	260	200	150	130
0·6	2·2	24	290		200	150	120	100
0·8	2·6	16·3	180		140	110	83	70
1·0	3·0	12·6	130		110	82	64	54
1·2	3·4	10·9	110			71	56	47
1·4	3·8	10·3	92			67	53	44
1·6	4·2	10·1	81				52	43
1·8	4·6	10·0	72				51	43
2·0	5·0	9·9	64				51	42
2·5	6·0	9·7	50					41
3·0	7·0	9·6	41					

No. 6.

PRELIMINARY EXPERIMENTS ON A CHEMICAL METHOD OF SEPARATING THE ISOTOPES OF LEAD.

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The usual methods used for the separation of the elements from one another depend upon reactions between ions, such as fractional crystallization and fractional precipitation. Reactions of this type are common to nearly all the elements, and, as the property of solubility of salts, upon which such reactions depend, often varies gradually among the elements, it is perhaps not surprising that there should be atoms which, while differing slightly in atomic weight, are so alike in all their chemical properties that they must be placed in the same position in the periodic table. Once, however, it is admitted that the atoms of any body have a different weight, it is difficult to imagine that they are absolutely identical in all chemical properties; and it would seem to be only a matter of finding the particular chemical reaction in which they show an appreciable difference in order to be able to effect their separation.

Now there is one property of the metallic elements which is of a very specialized character, and that is the property of forming organo-metallic compounds. This property is possessed by comparatively few metals, and elements standing close to one another in the periodic table show marked differences in the ease of formation and stability of their organic derivatives. Furthermore, the process of formation of the lead organic compounds is peculiar. Frankland and Lawrence[1] found that lead chloride reacts with zinc alkyl, half of the lead being precipitated, and the other half forming alkyl derivatives of tetravalent lead. Pfeiffer and Truskier[2] were the first to substitute the Grignard reagent for the organo-zinc compounds in this reaction, and they prepared various alkyl and aryl derivatives of lead by this means.

The reaction between lead chloride and the Grignard reagents is represented by the following equation:—

$$2\ PbCl_2 + 4\ MgRX = PbR_4 + 2\ MgCl_2 + 2\ MgX_2 + Pb.$$

Here, some of the lead atoms change their valency from two to four, while others pass into the elemental state. It occurred to us that it would be worth while trying whether the lead atoms of different weights showed any appreciable

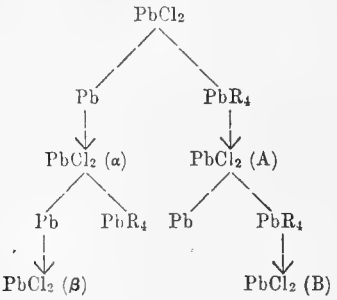
[1] J. C. S. 35 (1879), 244.

[2] Ber. 37 (1904), 1125.

choice in the direction they would take in this reaction, or, in other words, whether a separation of the isotopes of lead could be effected by its means.

It should be mentioned in this connexion that Hoffmann and Wolf[1] in 1907 acted on lead chloride containing radium D with magnesium phenyl bromide, and found that most of the radio-activity remained in the metallic lead.

Our scheme of work will be clear from the following diagram :—



A quantity of lead chloride is treated with the Grignard reagent, and the lead alkyl and the metallic lead are separated from one another, and converted into lead chloride. The two quantities of lead chloride are again separately treated with the Grignard reagent, and the resulting products separated as before. If there is any difference between the isotopes with regard to their tendency to form organic compounds, it will be seen that a repetition of this process, always using the metal on the extreme left and the organo-metallic compound on the extreme right of the diagram, will lead to an accumulation of one isotope at the extreme left and the other at the extreme right and the lead salts marked B and β in the diagram might possibly show a difference in the atomic weights of the lead which they contain.

As a result of some preliminary observations, we decided to use the ethyl compound of lead as the basis of our experiments. The synthesis of the magnesium compound with this group goes smoothly and easily without the formation of troublesome bye-products, while the lead alkyl could be easily purified as far as required by us. Grutner and Krause,[2] who first isolated the lead tetraethyl by this method, found that it was contaminated with unsaturated lead alkyls; but for our purpose this was of no consequence. We found that a quantitative yield of lead tetraethyl could be obtained by using one mol of lead chloride to three-and-a-half mols of magnesium ethiodide. If the proportion of lead chloride exceeded this, the yield diminished.

The lead chloride used was a sample kindly given to us by Messrs. Hopkin, Williams, & Co., London. It was recovered from the manufacture of meso-thorium, and therefore consisted of a mixture of the chlorides of ordinary lead and of lead from thorite. The salt was recrystallized and dried by heating in a current of hydrochloric acid gas.

The Grignard reagent was prepared in the usual way, from 104 grams of ethyl iodide and 16 grams of magnesium. Fifty-two grams of the dry lead chloride were then added gradually. When the lead chloride had all been added, the mixture was heated for four hours under a reflux condenser, air and moisture being excluded by means of a mercury seal. The reaction product was poured

[1] Ber. 40 (1907), 2425.

[2] Ber. 49 (1916), 1415.

slowly into water, and the aqueous mixture was extracted several times with ether. The ether solution was dried over calcium chloride; the ether evaporated, and the lead tetraethyl distilled in vacuo. A small residue of lead iodide remained in the distilling flask.

The lead tetraethyl was next converted into lead nitrate by dropping it slowly into hot dilute nitric acid; and the lead was precipitated from the solution as sulphate by adding sulphuric acid and alcohol. The lead sulphate was filtered off, washed, and dissolved in ammonium acetate; and the solution was saturated with sulphuretted hydrogen. The precipitated lead sulphide was filtered off, washed, and dissolved in hot hydrochloric acid; and the lead chloride which crystallized out was recrystallized from water containing hydrochloric acid. This lead chloride was dried and labelled "Lead chloride A."

The residue from the extraction of the lead alkyl compound, consisting of basic iodide of magnesium, metallic lead, and traces of the chloride and iodide of lead, was repeatedly boiled with a solution of sodium carbonate until the carbonate solution gave no reaction for halides with silver nitrate. It was then dissolved in nitric acid, treated with sulphuric acid and alcohol; and the resulting lead sulphate was converted into lead chloride by exactly the same process as that described above. It was recrystallized from water containing hydrochloric acid, dried, and labelled "Lead chloride α."

The A and the α chlorides were again made to react separately with the requisite quantities of magnesium ethiodide; and the organo-lead compound was in each case extracted as before. The metallic lead from the lead chloride α and the lead tetraethyl from the lead chloride A were then treated for conversion into their respective chlorides in exactly the same manner as had been done previously, and the two samples were labelled respectively "Lead chloride β" and "Lead chloride B."

Our next step was to find whether there was any appreciable difference between the atomic weights of the lead in the two samples. The method adopted for this purpose was that of relative atomic weight determination as used by Soddy and Hyman[1] in their comparison of the atomic weights of ordinary and thorite lead. A platinum boat was heated in a current of hydrochloric acid gas; and when the boat had cooled, the hydrochloric acid was displaced by dry air. The boat was weighed, and a quantity of the lead chloride under examination (a little over a gram) was placed in it. The lead chloride was fused in a current of dry hydrochloric acid; the hydrochloric acid was displaced by air when cold, and the boat and contents were weighed. The boat was then dropped into a stoppered bottle containing one litre of water and 5 c.c. of nitric acid (which had previously been distilled over silver nitrate), and the bottle was placed in a water bath kept at 60° C., and was shaken frequently. In this way all the lead chloride was brought into solution in about three hours. The boat was removed from the bottle, washed several times with distilled water, 100 c.c. of water being used altogether, and the washings being poured each time into the bottle. The boat was then dried in the steam oven, heated in dry hydrochloric acid, which on cooling was displaced by air, and was finally weighed again. A set of bottles, each containing one of the samples, was made up in this way, the same platinum boat being used throughout. In the first series of experiments three solutions of lead B and two of lead β were used. In the second series there were two of each kind.

When a set of solutions had been prepared, 200 c.c. of the same silver nitrate solution were added to each from the same pipette. The bottles were well shaken,

[1] J. C. S. 105 (1914), 1402.

and were left standing in the dark room in order to allow the precipitates to settle. On the next day the titration of the whole set was finished by dropping in the silver nitrate solution and watching by the aid of a red lamp for the appearance of a cloud. When no cloud appeared after one minute, a reading was taken. The addition of two drops then produced no further cloud. In the first series the silver nitrate was added from a dropping funnel, which was weighed before and after the experiment, and the volume added was calculated from the specific gravity of the solution. In the second series a one c.c. pipette, graduated in hundredths, and having a small syringe attached to regulate the dropping, was used. In both cases the volume of one drop was about 0·03 c.c.

In the first series of experiments the three B samples were titrated first, followed by the two β samples. In the second set the B and the β samples were alternated. The results of the experiments are tabulated below. The weight of lead chloride was obtained by subtracting the mean weight of the boat before and after the experiment from the weight of the boat and fused chloride. In No. 3 of the first series the weight of the boat varied by 0·4 milligram; and in No. 1 of the second series it varied by 0·5 milligram. In all the other experiments the variation in weight did not exceed 0·2 milligram. The final column of each table gives the volume of silver nitrate required per gram of lead chloride as calculated from the experiments.

First Series.

200 c.c. of silver nitrate solution added to each from pipette.
Specific gravity of silver nitrate solution, 1·005.

	Wt. of $PbCl_2$.	Wt. of $AgNO_3$ solution from burette.	Vol. of $AgNO_3$ solution calculated.	Total vol. of $AgNO_3$ solution added.	Vol. of $AgNO_3$ solution per gram of $PbCl_2$.
	grms.	grms.	c.c.	c.c.	c.c.
(1) Lead B, .	1·09175	2·4074	2·3954	202·3954	185·39
(2) ,, ,, .	1·09235	2·6126	2·5996	202·5996	185·47
(3) ,, ,, .	1·0910	2·5256	2·5130	202·5130	185·62
(4) Lead β, .	1·0893	2·4439	2·4317	202·4317	185·83
(5) ,, ,, .	1·09195	2·8557	2·8415	202·8415	185·76

Second Series.

	Wt. of $PbCl_2$.	Vol. of $AgNO_3$ solution.	Vol. of $AgNO_3$ solution per gram of $PbCl_2$.
	grms.	c.c.	c.c.
(1) Lead B, .	1·08875	201·36	184·94
(2) Lead β, .	1·0922	202·25	185·17
(3) Lead B, .	1·09275	202·06	184·91
(4) Lead β, .	1·09285	202·31	185·12

If we omit No. 3 of the first series, the maximum difference between two samples of the same kind in either series is ·08 c.c. per gram; whereas the minimum difference between two different samples is 0·18.

If we assume that the atomic weight of the lead β is 207·1, the mean ratio of the silver nitrate required per gram of lead β to that required per gram of lead B would give for the lead B an atomic weight of 207·4 in the first series, and of 207·3 in the second series.

Samples of the original lead chloride, together with the B and β chlorides, were sent to Professor John Nolan, of University College, Dublin, who very kindly examined their radio-activity, and reported that, while this property was weak in all three, the radio-activity of the β chloride was much greater than that of either the original or the B chloride. This confirms the observation of Hoffmann and Wolf.

Unless there is an unknown source of error, the relative atomic weight determinations indicate that the different isotopes of lead are not identical in their chemical behaviour towards the Grignard reagent. If this is so, a continuation of the process of chemical fractionation described above should produce such a difference in the atomic weights as would leave no doubt that a separation had taken place. Experiments in this direction are in progress.

No. 7.

THE LIGNITE OF WASHING BAY, CO. TYRONE.

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AND

JANE G. GILMORE, B.Sc.

(Plate III.)

[Read June 27. Printed August 28, 1922.]

Lignite or brown coal was found at various depths of the bore in isolated pieces embedded in the white clay; at a depth of 999 feet the deposit became a black mass. Much of the wood was very much crushed and twisted, to obliteration of its coniferous character, but for resin and a few pits.

Some scraps occur at 712 feet, 869 feet 6 inches, and at 909 feet, which are flaky, and have the appearance of charred wood. These pieces show the structure of the pits on the walls of tracheids and of the medullary rays better than the sections obtained from the solid pieces. The pits on the walls of the tracheids and the medullary rays are the same as in the solid material, but no resin parenchyma is found, with the exception of one piece at 909 feet. It seems that, in the charring, from whatever cause, the resin disappeared.

Although Dicotyledonous leaves are plentiful in the bore, no trace of Dicotyledonous wood has been observed. Goeppert (6) recorded the same fact with regard to the Tertiary Flora of Silesia.

The cross-section (Pl. III, figs. 1, 2) shows the wood much crushed and somewhat disorganized. The growth rings are very narrow, varying from 200 to 400μ; an occasional broad ring is seen. The spring wood consists of 10–30 rows of tracheids, which are fairly broad; the average size is 60μ; occasionally they reach 80μ, and in one case 100μ in diameter. They are thick-walled, and much flattened by pressure. The autumn wood occupies about a third of the ring.

The bordered pits (Pl. III, figs. 3, 4, 5, 6) have a circular pore, but are frequently somewhat flattened top and bottom or are horizontally extended in their outer contour. They are in single or double rows; frequently three rows occur, and in one tracheid four rows of pits were seen (Pl. III, fig. 6). When in two or more rows, they are opposite, and may be either closely packed or scattered. They measure 20–24μ in diameter, and when flattened measure about 4μ less. Between the rows of bordered pits are very distinct Sanio's bars, which are sometimes double. On the tangential walls of the spring and autumn tracheids small bordered pits are common (Pl. III, fig. 7) on the whole extent of the wall. According to Penhallow (14), pitting in this position is a primitive feature, and among recent Conifers is best seen in *Sequoia gigantea*, making it in this respect almost unique.

Resin canals are wanting. Resin parenchyma is very plentiful, and scattered throughout the wood. The average breadth of a resin cell is 50–60μ, and the

length is 1½ to 3 times the breadth. Short resin cells are found in wood, tending to form traumatic resin canals. The end walls of the resin cells are thin and unpitted (Pl. III, fig. 7).

Uniseriate medullary rays, only, are present, 2–20 cells high, each cell being 20–24μ in height. The horizontal and tangential walls are unpitted. The pits on the radial or lateral walls are (Pl. III, figs. 8, 9, 10, 11) usually in one, occasionally in two horizontal rows. The pits, 1–4 in the field, appear simple with oval pore, horizontally directed. They are 11–14μ in length and 5-8μ in breadth. At 712 feet the pits (Pl. III, fig. 11) were found to be bordered, so that the simple pits are probably due to bad preservation. The arrangement of the pits in the ray cells is very similar to that in *S. gigantea*, in which, however, the pits are always bordered. In the fossil and in *S. gigantea* pores which are slightly oblique may occasionally be seen, and occasionally 4–5 pits in two rows in the field occur.

In *S. sempervirens*, in common with previous observers, we find two horizontal rows of bordered pits with oblique pore in the cross-field is the normal arrangement.

The identification of the wood of living and fossil Conifers has for the past seventy years been the subject of much investigation. The similarity in structure in species which are far apart, judged by their external features, and the amount of variation which may occur in any one species, render the task difficult in the case of some living, but more so in that of fossil Conifers.

Recognizing the fact that leaf impressions and wood are hardly ever found together in the same deposit in organic continuity, Goeppert (7) in his Monographie der fossilen Coniferen, devised a classification of coniferous wood in which his composite genera united members of widely separated natural groups.

Our wood belongs to his Cupressinoxylon type, which is characterized by bordered pits on the radial walls of the tracheids in one row, or if in more than one row then opposite; resin canals wanting (or, if present, traumatic), and abundant wood parenchyma, containing resin.

Kraus, Kleeberg, Beust, Schroeter, Sanio, Schmalhausen, Conwentz, Knowlton, Penhallow, and others pursued their investigations in the hope of finding constant reliable diagnostic features for the different genera. In 1905 Gothan (8) showed that the pitting of the medullary rays is a very important feature which can be effectively utilized for identification when due regard is paid to the other characters of the wood. Gothan has founded the genus *Taxodioxylon* to include fossil members of the *Taxodineae* previously placed in the composite fossil genus *Cupressinoxylon*. Thus *Taxodioxylon sequoianum* includes Sequoia-like forms; T. taxodianum, *Taxodium*-like species.

Comparing our wood with the wood of living Conifers, we find it cannot be:—

> Juniper or *Libocedrus decurrens*—since the horizontal and tangential walls of the medullary ray cells are unpitted (11); or one of the
>
> Cupressineae—since the pits on the radial walls of the tracheids are often in more than two rows (16).

We conclude it is one of the Taxodineae.

Schroeter (18) found in 1880 that the wood of Sequoia and Taxodium could be distinguished by the fact that in Sequoia the horizontal walls of the resin cells are of uniform thickness, and not pitted or nodulose as in Taxodium. Schroeter evidently appreciated the diagnostic importance of this feature, as he mentions it

twice in his account of *Sequoia canadensis*. All subsequent observers overlooked his observation until Prill (*op. cit.*, s. 207), who has anticipated us in claiming credit for Schroeter for the discovery.

Our wood is not *Taxodium* or *Glyptostrobus*, nor indeed *Cryptomeria*, in which the cross-walls are slightly nodulose.

The species which have smooth cross-walls in the resin cells are *Sequoia*, *Athrotaxis*, and *Taiwania*. In *Taiwania* the pores of the medullary rays are oblique, sometimes almost vertical; we have not found *Athrotaxis* with medullary rays more than ten cells high, so that by a process of elimination we must conclude that our wood is *Sequoia*, and we believe it to be the wood of *S. Couttsiae* Heer, of which the leaves, cone, and seeds occur in the same bore, and have been already described by us (10). But for this association, it would be called *Taxodioxylon sequoianum* Gothan.

Heer (9) in his investigation of the Lignite of Bovey Tracey found resin plentiful, and satisfied himself that the wood was coniferous. He could make out little of its structure, but, from the occurrence of foliage and cones of *S. Couttsiae*, assumed that the wood belonged to that species.

Beust (2) in 1885 was a little more successful, and there is nothing in his account not in accordance with the now known characters of *Sequoia* wood. He states that *S. Couttsiae* is characterized by enormous quantities of resin, often seen in large lumps in the cells. Heer told Beust that he had found amber in large quantities in the wood.

In 1869, a few years after Heer's report on the Bovey Tracey Flora, Schenk (17) described the plant remains found in the lignite of Saxony near Leipzig, which has been assigned to the same age as Bovey Tracey—i.e., the Oligocene. It is to be regretted that Schenk did not publish figures to show the cross-walls of the resin parenchyma which he said were mostly evident, and to show the roundish pits of the medullary rays. Schenk said he would have placed such a wood in the composite genus *Cupressinoxylon* if found alone. He examined the structure of the wood, which was in connexion with foliage shoots like those of *S. gigantea*, and with cones like those of *S. sempervirens*, and satisfied himself that the fossil was identical with *S. Couttsiae* of Bovey Tracey.

Felix (4) holds that some of the lignite found in N.-W. Saxony and adjoining districts, and named *C. protolarix*, is undoubtedly *Sequoia Couttsiae* wood.

The lignites and silicified wood of Lough Neagh have been the subjects of conjecture and study for many years. Dr. Richard Barton (1) in 1751, in what was one of the first accounts of petrifactions in the British Isles, gives particulars of the abundance of fossil wood around Lough Neagh, and of the gift to the University near Dublin of specimens, one of which weighed 150 lbs. We have not seen this fossil, but have examined another piece of silicified wood in the Geological Museum of Trinity College. This specimen has a copper cap and label on which the inscription reads: "Brought from Lough Neagh, 1721, by Sir Wm. Fownes."

Unger (20), in 1847, named a sample of lignite from Lough Neagh, *Peuce Pritchardi*, and described it thus:—"Strata concentrica minus conspicua usque ad 1 mil met lata. Vasa leptoticha versus limitem annuli paulatim angustiora. Pori disciformes minuti contigui uni-biseriales. Radii medullores simplices rarius compositi 1–25 cellulis parenchymatis amplis formati. Ductus resiniferi copiosi. Ad Lough Neagh Angliae (Andw. Pritchard)." Goeppert renamed it *Pinites Pritchardi*, simply repeating Unger's description. Kraus placed it in his Cupressinoxylon group, and as such it has since been known.

	Breadth of Tracheids.	No. of Pits.	Pits in Tracheids.
Cupressinoxylon aequaele, Goepp (3) (Danzig).	56·2μ broad.	1 2 3 (4)	12–16μ
C. canadensis, Schroeter (18).	68μ = radial. 40μ = tangent.	1 2 (3)	8–19μ
S. Couttsiae, Schenk (17).		1 2 cleft-like.	
C. Holdenae, Seward (*op. cit.*, p. 194).		1 2	flattened and in contact.
C. McGeei, Knowlton (12).	to 80μ, average 68μ.	1 2 3	20–25μ. Small pits on tangent walls.
C. polyommatum, Cramer (3).		1 2 3 4 (5)	great no. of small pits on radial walls. 13·77μ av. 12·24μ, 15·3μ.
Rhizocupressinoxylon panniconum, Felix (*op. cit.*, p. 274).	radial breadth exceeds tangent.	1 2 3 (4)	outer border ellipse with greater axis parallel to med. ray, d = 18·3μ.
Pinites protolarix, Goepp, i (*op. cit.*, p. 218).	d = 75μ. Felix (*op. cit.*, s. 275).	1 2 3	20·7μ–24μ (Saarau) Felix (*op. cit.*, s. 275).
C. pulcrum, Cramer (3, p. 171).	slight striation.	1, 2 (3)	average 22·95μ. 18·36–27·5μ.
Taxodioxylon Sequoianum (Merck.-Schmal) Gothan		1, 2, 3	numerous, mod. large.
C. Wellingtonioides (Prill) Kraüsel. (1, s. 293).	spiral striations not very pronounced.	1 2	av. 22·95μ.
P. Pritchardi, Unger (20).		1 2	small touching.

Resin Canals.	Resin Parenchyma.	Med. rays, breadth and height.	Pitting of Medullary rays.
	copious with brown resin.	uniseriate, 1–8 cells high. 15·3–36·7μ = height of 1 ray cell.	1–8 elliptical, oblique.
	plentiful but variable.	uniseriate, 20μ = height of 1 ray cell.	1–4 oblique bordered pits in 1 horizontal row.
	cross walls evident.	uniseriate, 2–20 cells high.	roundish.
in summer wood. Tylosis in some canals.	present.	uniseriate, to 30 cells high.	2–4 fairly large, simple.
	abundant, 120–250 long, 50 broad, slight, narrower than tracheids.	uniseriate, 2–49 cells high.	1–2·3 oblong, oval, simple pits. 15 × 10μ.
	narrow.	uniseriate, 2–26 cells high.	2–4 large oval pits in field, horizontal.
		height varies.	transversely elliptical pores in two rows.
	copious.	2–40 cells high.	
		uniseriate, 4–14 cells high.	2 large oval pits, 1 in autumn wood, sometimes bordered. Rays without pits top and bottom.
	plentiful.	1–43 cells high.	2–7 horizontal pits in spring wood, elliptical, in two rows.
in spring wood in 1 ring.	crowded together, cells short.	biseriate, not rare, to 35 cells high.	1, 2 3, pore somewhat inclined and not broad.
	copious.	uniseriate rarely compound, 1–25 cells high.	

Macloskie (13) contributed an interesting paper in 1872, in which he gave for the first time a rough illustration of the wood. Seward (19) gives better illustrations of a Lough Neagh wood as Cupressinoxylon sp. Two slides in the Geological Laboratory of this College, labelled *Cupressinoxylon Pritchardi* Kraus, from Sandy Bay, show a wood which has the usual features of *Taxodioxylon*, and one resin passage or duct (Pl. III, fig. 12) at the end of the summer wood. This feature is of diagnostic importance, as *Sequoia* normally possesses resin cells only; but when injured, traumatic resin passages or cysts are found in the autumn wood in *S. sempervirens*, according to Krausel, in the spring wood according to Penhallow (*op. cit.*, p. 224). It would therefore appear, if these two preparations are correctly named, that *C. Pritchardi* Kraus may be nearly allied to, if not identical with, *Taxodioxylon Sequoianum* Gothan, and also with the lignite of the bore at Washing Bay.

Unfortunately, the diagnosis of Unger, repeated by Goeppert and later writers, is now inadequate, and the type material needs re-examination and description in the light of present-day views. Our 1721 specimen shows occasionally three rows of bordered pits in the radial walls of the tracheids, and cannot very well be, as Gardner (5) assumes is the case for Lough Neagh lignite, the wood of a *Cupressus*, which is distinguished by its mostly single row of bordered pits.

We are at present examining microscopically lignite material from various localities in Ireland, and need hardly say that we find it is not of uniform character. We may expect to find as lignite examples of all the Conifers recorded in the Irish Tertiary.

It needs little imagination to picture the presence of forests of Sequoia in N. Ireland, possibly contemporaneous with those in S. Devon at Bovey Tracey, the shores of the Baltic, the Rhine valley, Saxony, Silesia, and S. France. We may yet find in Ireland large deposits of lignite or brown coal of economic value like those abroad.

Among Cupressinoxylons the occurrence of 3–4 bordered pits on the radial walls of the tracheids is not common.

A comparative list of species which are like the Washing Bay lignite in many respects is shown in the table, pp. 62 and 63.

PLATE III.

Figs.

1, 2. Transverse section of stem (1002 W. 1). × 50.
(1002 W. 2). × 50.

3–6. Radial walls of tracheids showing bordered pits. × 50.

6. Shows four opposite pits on the wall of the tracheid (869′ 6″ W. 7).

7. Tangential section showing pits on tangential walls of tracheids, smooth cross walls of resin parenchyma, and balls of resin in the cells (1005 W. 1). × 250.

8, 9. 10. Pits of medullary rays, apparently simple. × 250.

11. Pits of medullary rays with border (712′). × 250.

12. Transverse section of *Cupressinoxylon Pritchardi* Kraus, showing resin duct or cyst.

1.

2.

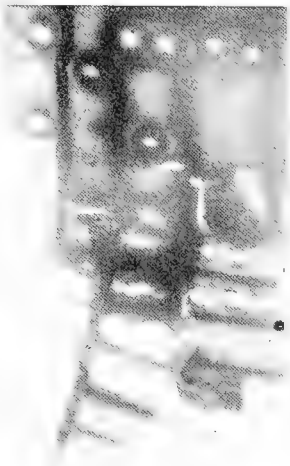
3.

4.

5.

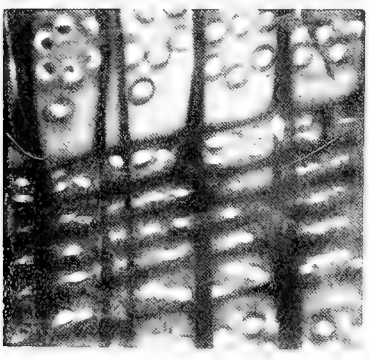
6.

7.

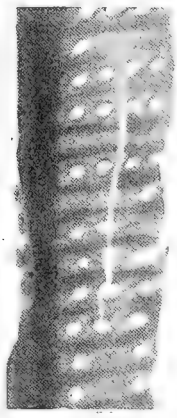


8.

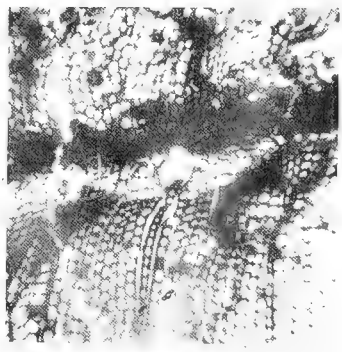
9.



10.



11.



12.

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No. 8.

LIBOCEDRUS AND ITS CONE IN THE IRISH TERTIARY.

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AND
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(Plate IV.)

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The present paucity of Conifers in the Irish Flora (Taxus and Juniperus) is most marked on comparing them with the extinct forms. We know that *Pinus*, *Sequoia*, *Cryptomeria*, and *Cupressus* once flourished on the mountain slopes of N.-E. Ireland. We propose to add to this list the genus Libocedrus. While *Sequoia* is now confined to West North America, *Cryptomeria* to Japan and East China, and Cupressus has its nearest representative in the Mediterranean region, *Libocedrus* has a much wider, though discontinuous, range, and is represented by eight species, of which three occur in America, one in S.-E. China, and the rest in New Zealand, New Caledonia, and New Guinea. It occurs to-day in much the same localities as *Sequoia* and *Cryptomeria*, but also ranges further southwards both in the Old and New World.

Mr. A. Deane, the Curator of the Public Art Gallery and Museum, Belfast, kindly lent us, for comparison with the flora of the Washing Bay Bore, the collections of Irish Tertiary fossils. Among them we discovered two slabs with cones and several slabs with foliage which came from the Interbasaltic beds of Ballypalady. We believe these to be the cones and foliage of *Libocedrus salicornioides*, Unger.

The cone (Pl. IV, figs. 1, 2) is oval oblong, 6·5 × 3 mm., borne on a short lateral shoot, and apparently shows two fertile scales at right angles to two sterile ones. The oval cone of a living *Libocedrus* consists of four, rarely six, scales in pairs at right angles. The outer lower pair is very short in *L. decurrens*. Each cone-scale is sub-apically mucronate. This mucro represents the free tip of the carpel of which the body is fused to the ovuliferous scale. In *L. plumosa* (*L. Doniana*, Pl. IV, fig. 3) the sterile pair of scales is better developed than in *L. decurrens*, and about one-third the length of the fertile pair, each scale having at its centre, not sub-apically, a pronounced recurved mucro. In our fossil a fertile scale is shown in surface view. The projection on the right may be either a sterile scale at right angles to it or the mucro of such a scale of the same length as the fertile one. If this latter view be accepted, *L. plumosa* would be intermediate in this respect between *L. decurrens* and the fossil. In an attempt at restoration of tissue we got scraps of wood showing bordered pits in the tracheid (Pl. IV, fig. 4) and medullary rays with small pits (Pl. IV, fig. 5). As far as they go, these scraps and one from a foliage shoot (Pl. IV, fig. 7) agree with the structure of the living *Libocedrus* wood.

We also found a fusiform sclerotic idioblast 294 × 36μ. In *L. salicornioides* from Leoben in Stygia we found an idioblast almost identical. Examination of fresh material of recent species of *Libocedrus* showed similar sclerotic cells in some of them, e.g., in *L. macrolepis*. Though such cells are not by any means confined to *Libocedrus*, it is worthy of note that the fossil specimens agree with one another and with the living *Libocedrus* species in their possession. This *Libocedrus*-like cone is in continuity with a shoot which shows the characteristics of the widely recorded fossil named *L. salicornioides*, specimens of which we have from the same locality.

We had already examined and identified the Interbasaltic *Libocedrus* material when unexpected confirmation was supplied by an interesting find in the Washing Bay core (Pl. IV, fig. 8). The joint-like scrap 7 × 4 mm., represents an elongated grooved internode and a node flattened out, owing probably to the insertion of two opposite branches. The two lateral adpressed leaves with their decurrent bases are observable. One of the short, obtuse, slightly ridged, facial leaves is visible. To the left the basal joint of a lateral branch is recognizable. We have introduced for comparison a scrap of *Libocedrus decurrens* (Pl. IV, fig. 9). Fortunately the fossil yielded a little tissue which on restoration (fig. 10) shows an epidermis of oblong and polygonal cells with straight, simply pitted walls. Stomata are in single ribands about three cells apart. Stoma and ridge measure 26–36μ.

The usually solitary nucleus-like body seen on each cell represents a papilla projecting into space from the outer wall of the epidermal cell. Similar papillae occur in *L. decurrens*, *L. macrolepis*, *Thuja occidentalis*, and *Callitris quadrivalvis*, but not in *L. plumosa*, *L. Bidwilli*, or *Biota orientalis*. In *L. chilensis* and *Callitris robusta* there are several in each cell. Berry (1) has found similar papillae in *Frenelopsis ramosissima*; and Kräusel (10) notes them in his recent description of the epidermis and stomata of *L. salicornioides*. The waviness of the wall mentioned in his first account was afterwards (11) found by him to be a secondary artificial feature, due to the same cause as that resulting in the spiral striation of the tracheids of many Conifers, and in consequence of no systematic value.

Our material is in general agreement with the Silesian material. The depression of the stomata naturally lowers transpiration, and this is still further lessened by the raised epidermal ring or fence which acts as a chimney or funnel. This is a xerophytic feature found in a sclerophyllous flora, of which we have many indications in the fossil Dicotyledonous leaves of Washing Bay still to be recorded.

We hoped that a comparison with other Conifers of the epidermis and stomata of our fossil, though surface views only of it were available, would throw additional light on its affinities. Hildebrand (9) found in all the many Conifers he examined that the stomata are depressed below the general level of the epidermis; that the shape of this depression, pit, or external chamber thus formed varied and was of systematic value. In the great majority of Conifers the one or two rows or rings of epidermal cells surrounding the sunk stoma have their external walls at the same level as the rest of the epidermis. In a few cases they have their external wall below the level. This holds true for *Araucaria*, *Sequoia*, most species of *Abies* and *Picea*. A third type, found more especially in the *Cupressineae*, shows the stoma ringed by a wall formed of the surrounding 4–6 epidermal cells raised above the level of the rest of the epidermis. The wall in some cases, as in *Thuja plicata*, is due to the papillate arched thickening of the outer wall of the cell, which itself, however, is not raised above the general level. The projecting wall

is occasionally, as in *Dammara* and *Libocedrus plumosa* (*L. Doniana*), marked off from the general epidermis by an apparent groove or depression. In all the six species of Libocedrus we examined, except *L. chilensis*, it is of interest to note that the rampart or raised wall is observable, as it is also in our fossil. A similar wall occurs in *Thuja occidentalis*, *Callitris quadrivalvis*, and *Fokienia Hodgkinsi*. The shape of the external chamber in surface view is oblong to square in the fossil as a rule. The same shape is met with in *L. decurrens*. In *L. chilensis*, *L. Doniana*, *L. tetragona*, and *T. occidentalis* the prevailing shape is oblong. *Callitris quadrivalvis*, *C. robusta*, and *Libocedrus macrolepis* show quadrangular or polygonal spaces, twice the size of the fossil ones. *L. Bidwilli* has smaller square-shaped spaces.[1] As cross-sections of the fossil are not available, no comparison of it with the living forms as regards the shape of the outer stomatal chamber, as seen at right angles to the surface, is possible.

Taking into consideration the elongated internode, the expanded whorl, the features represented by the epidermis, stomata, and papillae, we refer the Washing Bay fossil to *Libocedrus salicornioides*. It comes near *L. decurrens* and *L. chilensis*.

Seward (16) has proposed the term *Cupressinocladus* to include fossil vegetative shoots of apparently *Cupressineous* affinity, whose generic character, especially in the absence of cones, is doubtful. As a receptacle for primitive forms of a group, not yet differentiated out into the generic types of to-day, such a term is distinctly useful. In the adoption of such an all-embracing term, there is danger of placing under it, too readily, forms of recognisably distinct generic character, resulting in the introduction of an unnecessary vagueness into our knowledge of the geographical origin and distribution of forms. This seems to be the case with the fossil species of *Libocedrus*, as all the recorded species are referred by Seward to *Cupressinocladus*.

Newberry (13) regards the earliest species known, *Libocedrus cretacea* Hr., of Greenland, as a synonym of his *Thuya cretacea*. It is, however, evident from the illustrations that the two are not identical. Heer's fossil has the *Libocedrus* foliage and habit, Newberry's the Thuya characters. In *Libocedrus* there is intercalated between each false whorl, formed of two pairs of opposite decussate leaves (one pair lateral, the other median or facial), a zone which has been variously described as an elongated internode, an enlarged node, or as derived from the decurrent leaf-bases. *Libocedrus* has thus a more or less jointed stem, in which each joint consists of an internode covered by the decurrent bases of the false whorl of the four more or less adpressed leaves attached at its upper end. Newberry notes this difference in character of the foliage in *Libocedrus* and *Thuya* in his text, but overlooks or ignores it in his comparison of his figure of *T. cretacea* (*op. cit.*, Pl. X, fig. 1a) with that of Heer of *L. cretacea* (*op. cit.*, Pl. XXIX, fig. 3).

So far as we know, the only other *Cupressineous* genus which shows this intercalated elongation of the internode in its youngest twigs is *Fokienia*, Henry and Thomas (8). This genus from East China shows the foliage of *Libocedrus macrolepis* combined with the cone of Cupressus. In *L. tetragona*, which is nearer *Thuya* in habit, the intercalation takes place between each pair of facial and of lateral leaves. A joint in it thus consists of only one pair of divaricate, not adpressed, leaves (either facial or lateral). *Libocedrus* has been described by Sargent (15) as "perhaps too closely connected with Thuya to be considered generically distinct." Such a view must be still more applicable to early Tertiary

[1] We are indebted to Professor A. Henry for recent material of *Libocedrus* for this comparison.

forms. The seeds of *Libocedrus*, *Thuya*, and *Biota* are, however, easily distinguishable, being one-sidedly, two-sidedly, or not at all winged, respectively.

The earliest recorded *Libocedrus*, *L. cretacea*, Heer (4), is from the Upper Cretaceous beds of Atane in Greenland. Its lateral leaves are basally united for some distance. All the leaves are completely adpressed, giving the twigs, which are opposite and 2 mm. wide, parallel sides. Though Heer lays stress on the oppositeness of the lateral branches, the twig he figures (*op. cit.*, Tf. xliii, fig. 1d) shows alternate branches only. Very similar to *L. cretacea* and more highly suggestive of *L. decurrens* of California is *L. Sabiniana*, Heer, from the Spitzbergen Miocene (5). The foliage and opposite branches (most *Cupressineae* have alternate branches) suggest *Libocedrus*. This view is practically confirmed by the occurrence in the same beds of seeds which have the characteristic oblique almost one-sided wing of the *Libocedrus* seed, well shown in Heer's illustrations. In his elaborate investigation of fossil woods from Greenland, Beust (2) finds, by comparison of the fossil and recent woods, that Libocedrus was a common tree in the Lower Miocene of Greenland. His identification of the Greenland fossil as *Libocedrus* is not accepted by Schenk (18). The detailed illustrated account of *L. Sabiniana* occurs in the second volume of Heer's Flora fossilis Arctica (5), but appears to have been overlooked by Seward (15), as his rejection of Heer's identification of the fossil as *Libocedrus* is based on a short note in the seventh volume (6), almost confined to a statement by Heer that the fossil from the Atane Tertiary beds in Greenland agrees with that (the type) from Spitzbergen. He adds that a cone-scale from Hare Island probably belongs to *L. Sabiniana*. *L. gracilis*, Hr. (7), which is also recorded from the Miocene of Spitzbergen, is more like *L. plumosa* (*L. Doniana*) of New Zealand in habit, and *L. Chilensis* of South America in its associated cone-scales.

The most widely distributed fossil assigned to *Libocedrus* is *L. salicornioides* Unger (17). It occurs in numerous localities in the Tertiary beds of Europe, from the Eocene to the Upper Miocene. Opinions have differed considerably as to its interpretation. Thus Saporta (13) finds his material from Armissan in all points identical with the type *Thuytes salicornioides* Unger from Croatia, but adds it is not at all certain that it is a *Libocedrus*. More perfectly preserved fertile specimens may, he thinks, show it is *Viscum*, for which he supplies a then appropriate diagnosis. The occurrence of *L. salicornioides* in the Interbasaltic beds of North-East Ireland is not surprising, as the species is known from the amber beds of the Baltic and from Central Europe. Gardner (3) reported the genus doubtfully as *Libocedrus adpressa* sp. n. from the Woolwich beds at Bromley in Kent. The evidence has since been rejected as insufficient (*op. cit.*, p. 308). It is of interest to add that Ludwig (12) records *Libocedrus salicornioides* from below the columnar basalt of Holzhausen. This material, like ours from Washing Bay, was found in the form of single joints.

PLATE IV.

1. Cone. (× 3.) (A. 64A.)
2. Cone. (× 3.) (A. 64B.)
3. Cone of *L. plumosa* (*L. Doniana*). (× 3.)
4. Tracheids with bordered pits. (× 250.) (A. 64A.)
5. Medullary rays with small pits. (× 250.) (A. 64A.)
6. *L. salicornioides*, Unger. (× 3.) (A. 64A.)
7. Tracheid and medullary ray from foliage shoot. (× 250.) (A. 61.)
8. *L. salicornioides* from Washing Bay. (× 3.) (904′ 6″.)
9. *L. decurrens.* (× 3.)
10. Epidermis of *L. salicornioides* showing stomata and papillae. (× 250.) (904′ 6″ b^3.)

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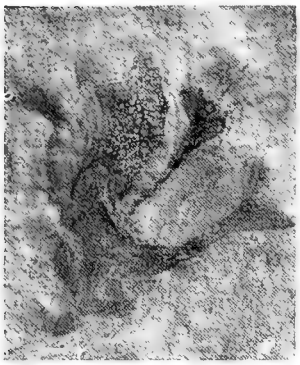

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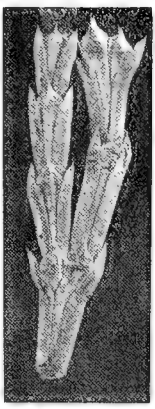

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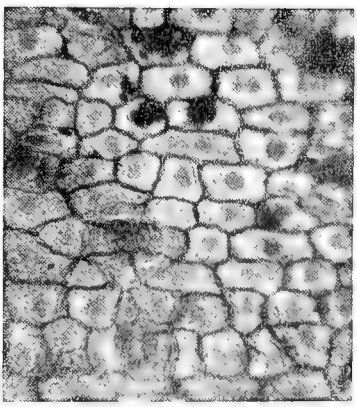


10.

JOHNSON AND GILMORE.

No. 9.

THE ELECTRICAL DESIGN OF A.C. HIGH TENSION TRANSMISSION LINES.

By H. H. JEFFCOTT.

[Read June 27. Printed August 4, 1922.]

1. The recent Reports on the Water-Power Resources of the British Islands have directed attention to the very important economic possibilities of hydro-electric developments in these countries.

Compared with the total estimated power available, very little has yet been done in the direction of harnessing the rivers. It is hoped that, as a result of the recent investigations, active steps will be taken to bring about a great extension in the development of this valuable national asset.

It appears, therefore, to be opportune to direct attention to some of the varied problems that are met with in hydro-electric engineering.

The present paper gives an account of a method for calculating the performance of high tension transmission lines, based on direct evaluation by complex quantities. In it the phase angles and magnitudes of the various vector quantities have not to be separately considered, and the process is almost entirely arithmetical. The method is straightforward, and can largely be systematized into an arithmetical routine, which greatly simplifies the procedure.

The method is illustrated in its application to three-phase overhead transmission lines. It may also be applied to single-phase working.

2. The problem of the electrical design of a transmission line presents itself usually in this way. At one site—the sending end—A.C. electric power is generated, transformed to high tension, and put into the line.

At a second site—the receiving end—the power is to be employed, and usually step-down transformers are located. The number of phases, the frequency, the voltage, and the power to be delivered are known at the receiving end. The power factor of the load is also given, or is approximately estimated. The hours during which power is to be used and the fluctuations in the demand throughout the year are also presumed to be known approximately.

The distance between the two sites is measured, and allowance is made for increased length of the wires due to sag, and also to irregularities in direction of the line both horizontally and vertically.

It is then required to find the best size of conductor to use in the line, and, the size having been decided on, to determine the efficiency of transmission and the variation of voltage at the receiving end under varying load.

3. The choice of size of conductor is governed largely by the consideration that the total annual cost involved in its use shall be the least possible. It must always be provided, however, that the size of the conductor falls within the limits imposed by electrical considerations, such as ohmic heating, voltage

regulation, and corona loss, and also by mechanical considerations, such as the size of wire or cable convenient for manufacture, and its mechanical strength necessitated by its employment as an overhead line exposed to wind and weather. In long spans the question of mechanical strength becomes of great importance.

The annual cost involved in the use of a particular size of conductor (assuming that the electrical and mechanical conditions are satisfied) depends mainly on two items. The first of these is the annual value of the power lost in the line, which is greater the smaller the size of the conductor, and which also depends on the current to be transmitted, and its fluctuations in value at different times throughout the year.

The second item is the interest and depreciation on the cost of the conductor; and this is greater the larger the size of conductor employed. In the case of overhead lines the supporting poles or towers will in general increase in size to a slight extent with increase in weight of the line; but this can probably be sufficiently allowed for by a slight increase in the basic rate for cost of conductor, thus regarding the fluctuating part of the cost of poles as a small percentage added to the cost of the conductor proper.

The best size of conductor then is that for which the sum of these two items of annual cost is as small as possible; and this consideration leads to a mathematical determination of the best area of cross-section.

4. When the size of conductor is known, it is necessary to determine its electrical performance. The line has resistance, inductance, capacity, and leakance. In long distance lines it is necessary to take all these factors into account. In short lines, however, it is usually permissible to neglect the effects of capacity and leakance, with the result that the calculations are greatly simplified.

The solution of the long distance high tension transmission problem commences with Heaviside's general differential equations for the line. Their solution leads to hyperbolic functions of vector quantities. The method to be described below deals directly with these functions, and manipulates them by the laws of complex quantities. The process is illustrated by numerical examples, which indicate how the work can be systematically arranged.

As a result of the calculation of the electrical performance of the line, it may be found, for example, that the voltage regulation is so bad that a change of size of the conductor must be made from that which otherwise gives theoretically the most economic performance. In other cases, the use of auxiliary apparatus, such as synchronous condensers, may be necessary. When the voltage is high and the size of conductor small, the corona loss may become relatively so great that a modification of the design is essential.

5. It is necessary now to set out symbols for the several quantities involved in the calculations. The following notation will be adopted :—

Let x be the distance in miles measured from the sending end to any point in the line where, at a particular instant t seconds from some zero of time, the instantaneous electrical pressure to neutral is v, and the current is c.

Let the length of the line between stations be l miles, and the line be of overhead type. We will suppose that the system is three-phase or single-phase, with a frequency of f cycles per second.

Let the circular conductor (solid or stranded) be of radius a inches, and of cross-sectional area A square inches.

Let the conductors be equally spaced at b inches mutually apart. For each conductor let the resistance be R ohms per mile; the inductance be L henrys per

mile; the leakage conductance be K mhos. per mile; and the permittance or capacity be S farads per mile.

Let V volts be the effective value of the voltage between one conductor and neutral; let C amperes be the effective value of the current per line; and let P watts be the power transmitted per line. Let suffixes 1 and 2 applied to V, C, P indicate their values at the sending end and receiving end of the line respectively.

Let V_c be the disruptive critical voltage to neutral; and let Q watts be the corona loss of power per line. Let θ be the temperature in degrees centigrade of the atmosphere surrounding the line; and h be the barometric pressure in inches of mercury.

Let $\delta = \dfrac{9{\cdot}96h}{273 + \theta}$, and let γ be the surface condition factor for the conductor.

Further, let $\cos\phi$ be the power-factor of the load, let the efficiency of transmission be denoted by η, and the voltage regulation fraction from no to full load by ρ.

Write
$$i = \sqrt{-1}, \quad p = 2\pi f,$$
$$m = \sqrt{(R + ipL)(K + ipS)},$$
and
$$n = \sqrt{\frac{R + ipL}{K + ipS}}.$$

Let the value of power be s pence per kilowatt hour, the value of conductor material be q pence per lb., and the rate of interest and depreciation on the line be r per annum.

6. We will require to use certain identical relations connecting complex quantities, and these are written down here for reference.

$$(a + ix) + (b + iy) = (a + b) + i(x + y),$$
$$(a + ix) - (b + iy) = (a - b) + i(x - y),$$
$$n(a + ix) = na + inx,$$
$$(a + ix)(b + iy) = (ab - xy) + i(bx + ay),$$
$$\frac{a + ix}{b + iy} = \frac{ab + xy}{b^2 + y^2} + i\frac{bx - ay}{b^2 + y^2}.$$

$$(a + ix)^2 = (a^2 - x^2) + i(2ax),$$
$$\sqrt{a + ix} = \frac{1}{\sqrt{2}}\left\{\sqrt{a \pm \sqrt{a^2 + x^2}} + i\sqrt{-a \pm \sqrt{a^2 + x^2}}\right\},$$
$$\sqrt{a - ix} = \frac{1}{\sqrt{2}}\left\{\sqrt{a \pm \sqrt{a^2 + x^2}} - i\sqrt{-a \pm \sqrt{a^2 + x^2}}\right\},$$
$$\cosh(a + ix) = \cosh a \cos x + i \sinh a \sin x,$$
$$\sinh(a + ix) = \sinh a \cos x + i \cosh a \sin x,$$
$$\cosh x = 1 + \frac{x^2}{2!} + \frac{x^4}{4!} + \frac{x^6}{6!} + \ldots$$
$$\sinh x = x + \frac{x^3}{3!} + \frac{x^5}{5!} + \frac{x^7}{7!} + \ldots$$

$$\cos x = 1 - \frac{x^2}{2!} + \frac{x^4}{4!} - \frac{x^6}{6!} + \ldots$$

$$\sin x = x - \frac{x^3}{3!} + \frac{x^5}{5!} - \frac{x^7}{7!} +$$

7. The constants for the single-phase or symmetrical three-phase line are determined by the following formulæ. They refer to one conductor:—

$R = \frac{\cdot 043}{A}$ ohms per mile for copper; or $\frac{\cdot 070}{A}$ ohms per mile for aluminium.

$L = \left(8 + 74{\cdot}1 \log_{10} \frac{b}{a}\right) \times 10^{-5}$ henrys per mile.

$K = 5 \times 10^{-9}$ mhos per mile, approximately.

$S = \frac{3{\cdot}88 \times 10^{-8}}{\log_{10} \frac{b}{a}}$ farads per mile.

8. We commence with the determination of the best size of conductor.

First we seek to determine the amount of power lost in the year and its value. The average loss of power in the line for the year depends, not on the average current, but on the square root of mean square of the current.

We require to know this square root of mean square value of the current for the year.

Let it be C amperes per line. If the demand for power is steady and continuous night and day throughout the year, then C is the value of the current transmitted.

But in general the power demand is not steady and continuous, and it is necessary to estimate the square root of mean square value of the current transmitted.

Thus, for example, if the various values of the current during the year, placed in order of magnitude for their appropriate times, give a straight line, having an arithmetical mean value k and extreme variations of k' from the mean, the effective value of the current may be found thus:—

Let y be the value of the current at any time t. Then

$$y = (k - k') + 2k' \frac{t}{T},$$

where T is the whole period of time or year. The root mean square value of y is

$$\sqrt{\frac{1}{T}\int_0^T y^2 dt} = \sqrt{k^2 + \tfrac{1}{3}k'^2}.$$

As another example: if the variations of the current transmitted throughout the year follow a sine law with a complete period of a year, or if the various values of the current during the year, placed in order of magnitude for their appropriate times, are equivalent to a sine load, we obtain $\sqrt{k^2 + \frac{1}{2}k'^2}$ for the effective value of the current. Here k is the arithmetical mean value of the current for the year, and k' is the amplitude of the sine curve. Then

$$y = k + k' \sin \frac{2\pi t}{T},$$

and the root mean square value of y is

$$\sqrt{\frac{1}{T}\int_0^T y^2 dt} = \sqrt{k^2 + \tfrac{1}{2}k'^2}.$$

Under the assumptions made, this is the effective value of the current so far as power loss in the line throughout the year is concerned.

And similarly for other cases, the effective value of the current must be estimated from the load and time diagram.

For present purposes the average power loss throughout the year is taken as C^2R as a first approximation. If desired, more exact figures, resulting from the determination of the electrical performance of the line, including leakage and corona effects, may be used afterwards for greater accuracy.

Let us suppose copper is the material chosen for the line.

Then $R = \dfrac{\cdot 043\, l}{A}$ ohms.

Hence power loss per line = $C^2R = \cdot 043 \dfrac{C^2 l}{A}$ watts; and the annual value of this power is $\cdot 376\, C^2 \dfrac{l}{A} s$ pence.

The weight of copper is $20{,}300\, Al$ lbs. The annual charge for interest and depreciation on the copper is therefore $20{,}300\, Alqr$ pence. The total annual cost in pence is then $\cdot 376 C^2 \dfrac{l}{A} s + 20{,}300\, Alqr$, and this is to be a minimum.

Hence $\dfrac{C^2 s}{A} + 53{,}900\, Aqr$ is to be a minimum. To satisfy this requirement we find A is given by

$$\frac{C^2 s}{A^2} - 53{,}900\, qr = 0,$$

or

$$A^2 = \cdot 0000185 C^2 \frac{s}{qr},$$

or

$$A = \cdot 0043 C \sqrt{\frac{s}{qr}}.$$

If $q = 15$ pence per lb., $r = 8$ per cent., $s =$ one penny per unit, we obtain $A = \cdot 00392\, C$.

Similarly for other values of q, r, s.

This equation gives us the most economic sectional area of copper conductor, and it will be noted that the current density in the conductor is quite small, so that there is no risk of overheating.

If aluminium be used for the line, we have $R = \dfrac{\cdot 070\, l}{A}$ ohms. The weight of aluminium is $6120\, Al$ lbs. Hence we find that $\dfrac{C^2 s}{A} + 10{,}000\, Aqr$ is to be a minimum. Therefore $A = \cdot 010\, C \sqrt{\dfrac{s}{qr}}$ for aluminium conductor.

9. We may now presume that we know the size of conductor to be used. We seek to determine its electrical performance.

The equations connecting the voltage v and current c at any point distant x from the sending end of the line are

$$-\frac{dv}{dx} = \left(R + L\frac{d}{dt}\right)c,$$
$$-\frac{dc}{dx} = \left(K + S\frac{d}{dt}\right)v.$$

We are mainly concerned with the space variations of v and c along the wire. We suppose a sine wave of voltage to be impressed on the line at the sending end.

Differentiating the first of these equations with respect to x and using the second, we obtain

$$\frac{d^2v}{dx^2} = \left(R + L\frac{d}{dt}\right)\left(K + S\frac{d}{dt}\right)v.$$

We may write ip for $\frac{d}{dt}$ in these equations, changing v, the instantaneous voltage, into v_0, the maximum voltage at the position considered, so that

$$\frac{d^2v_0}{dx^2} = (R + ipL)(K + ipS)v_0 = m^2v_0.$$

The solution of this equation is

$$v_0 = A_0e^{mx} + B_0e^{-mx}.$$

If V and C be the effective values of voltage and current at the position x, we have

$$V = Ae^{mx} + Be^{-mx},$$

where A and B are constants independent of x.

Also $$-\frac{dv_0}{dx} = (R + ipL)c_0 \qquad \text{and} \qquad \frac{dv_0}{dx} = m(A_0e^{mx} - B_0e^{-mx}).$$

Hence $$c_0 = \frac{m}{R + ipL}(-A_0e^{mx} + B_0e^{-mx}).$$

But $$\frac{R + ipL}{m} = n, \qquad \therefore \quad nc_0 = -A_0e^{mx} + B_0e^{-mx}.$$

and $$nC = -Ae^{mx} + Be^{-mx}.$$

At the sending end of the line $x = 0$, and therefore

$$V_1 = A + B, \qquad nC_1 = -A + B;$$
$$\therefore\ 2A = V_1 - nC_1,$$
$$2B = V_1 + nC_1.$$

Hence

$$V = \tfrac{1}{2}V_1(e^{mx} + e^{-mx}) - \tfrac{1}{2}nC_1(e^{mx} - e^{-mx}) = V_1\cosh mx - nC_1\sinh mx.$$

Likewise

$$C = \tfrac{1}{2}C_1(e^{mx} + e^{-mx}) - \frac{V_1}{2n}(e^{mx} - e^{-mx}) = C_1\cosh mx - \frac{V_1}{n}\sinh mx.$$

Put $x = l$, and we find

$$V_2 = V_1 \cosh ml - nC_1 \sinh ml,$$

$$C_2 = C_1 \cosh ml - \frac{V_1}{n} \sinh ml.$$

These equations give

$$V_1 = V_2 \cosh ml + nC_2 \sinh ml,$$

$$C_1 = C_2 \cosh ml + \frac{V_2}{n} \sinh ml,$$

which may also be obtained similarly to the foregoing equations by putting $x = l$ in the general equations which determine A and B.

The power put in per phase is $P_1 = V_1 C_1 \cos(\phi_1 - \phi_1')$, where ϕ_1 and ϕ_1' are the phase angles of V_1 and C_1 respectively referred to the phase of C_2 as standard;

$$\therefore P_1 = V_1 \cos \phi_1 \times C_1 \cos \phi_1' + V_1 \sin \phi_1 \times C_1 \sin \phi_1'.$$

If the receiving end is open,

$$C_2 = 0, \quad V_1' = V_2' \cosh ml, \quad \text{and} \quad C_1' = \frac{V_2'}{n} \sinh ml,$$

which is the charging current.

If the receiving end is short-circuited, $V_2 = 0$. Then

$$V_1'' = nC_2'' \sinh ml \quad \text{and} \quad C_1'' = C_2'' \cosh ml.$$

We thus find for the voltage regulation at the receiving end that the ratio of the voltage at the receiving end, on throwing off the load, to the full load voltage is

$$\rho_2 = \frac{V_2'}{V_2} = \frac{V_1}{V_2 \cosh ml}, \text{ since } V_1 = V_1',$$

the transmitting voltage remaining constant.

The efficiency is the ratio of the power taken from the line at the receiving end to that put in at the sending end, or $\eta = \frac{P_2}{P_1}$.

10. We now turn attention to corona loss, or the loss of power from the line occasioned by the discharge from the conductor due to ionization of the surrounding atmosphere. Above a certain disruptive critical voltage this loss can take place. The magnitude of the loss depends on the amount by which the line voltage exceeds the disruptive critical voltage, and, to some extent, on the temperature and pressure of the atmosphere. For single-phase and symmetrical three-phase lines it has been shown by Peek (Trans. Am. Inst. El. Eng., vol. xxx, 1911) to be expressible in the form

$$Q = 5{\cdot}53 \times \frac{fl}{\delta} \times \sqrt{\frac{a}{b}} \times (V - V_c)^2 \times 10^{-6} \text{ watts},$$

where the disruptive critical voltage V_c is given by

$$V_c = 123{,}400\ \gamma \delta a \log_{10} \frac{b}{a}$$

effective volts to neutral.

δ depends on the temperature T and pressure h of the atmosphere surrounding the line, and is given by

$$\delta = \frac{9{\cdot}96h}{273 + \theta},$$

if θ is expressed in degrees centigrade and h in inches of mercury.

γ is a factor depending on the roughness of surface of the conductor, and is 1 for polished round wires, 0·98 to 0·93 for roughened or weathered wires, 0·87 to 0·83 for seven-strand cable.

a, b, f, l, V have the same significations as before.

11. We can now set out in systematic form the scheme of calculation of a single-phase or symmetrical three-phase line.

We divide the scheme into five sections—(1) a statement of the data of the problem, (2) the determination of the most economic section of conductor, (3) the calculation of the line constants, (4) the calculation of the electrical quantities required, and (5) the determination of the corona loss.

(1) *Data* for one line.

Frequency, f cycles per second.
Voltage between wires at receiving end.
Power delivered.
Power factor of load, $\cos \phi$.
Effective average value of current C' delivered throughout the year.
Length of line, l miles.
Conductors spaced b inches mutually apart.
Cost of conductor material, q pence per lb., and rate of interest and depreciation thereon, r per annum.
Value of power, s pence per unit.
Average atmospheric temperature, θ degrees centigrade, and pressure, h inches of mercury.

(2) *Determination of the most economical size of conductor.* (See paragraph 8.)

For copper, $A' = {\cdot}0043\ C' \sqrt{\frac{s}{qr}}$ sq. inches.

For aluminium, $A' = {\cdot}010\ C' \sqrt{\frac{s}{qr}}$ sq. inches.

Choose the nearest standard size of conductor A sq. inches.

$a = \sqrt{\frac{A}{\pi}}$ inches for solid conductor.

(3) *Calculation of line constants.*

$R = \frac{{\cdot}043}{A}$ ohms per mile for copper $= \frac{{\cdot}070}{A}$ ohms per mile for aluminium.

$L = \left(8 + 74{\cdot}1 \times \log_{10} \frac{b}{a}\right) \times 10^{-5}$ henrys per mile.

$K = 5 \times 10^{-9}$ mhos per mile.

$$S = \frac{3{\cdot}88 \times 10^{-8}}{\log_{10}\frac{b}{a}} \text{ farads per mile.}$$

$$p = 2\pi f.$$

$$m = \sqrt{(R + ipL)(K + ipS)}.$$

$$n = \sqrt{\frac{R + ipL}{K + ipS}}.$$

$$\cosh ml = \cosh(x + iy) = \cosh x \cos y + i \sinh x \sin y = \xi + i\zeta,$$

$$\sinh ml = \sinh(x + iy) = \sinh x \cos y + i \cosh x \sin y.$$

$$\cosh x = 1 + \frac{x^2}{2!} + \frac{x^4}{4!} + \frac{x^6}{6!} + \ldots.$$

$$\sinh x = x + \frac{x^3}{3!} + \frac{x^5}{5!} + \frac{x^7}{7!} + \ldots.$$

$n \sinh ml.$

$$\frac{\sinh ml}{n}.$$

(4) *Calculation of electrical quantities.*

Refer all vector quantities to the phase of the current C_2 as standard.

The phase angles of the various vector quantities may readily be obtained if desired, since we know the components of the complex quantities.

For a single-phase line :—

V_2 = ½ × voltage between wires at receiving end $= \lambda + i\mu$, where $\mu = \lambda \tan \phi$.

P_2 = ½ × total power delivered.

$$C_2 = \frac{P_2}{V_2 \cos \phi}.$$

In a symmetrical three-phase line :—

V_2 = voltage to neutral at receiving end $= \frac{1}{\sqrt{3}}$ × voltage between wires at receiving end $= \lambda + i\mu$, where $\mu = \lambda \tan \phi$.

P_2 = power transmitted per phase = ⅓ × total power delivered.

C_2 = current per phase delivered $= \frac{P_2}{V_2 \cos \phi}$.

Then

$$V_1 = V_2 \cosh ml + nC_2 \sinh ml = \alpha + i\beta.$$

Voltage to neutral at sending end $= \sqrt{\alpha^2 + \beta^2}$.

$$C_1 = C_2 \cosh ml + \frac{V_2}{n} \sinh ml = \chi + i\psi.$$

Current per phase at sending end $= \sqrt{\chi^2 + \psi^2}$.

$P_1 = \alpha\chi + \beta\psi$.

η = efficiency $= \frac{P_2}{P_1}$.

$$V_2' = \frac{V_1}{\cosh ml} = \frac{\alpha + i\beta}{\xi + i\zeta} = \sigma + i\tau.$$

$$V_2 = \lambda + i\mu.$$

$$\rho_2 = \frac{V_2'}{V_2} = \sqrt{\frac{\sigma^2 + \tau^2}{\lambda^2 + \mu^2}}.$$

(5) *Determination of corona loss.*

For a single-phase or symmetrical three-phase system

γ = 1 for polished round wires,
= ·98 to ·93 for roughened or weathered wires,
= ·87 to ·83 for seven-strand cables.

$$\delta = \frac{9{\cdot}96h}{273 + \theta}.$$

$$V_c = 123{,}400\,\gamma\delta a \log_{10}\frac{b}{a} \text{ volts.}$$

$$\text{Loss } Q = 5{\cdot}53 \times \frac{fl}{\delta} \times \sqrt{\frac{a}{b}} \times (V - V_c)^2 \times 10^{-5} \text{ watts per line.}$$

12. When the line is comparatively short and the voltage not especially high, the capacity and leakage of the line may be neglected. The calculations then become greatly simplified. The efficiency and regulation are calculated by the following formulæ:—

$$V_1^2 = (V_2 \cos\phi + CRl)^2 + (V_2 \sin\phi + CpLl)^2.$$

$$\eta = \frac{1}{1 + \frac{CRl}{V_2 \cos\phi}}.$$

$$\rho_2 = \frac{V_1}{V_2}.$$

13. Illustrations will now be given of the procedure in particular cases. It is convenient to follow the routine arrangement of the calculations employed below.

(1) *Data* for one line.

Line, symmetrical three-phase.
Frequency, 50 cycles per second.
Voltage between wires at receiving end, 66,000 volts.
Greatest power delivered, 8,250 kw.
Power factor of load, $\cos\phi$ = ·85.
Effective average value of current delivered throughout a year, 31 amps. per phase, being 1·225 × mean amps. for year, since the load-curve is approximately equivalent to a sine-curve in which $k' = k$. (See paragraph 8.)
Length of line, l = 25 miles.
Conductors of copper, spaced b = 108 inches mutually apart.
Cost of conductor material, q = 15 pence per lb.
Interest and depreciation rate thereon, r = ·08.
Value of power, s = 1 penny per unit.
Average atmospheric temperature, θ = 20° C., and barometric pressure, h = 30 inches.

(2) *Determination of most economic size of conductor.*

$C' = 31$ amps.

$$A' = \cdot0043 \times 31 \times \sqrt{\frac{1}{15 \times \cdot08}} = \cdot122 \text{ sq. inch.}$$

Choose conductor 19/13, having $A = \cdot129$ sq. inch; $\therefore a = 0\cdot23$ inch.
The current transmitted at maximum load is 85 amperes, and so the current density in the conductor is allowable.

(3) *Calculation of line constants.*

$$R = \frac{\cdot043}{A} = \cdot34 \text{ ohms per mile.}$$

$b = 108$ inches, $a = \cdot23$ inch.

$$\frac{b}{a} = 470\,; \quad \log_{10}\frac{b}{a} = 2\cdot67.$$

$$L = (8 + 74\cdot1 \times 2\cdot67) \times 10^{-5} = 2\cdot06 \times 10^{-3} \text{ henrys per mile.}$$

$$K = 5 \times 10^{-9} \text{ mhos per mile.}$$

$$S = \frac{3\cdot88 \times 10^{-8}}{2\cdot67} = 1\cdot45 \times 10^{-8} \text{ farads per mile.}$$

$$p = 2\pi \times 50 = 314\cdot16.$$

$$pL = \cdot648.$$

$$pS = 4\cdot56 \times 10^{-6}.$$

$$m = \sqrt{(\cdot34 + i \times \cdot648)\,(5 \times 10^{-9} + i \times 4\cdot56 \times 10^{-6})}.$$

Multiplying and evaluating these complex quantities by the rules given in paragraph 6, we find

$$m = \sqrt{-2\cdot958 + i \times 1\cdot558} \times 10^{-3} = \cdot000437 + i \times \cdot00177.$$

$$n = \sqrt{\frac{\cdot34 + i \times \cdot648}{5 \times 10^{-9} + i \times 4\cdot56 \times 10^{-6}}} = 100\sqrt{14\cdot24 - i \times 7\cdot47}$$

$$= 388 - i \times 96\cdot2.$$

$$ml = 25 \times (\cdot000437 + i \times \cdot00177) = \cdot01092 + i \times \cdot0442.$$

$$\cosh ml = \cosh \cdot01092 \cos \cdot0442 + i \sinh \cdot01092 \sin \cdot0442.$$

$$\cosh \cdot01092 = 1 + \cdot00006 = 1\cdot00006.$$

$$\sinh \cdot01092 = \cdot01092\,(1 + \cdot00002) = \cdot01092.$$

$$\cos \cdot0442 = \cdot999.$$

$$\sin \cdot0442 = \cdot0442.$$

Hence

$$\cosh ml = 1\cdot00006 \times \cdot999 + i \times \cdot01092 \times \cdot0442 = \cdot999 + i \times \cdot000483.$$

$$\sinh ml = \sinh \cdot01092 \cos \cdot0442 + i \times \cosh \cdot01092 \sin \cdot0442$$

$$= \cdot01092 \times \cdot999 + i \times 1\cdot00006 \times \cdot0442$$

$$= \cdot01091 + i \times \cdot0442.$$

$$n \sinh ml = (388 - i \times 96\cdot2)\,(\cdot01091 + i \times \cdot0442) = 8\cdot49 + i \times 16\cdot1.$$

$$\frac{\sinh ml}{n} = \frac{\cdot01091 + i \times \cdot0442}{388 - i \times 96\cdot2} = (-\cdot0625 + i \times 113\cdot9) \times 10^{-6}.$$

(4) *Calculation of electrical quantities.*

Refer all vector quantities to C_2 as standard.

$$V_2 = \frac{1}{\sqrt{3}} \times 66{,}000 = 38{,}000 \text{ volts at } \cos\phi = \cdot 85, = 32{,}300 + i \times 20{,}000.$$

$$P_2 = \tfrac{1}{3} \times 8{,}250 = 2{,}750 \text{ kw.}$$

$$C_2 = \frac{2{,}750{,}000}{38{,}000 \times \cdot 85} = 85 \text{ amps.}$$

$$V_1 = V_2 \cosh ml + nC_2 \sinh ml.$$

$$V_2 \cosh ml = (32{,}300 + i \times 20{,}000)(\cdot 999 + i \times \cdot 000483) = 32{,}260 + i \times 19{,}996.$$

$$C_2 n \sinh ml = 85 \times (8 \cdot 49 + i \times 16 \cdot 1) = 722 + i \times 1{,}370.$$

$$\therefore \quad V_1 = 32{,}982 + i \times 21{,}366 = 39{,}300 \text{ volts to neutral at sending end.}$$

$$C_1 = C_2 \cosh ml + \frac{V_2}{n} \sinh ml.$$

$$C_2 \cosh ml = 85 \times (\cdot 999 + i \times \cdot 000483) = 84 \cdot 9 + i \times \cdot 0411.$$

$$\frac{V_2}{n} \sinh ml = (32{,}300 + i \times 20{,}000)(- \cdot 0625 + i \times 113 \cdot 9) \times 10^{-6} = - 2 \cdot 28 + i \times 3 \cdot 679.$$

$$\therefore \quad C_1 = 82 \cdot 62 + i \times 3 \cdot 72 = 82 \cdot 8 \text{ amps. per phase at sending end.}$$

$$P_1 = 32{,}982 \times 82 \cdot 62 + 21{,}366 \times 3 \cdot 72 \text{ watts} = 2{,}725{,}000 + 79{,}500 = 2804 \text{ kw.}$$

$$\eta = \frac{2{,}750}{2{,}804} = 0 \cdot 98.$$

$$V_2' = \frac{32{,}982 + i \times 21{,}366}{\cdot 999 + i \times \cdot 000483} = 33{,}020 + i \times 21{,}370.$$

$$\rho_2 = \sqrt{\frac{33{,}020^2 + 21{,}370^2}{32{,}300^2 + 20{,}000^2}} = 1 \cdot 035.$$

It is thus seen that the electrical performance, calculated for maximum load, is very satisfactory.

(5) *Determination of corona loss.*

Take $\gamma = \cdot 83$ for 19/13 cable.

$$\delta = \frac{9 \cdot 96 \times 30}{273 + 20} = 1 \cdot 02.$$

$$V_c = 123{,}400 \times \cdot 83 \times 1 \cdot 02 \times \cdot 23 \times 2 \cdot 67 = 64{,}300.$$

As the working voltage to neutral (39,300) is less than this figure, there will be no corona loss.

14. Seeing that the example in the last paragraph represents a comparatively short line, and the voltage is not extremely high, it is likely the simpler formulæ of paragraph 12, in which K and S are omitted, would lead to close results.

This may be verified.

$$V_1^2 = (38{,}000 \times \cdot 85 + 85 \times \cdot 34 \times 25)^2 + (38{,}000 \times \cdot 527 + 85 \times \cdot 648 \times 25)^2$$

$$= (32{,}300 + 722)^2 + (20{,}000 + 1{,}375)^2 = 1 \cdot 546 \times 10^9;$$

$$\therefore \quad V_1 = 39{,}300.$$

$$\eta = \frac{1}{1 + \frac{722}{32{,}300}} = \cdot 978.$$

$$\rho_2 = \frac{39{,}200}{38{,}000} = 1\cdot 034.$$

Thus these results are in close agreement with those of paragraph 13.

15. As an example of long distance transmission, we will take the same data as in paragraph 13, with the exception of the length of line, which we will now suppose to be 200 miles.

The calculation of the several quantities proceeds exactly as before till we come to ml. Then

(3) $ml = 200 \times (\cdot 000437 + i \times \cdot 00177) = \cdot 0874 + i \times \cdot 354.$

$\cosh ml = \cosh \cdot 0874 \cos \cdot 354 + i \sinh \cdot 0874 \sin \cdot 354.$

$\cosh \cdot 0874 = 1 + \cdot 003819 + \cdot 0000006 = 1\cdot 00382.$

$\sinh \cdot 0874 = \cdot 0874\ (1 + \cdot 001273) = \cdot 08751.$

$\cos \cdot 354 = \cdot 9380.$

$\sin \cdot 354 = \cdot 3467.$

$\therefore \cosh ml = 1\cdot 00382 \times \cdot 938 + i \times \cdot 08751 \times \cdot 3467 = \cdot 9416 + i \times \cdot 03034.$

$\sinh ml = \sinh \cdot 0874 \cos \cdot 354 + i \times \cosh \cdot 0874 \times \sin \cdot 354$

$= \cdot 08751 \times \cdot 938 + i \times 1\cdot 00382 \times \cdot 3467 = \cdot 0821 + i \times \cdot 348.$

$n \sinh ml = (388 - i \times 96\cdot 2)\ (\cdot 0821 + i \times \cdot 348) = 65\cdot 32 + i \times 127\cdot 1$

$$\frac{\sinh ml}{n} = \frac{\cdot 0821 + i \times \cdot 348}{388 - i \times 96\cdot 2} = (-\ 10\cdot 18 + i \times 894\cdot 3) \times 10^{-6}.$$

(4) *Calculation of electrical quantities.*

V_2, P_2, C_2 are the same as in paragraph 13.

$V_1 = V_2 \cosh ml + nC_2 \sinh ml.$

$V_2 \cosh ml = (32{,}300 + i \times 20{,}000)\ (\cdot 9416 + i \times \cdot 03034) = 29{,}810 + i \times 19{,}810.$

$C_2 n \sinh ml = 85 \times (65\cdot 32 + i \times 127\cdot 1) = 5{,}550 + i \times 10{,}800.$

$\therefore V_1 = 35{,}360 + i \times 30{,}610 = 46{,}750$ volts to neutral at sending end.

$$C_1 = C_2 \cosh ml + \frac{V_2}{n} \sinh ml.$$

$C_2 \cosh ml = 85\ (\cdot 9416 + i \times \cdot 03034) = 80\cdot 03 + i \times 2\cdot 579.$

$$V_2 \frac{\sinh ml}{n} = (32{,}300 + i \times 20{,}000)\ (-\ 10\cdot 18 + i \times 894\cdot 3) \times 10^{-6}$$

$$= -\ 18\cdot 22 + i \times 28\cdot 68.$$

$\therefore C_1 = 61\cdot 81 + i \times 31\cdot 26 = 69\cdot 3$ amps. per phase at sending end.

$P_1 = 35{,}360 \times 61\cdot 81 + 30{,}610 \times 31\cdot 26 = 2{,}183{,}000 + 957{,}000$ watts

$= 3{,}140$ kw.

$$\eta = \frac{2{,}750}{3{,}140} = \cdot 876.$$

$$V_2' = \frac{35{,}360 + i \times 30{,}610}{\cdot 9416 + i \times \cdot 03034} = 38{,}470 + i \times 31{,}250.$$

$$\rho_2 = \sqrt{\frac{38{,}470^2 + 31{,}250^2}{32{,}300^2 + 20{,}000^2}} = 1\cdot 305.$$

(5) The corona loss is nil, as the disruptive critical voltage exceeds the operating voltage.

Owing to the poor efficiency and bad regulation, it would be better to redesign the line for a higher voltage.

16. Using the approximate method of paragraph 12 for the example of the last paragraph, we obtain the following results:—

$$V_1^2 = (38{,}000 \times \cdot 85 + 85 \times \cdot 34 \times 200)^2 + (38{,}000 \times \cdot 527 + 85 \times \cdot 648 \times 200)^2$$
$$= (32{,}300 + 5{,}780)^2 + (20{,}000 + 11{,}000)^2 = 2 \cdot 411 \times 10^9.$$

$$\therefore V_1 = 49{,}100.$$

$$\eta = \frac{1}{1 + \frac{5780}{32300}} = \cdot 849.$$

$$\rho_2 = \frac{49{,}100}{38{,}000} = 1 \cdot 29.$$

Comparing these results with those of paragraph 15, we find:—

	Par. 15	Par. 16
Voltage to neutral at sending end,	46,750	49,100
Efficiency at greatest load,	·876	·849
Regulation fraction,	1·305	1·29

These results are somewhat discordant, and consequently the more accurate method of paragraph 15 is preferable here, except for a rough approximation.

17. Data as in paragraph 15, but let voltage between wires at receiving end be 100,000 volts, and spacing between conductors be 120 inches.

(2) For the same power delivered, the effective mean current is now

$$C' = 31 \times \frac{66}{100} = 20 \cdot 5 \text{ amps.}$$

The most economic size is then

$$A' = \cdot 00392 \times 20 \cdot 5 = \cdot 0805 \text{ sq. inch.}$$

Choose conductor 19/14, having $A = \cdot 097$ sq. inch, and $a = 0 \cdot 20$ inch.

(3) *Calculation of line constants.*

$$R = \frac{\cdot 043}{A} = \cdot 45.$$

$$a = \cdot 2, \quad b = 120.$$

$$\frac{b}{a} = 600, \quad \log_{10} \frac{b}{a} = 2 \cdot 78.$$

$$L = (8 + 74 \cdot 1 \times 2 \cdot 78) \times 10^{-5} = 2 \cdot 14 \times 10^{-3}.$$

$$K = 5 \times 10^{-9}.$$

$$S = \frac{3 \cdot 88 \times 10^{-8}}{2 \cdot 78} = 1 \cdot 395 \times 10^{-8},$$

$$p = 314{\cdot}16.$$
$$pL = {\cdot}673.$$
$$pS = 4{\cdot}4 \times 10^{-6}.$$
$$m = \sqrt{({\cdot}45 + i \times {\cdot}673)({\cdot}005 + i \times 4{\cdot}4)} \times 10^{-3}$$
$$= \sqrt{-2{\cdot}958 + i \times 1{\cdot}983} \times 10^{-3} = {\cdot}000548 + i \times {\cdot}001806.$$
$$n = \sqrt{\frac{{\cdot}45 + i \times {\cdot}673}{{\cdot}005 + i \times 4{\cdot}4}} \times 10^{3} = \sqrt{{\cdot}153 - i \times {\cdot}102} \times 10^{3} = 410 - i \times 124{\cdot}4$$
$$ml = 200\,({\cdot}000548 + i \times {\cdot}001806) = {\cdot}1096 + i \times {\cdot}3612.$$
$$\cosh ml = \cosh {\cdot}1096 \cos {\cdot}3612 + i \sinh {\cdot}1096 \sin {\cdot}3612.$$
$$\cosh {\cdot}1096 = 1 + {\cdot}0060 + {\cdot}000006 = 1{\cdot}0060.$$
$$\sinh {\cdot}1096 = {\cdot}1096 + {\cdot}00022 = {\cdot}10982.$$
$$\cos {\cdot}3612 = {\cdot}9354.$$
$$\sin {\cdot}3612 = {\cdot}3535.$$
$$\cosh ml = 1{\cdot}006 \times {\cdot}9354 + i \times {\cdot}10982 \times {\cdot}3535 = {\cdot}941 + i \times {\cdot}0388.$$
$$\sinh ml = {\cdot}10982 \times {\cdot}9354 + i \times 1{\cdot}006 \times {\cdot}3535 = {\cdot}1027 + i \times {\cdot}356.$$
$$n \sin ml = (410 - i \times 124{\cdot}4)({\cdot}1027 + i \times {\cdot}356) = 86{\cdot}4 + i \times 133{\cdot}2.$$
$$\frac{\sinh ml}{n} = \frac{{\cdot}1027 + i \times {\cdot}356}{410 - i \times 124{\cdot}4} = (-12{\cdot}27 + i \times 865) \times 10^{-6}.$$

(4) *Calculation of electrical quantities.*

$$V_2 = \frac{100{,}000}{\sqrt{3}} = 57{,}700 \quad \text{at } \cos\phi = {\cdot}85, = 49{,}000 + i \times 30{,}400.$$
$$P_2 = \frac{8{,}250}{3} = 2{,}750 \text{ kw.}$$
$$C_2 = \frac{2{,}750{,}000}{49{,}000} = 56{\cdot}1 \text{ amps.}$$
$$V_1 = V_2 \cosh ml + nC_2 \sinh ml.$$
$$V_2 \cosh ml = (49{,}000 + i \times 30{,}400)({\cdot}941 + i \times {\cdot}0388) = 44{,}920 + i \times 30{,}500.$$
$$C_2 n \sinh ml = 56{\cdot}1 \times (86{\cdot}4 + i \times 133{\cdot}2) = 4{,}850 + i \times 7{,}480.$$
$$\therefore \quad V_1 = 49{,}770 + i \times 37{,}980 = 62{,}600 \text{ volts to neutral at sending end.}$$
$$C_1 = C_2 \cosh ml + \frac{V_2}{n} \sinh ml.$$
$$C_2 \cosh ml = 56{\cdot}1\,({\cdot}941 + i \times {\cdot}0388) = 52{\cdot}8 + i \times 2{\cdot}18.$$
$$\frac{V_2}{n} \sinh ml = (49{,}000 + i \times 30{,}400)(-12{\cdot}27 + i \times 865) \times 10^{-6} = -26{\cdot}9 + i \times 42{\cdot}08.$$
$$\therefore \quad C_1 = 25{\cdot}9 + i \times 44{\cdot}26 = 51{\cdot}3 \text{ amps. per phase.}$$
$$P_1 = 49{,}770 \times 25{\cdot}9 + 37{,}980 \times 44{\cdot}26 = 1{,}290{,}000 + 1{,}680{,}000 = 2{,}970 \text{ kw.}$$
$$\eta = \frac{2{,}750}{2{,}970} = {\cdot}926.$$
$$V_2' = \frac{49{,}770 + i \times 37{,}980}{{\cdot}941 + i \times {\cdot}0388} = 54{,}450 + i \times 38{,}150.$$
$$\rho_2 = \sqrt{\frac{54{,}450^2 + 38{,}150^2}{49{,}000^2 + 30{,}400^2}} = 1{\cdot}152.$$

(5) *Determination of corona loss.*

$$\gamma = 0{\cdot}83 \text{ for } 19/14 \text{ cable.}$$

$$\delta = 1{\cdot}02.$$

$$V_c = 123{,}400 \times {\cdot}83 \times 1{\cdot}02 \times {\cdot}2 \times 2{\cdot}78 = 58{,}000.$$

$$Q = 5{\cdot}53 \times \frac{50 \times 200}{1{\cdot}02} \times \frac{1}{24{\cdot}5} \times (62{,}600 - 58{,}000)^2 \times 10^{-6} = 46{,}800 \text{ watts}$$

$$= 46{\cdot}8 \text{ kw.}$$

We now revise the efficiency estimate. The approximate total power put in is

$$2{,}970 + 47 = 3{,}017.$$

$$\eta = \frac{2{,}750}{3{,}017} = {\cdot}912.$$

18. As a further example we will assume a still higher voltage, with a view to illustrating the great increase in corona loss at high voltages.

Data as in paragraph 15, but let the voltage between wires at the receiving end be 132,000 volts, and the spacing between conductors be 120 inches.

(2) For the same power delivered, the effective mean current per phase is now (see paragraph 13) halved, or $C' = 15{\cdot}5$ amps.

The most economic size is then

$$A' = {\cdot}00392 \times 15{\cdot}5 = {\cdot}061 \text{ sq. inch.}$$

Choose conductor 7/12 having $A = {\cdot}0606$ sq. inch. $\therefore\ a = {\cdot}156$ inch.

(3) *Calculation of line constants.*

$$R = \frac{{\cdot}043}{A} = {\cdot}71.$$

$$a = {\cdot}156, \quad b = 120, \quad \frac{b}{a} = 770.$$

$$\log_{10}\frac{b}{a} = 2{\cdot}89.$$

$$L = (8 + 74{\cdot}1 \times 2{\cdot}89) \times 10^{-5} = 2{\cdot}22 \times 10^{-3}.$$

$$K = 5 \times 10^{-9}.$$

$$S = \frac{3{\cdot}88 \times 10^{-8}}{2{\cdot}89} = 1{\cdot}343 \times 10^{-8}.$$

$$p = 2\pi \times 50 = 314{\cdot}16.$$

$$pL = {\cdot}699.$$

$$pS = 4{\cdot}23 \times 10^{-6}.$$

$$m = \sqrt{({\cdot}71 + i \times {\cdot}699)\,(5 \times 10^{-9} + i \times 4{\cdot}23 \times 10^{-6})}.$$

$$= \sqrt{-2{\cdot}96 + i \times 3{\cdot}01} \times 10^{-3} = {\cdot}000794 + i \times {\cdot}00189.$$

$$n = \sqrt{\frac{{\cdot}71 + i \times {\cdot}699}{5 \times 10^{-9} + i \times 4{\cdot}23 \times 10^{-6}}}$$

$$= \sqrt{{\cdot}165 - i \times {\cdot}168} \times 10^{3} = 448 - i \times 188.$$

$$ml = 200\,(\cdot000794 + i \times \cdot00189) = \cdot159 + i \times \cdot378.$$
$$\cosh ml = \cosh \cdot159 \cos \cdot378 + i \sinh \cdot159 \times \sin \cdot378.$$
$$\cosh \cdot159 = 1 + \cdot01265 + \cdot000027 = 1\cdot01268.$$
$$\sinh \cdot159 = \cdot159 + \cdot00067 + \cdot0000008 = \cdot15967.$$
$$\cos \cdot378 = \cdot9294.$$
$$\sin \cdot378 = \cdot3691.$$
$$\cosh ml = 1\cdot01268 \times \cdot9294 + i \times \cdot15967 \times \cdot3691 = \cdot941 + i \times \cdot059.$$
$$\sinh ml = \cdot15967 \times \cdot9294 + i \times 1\cdot01268 \times \cdot3691 = \cdot1482 + i \times \cdot374.$$
$$n \sinh ml = (448 - i \times 188)(\cdot1482 + i \times \cdot374) = 136\cdot8 + i \times 139\cdot6.$$
$$\frac{\sinh ml}{n} = \frac{\cdot1482 + i \times \cdot374}{448 - i \times 188} = (-16\cdot07 + i \times 826) \times 10^{-6}.$$

(4) *Calculation of electrical quantities.*

$$V_2 = \frac{132{,}000}{\sqrt{3}} = 76{,}000, \text{ at } \cos \phi = \cdot85, = 64{,}600 + i \times 40{,}000.$$
$$P_2 = \frac{8{,}250}{3} = 2{,}750 \text{ kw.}$$
$$C_2 = \frac{2{,}750{,}000}{76{,}000 \times \cdot85} = 42\cdot5 \text{ amps.}$$
$$V_1 = V_2 \cosh ml + nC_2 \sinh ml.$$
$$V_2 \cosh ml = (64{,}600 + i \times 40{,}000)(\cdot941 + i \times \cdot059) = 58{,}440 + i \times 41{,}450.$$
$$C_2 n \sinh ml = 42\cdot5\,(136\cdot8 + i \times 139\cdot6) = 5{,}810 + i \times 5{,}930.$$
$$\therefore V_1 = 64{,}250 + i \times 47{,}380 = 79{,}900 \text{ volts to neutral at sending end.}$$
$$C_1 = C_2 \cosh ml + \frac{V_2}{n} \sinh ml.$$
$$C_2 \cosh ml = 42\cdot5\,(\cdot941 + i \times \cdot059) = 40 + i \times 2\cdot51.$$
$$\frac{V_2}{n} \sinh ml = (64{,}600 + i \times 40{,}000)(-16\cdot07 + i \times 826) \times 10^{-6}$$
$$= -34\cdot08 + i \times 52\cdot76.$$
$$\therefore C_1 = 5\cdot92 + i \times 55\cdot27 = 55 \text{ amps. per phase at sending end.}$$
$$P_1 = 64{,}250 \times 5\cdot92 + 47{,}380 \times 55\cdot27 = 380{,}000 + 2{,}620{,}000 = 3{,}000 \text{ kw.}$$
$$\eta = \frac{2{,}750}{3{,}000} = \cdot917.$$
$$V_2' = \frac{64{,}250 + i \times 47{,}380}{\cdot941 + i \times \cdot059} = 71{,}300 + i \times 45{,}800.$$
$$\rho_2 = \sqrt{\frac{71{,}300^2 + 45{,}800^2}{64{,}600^2 + 40{,}000^2}} = 1\cdot112.$$

(5) *Determination of corona loss.*

$$\gamma = \cdot83 \text{ for } 7/12 \text{ cable.}$$
$$\delta = 1\cdot02.$$
$$V_c = 123{,}400 \times \cdot83 \times 1\cdot02 \times \cdot156 \times 2\cdot89 = 47{,}000.$$
$$Q = 5\cdot53 \times \frac{50 \times 200}{1\cdot02 \times 27\cdot75} \times (79{,}900 - 47{,}000)^2 \times 10^{-6} = 2{,}120 \text{ kw.}$$

The input at sending end, irrespective of corona loss, is 3,000 kw. Hence approximately

$$\eta = \frac{2{,}750}{3{,}000 + 2{,}120} = \cdot 537.$$

Thus the efficiency is greatly reduced by corona.

Here the loss from the line due to corona discharge is so great that the value of the leakage conductance K chosen above must be increased if a better determination of the performance of the line be required.

If so high a voltage as 132,000 be desired, it would be necessary to use a conductor of larger diameter for the sake of reducing the corona loss. Possibly aluminium might be employed instead of copper.

No. 10.

THE OCCURRENCE OF HELIUM IN THE BOILING WELL AT ST. EDMUNDSBURY, LUCAN.

By A. G. G. LEONARD, F.R.C.Sc.I., Ph.D., F.I.C.,

AND

A. M. RICHARDSON, A.R.C.Sc.I., A.I.C.

(Plate V.)

[Read May 23. Printed August 29, 1922.]

The water from this well and the gases evolved from it were examined by Adeney (Proc. Roy. I. Acad., 1906). He found the gas to contain 97·9 per cent. nitrogen, 2·1 per cent. carbon dioxide. On the suggestion of Professor Adeney, we have examined the gas for the presence of rare gases.

The gases rising from the bottom of the well and giving it the appearance of ebullition were collected by the method of Ramsay and Travers (Proc. Roy. Soc., **60**, p. 442, 1897). Quantities of about eight litres were collected at a time. The gases were transferred in the laboratory to glass gas-holders.

Experimental.

Some preliminary experiments were first carried out in the preparation of argon by sparking atmospheric nitrogen with oxygen over caustic potash, and later by passing atmospheric nitrogen over red-hot calcium turnings. When using calcium for this purpose, it is necessary to heat the tube fairly strongly, and pump off any gases evolved, before commencing the combustion, as calcium turnings which are not quite fresh develop a surface film of hydroxide which loses water on heating, and this interacts with the calcium giving off hydrogen.

A qualitative examination of the gases from the well having definitely shown the presence of argon and helium, a quantitative determination was undertaken This was carried out by the removal of the bulk of the nitrogen by heated calcium and subsequent removal of argon and traces of nitrogen by charcoal immersed in liquid air.

A separating funnel of capacity 838 c.cs. was fitted with a rubber stopper carrying two tubes with glass taps, one of which connected with the gas reservoir, and the other with the rest of the system. The stoppered stem of the funnel was connected by rubber tubing with a reservoir of water. By this arrangement it was possible without dis connection to admit several quantities of moist gas, measured at atmospheric temperature and pressure, to the apparatus. The calcium tube was

packed with 25-30 grams of calcium turnings, and about 9 inches of its length at the end with copper oxide to eliminate hydrogen. The gas passed from the funnel through tubes containing $CaCl_2$ and P_2O_5, bubbled through sulphuric acid, and then passed over heated calcium, the issuing gas again bubbling through sulphuric acid, which served to indicate the speed of the current of gas. The gas was then drawn into an automatic Sprengel pump, and delivered into a small gas-holder. Before passage of the gas the apparatus was exhausted with a filter pump, while the calcium tube was heated to eliminate any hydrogen evolved by interaction of the calcium with any water vapour present, and finally exhausted by the Sprengel pump. In all places, where possible, connections were made by fusing the glass tubes together. In the few places where this was not possible the rubber joints were varnished with shellac and luted with melted black rubber. The exhaustion having been completed and the apparatus proved air-tight, the measuring funnel was filled with gas and adjusted to atmospheric pressure. The calcium was then heated and the gas passed over slowly, the pressure in the apparatus not rising above a few mms., and the residual gas being pumped off continuously until exhaustion was complete. The residual gas was now passed over heated copper oxide to remove hydrogen, and over phosphorus pentoxide, and allowed to remain fifteen minutes in contact with charcoal immersed in liquid air, which removes all gases except helium and hydrogen. The charcoal tube was previously heated to 200° C. and exhausted. From the charcoal the gas was then pumped off by a Töpler pump and collected for measurement. It was found that a further treatment with charcoal in liquid air did not appreciably affect the final volume. Three estimations of the helium content were made, but the other gaseous constituents were collected together in the one charcoal tube. The volume of helium was measured by passing it into a capillary tube connected with a mercury reservoir, adjusting to atmospheric pressure, marking the level, subsequently filling the space occupied by the gas with mercury, and then weighing the mercury.

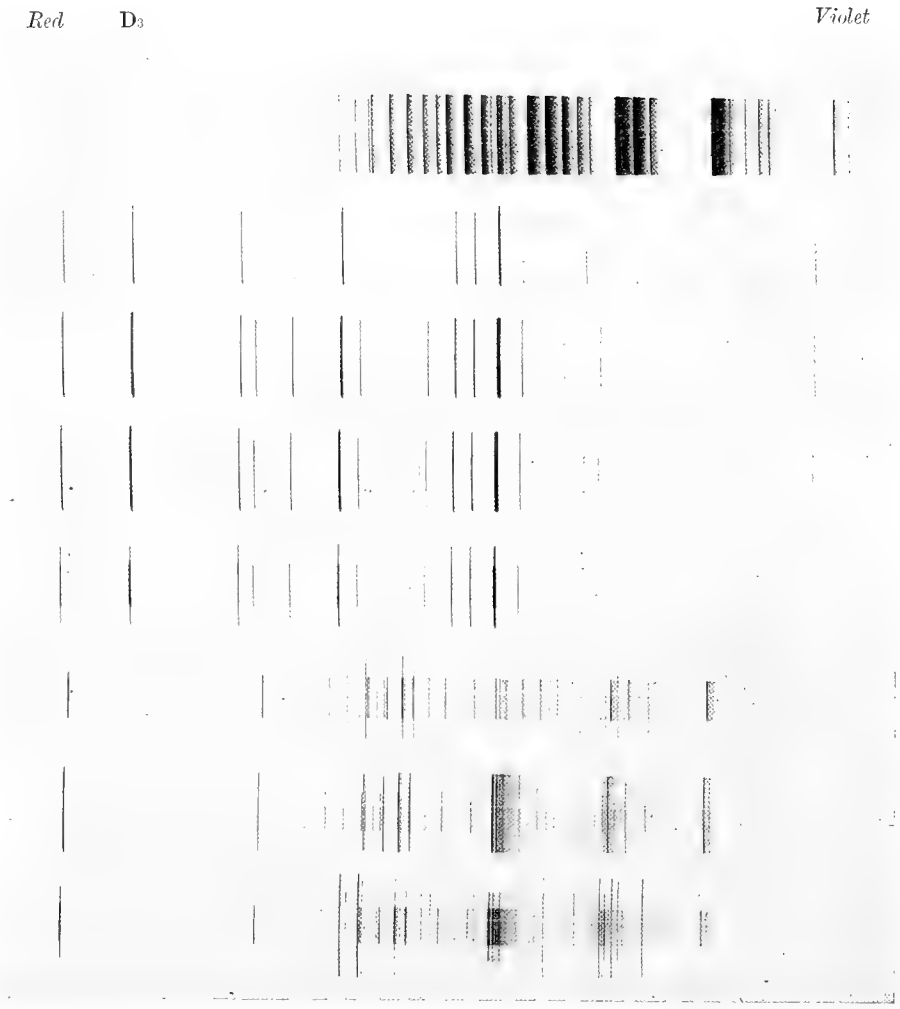
The charcoal tube was now heated to 200° C., and the gases pumped off and collected, when they were again passed over heated calcium and copper oxide. The volume of gas remaining was then measured. On examining the spectrum of this gas it showed the lines of argon strongly with some hydrogen lines.

Results.

The following table gives the results obtained, all volumes being reduced to N.T.P. and expressed in c.cs. The argon was estimated by uniting the residual gases from the three experiments :—

Vol. of gas taken.	Vol. after treatment with Calcium.	Vol. of Helium.	Vol. of Argon.	Percentage of Helium.	Percentage of Argon.
788	9·58	0·566	24·48	0·072	0·95
785	14·16	0·550		0·070	
785	24·09	0·620		0·079	

Some determinations by other experimenters of helium and argon in natural gas from other places are given for comparison, the values for nitrogen and carbon-dioxide in the gas from Lucan being taken from Adeney's analysis.



Crude gas (W).

He (W).

He (P).

He (W), | . He (P), | .

He (W), | . He (P), | .

A (W), | . A (P), | .

A (W), | . A (P), | .

A (W), | . A (P), | . H, | .

(W) from well ; (P) purchased.

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	Oxygen.	Helium.	Argon.	Nitrogen.	Carbon-dioxide.
Aix-les-Bains, France, . .	0·0	0·03	1·18	94·99	4·00
Badgestein, Austria, . .	1·4	0·169	1·18	97·25	trace
Bath, England, . . .	—	0·12	—	—	—
Caldellas, Portugal, . .	2·4	0·017	1·14	96·40	0·0
Kansas, U. S. A., . . .	—	0·06	—	—	—
Lucan (St. Edmundsbury), Ireland,	0·0	0·074	0·95	96·88	2·10
Santinay, France, . . .	—	10·16	—	—	—
Texas, U. S. A., . . .	—	10·0	—	—	—

Spectroscopic examination of the gases.

The photographed vacuum tube spectra are shown in Plate V.

The spectrum of the original gas from the well is shown in No. 1. It gives the characteristic band spectrum of nitrogen, the helium and argon lines being completely absent. Nos. 2 and 3 are the spectra of helium from the well and of purchased helium respectively, while No. 4 shows the spectrum of the purchased helium (long) superimposed on that of the helium from the well (short).

Eleven lines (dotted) appear in the latter, which are not visible in the spectrum of the purchased gas.

They have been identified as lines of hydrogen, mercury, and argon.

In No. 5 the purchased argon lines are photographed (long) with those of argon from the well (short) superimposed. The bands of nitrogen appear faintly in the latter, together with some hydrogen lines. The coincidence of these lines with those of hydrogen is shown in No. 8, where the purchased argon is photographed full length, hydrogen two-thirds length, and argon from the well short. On examination of the latter spectrum with a lens, all the lines are seen to be continued into the hydrogen lines or those of the purchased argon. In No. 7 the spectrum of argon from the well (long) is superimposed on that of the purchased argon (short); the lines appearing in the latter are some of the lines of the blue spectrum of argon, which developed owing to variation in the tension of the contact-breaker of the coil.

As the gases in the process of purification were passed more than once over heated copper oxide for removal of hydrogen, it is possible that the occurrence of hydrogen in the gases after separation is due to the presence of phosphorous anhydride in the commercial phosphoric anhydride employed for desiccation (Manley, Journ. Chem. Soc., 1922, 331.)

Radioactivity of the water from the well.

A test of the radioactivity of the water, kindly carried out by Dr. J. H. J. Poole, indicated the presence of about $0{\cdot}01 \times 10^{-12}$ gram of radium per c.c., i.e. rather less than the average for sea-water and rather more than that for river-water.

The authors desire to express their thanks to Dr. R. Leeper, who granted them facility of access to the well.

CHEMICAL LABORATORY,
ROYAL COLLEGE OF SCIENCE,
DUBLIN.

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No. 11.

ON THE DETONATING ACTION OF α PARTICLES.

By H. H. POOLE, M.A., Sc.D.,

Chief Scientific Officer, Royal Dublin Society.

[Read November 28. Printed December 7, 1922.]

It has recently been shown[1] that α particles are capable of causing the detonation of iodide of nitrogen. This observation suggests two questions of some interest, the first being concerned with the very small probability of explosion—only one detonation occurring on the average per 10^7 to 10^8 α particles, and the second with the possibility of danger arising from a similar effect with detonators and explosives in common use. This suggestion has already been put forward by the writer,[2] but all the evidence recorded below appears to be of a reassuring nature.

Taking first the consideration of the manner in which detonation occurs, it is obvious that mere ionisation of a molecule of the iodide is incapable of bringing it about, as in that case the first α particle striking the material would cause an explosion. Two possible explanations of the very small probability effect suggest themselves. Detonation may be caused by the combined effect of two or more α particles passing in rapid succession through the same very small region, and thus raising the local temperature to the detonation point. On this hypothesis the chance of an explosion occurring in a given time should be proportional at least to the square, and probably to a higher power, of the concentration of α particles. If, on the other hand, a single α particle is capable of causing detonation, probably by collision with an atomic nucleus, the chance of explosion should be directly proportional to the concentration, and the average number of particles per explosion should be independent of the concentration.

Several series of observations on this point have been carried out, sets of specimen patches of iodide on filter-paper being prepared, and detonated by placing a small wire loop coated with RaC in the vicinity. The time in seconds (t) that elapsed before detonation was noted. The number (n) of α particles striking the patch per second was estimated from the known activity of the source (obtained by γ-ray test and the law of decay) and the geometrical conditions, allowance being made for small differences in the areas of the test

[1] G. H. Henderson, *Nature*, June 10, 1922.

[2] *Nature*, July 29, 1922.

patches. As the activity decayed, the average interval before detonation increased, as shown below, where a typical set of results is recorded:—

n	t	nt
$2{\cdot}74 \times 10^7$	0·4	$1{\cdot}1 \times 10^7$
2·45 „	2·4	5·9 „
1·79 „	4·6	8·2 „
1·84 „	1·2	2·2 „
$4{\cdot}00 \times 10^6$	2·8	1·1 „
2·98 „	16·6	4·9 „
2·39 „	11·6	2·8 „
1·76 „	26·2	4·6 „

Mean diameter of specimen patch, 1·75 cms. Mean distance of source from specimen, 1·1 cms. in every case.

An interval of over an hour elapsed between the fourth and fifth tests, so that the concentration of α particles only averages about one-tenth as great in the last four tests as in the first four. The concentration per square centimetre is nearly, but not quite, proportional to n, as small variations occurred in the areas of the patches. The average value of nt for the first four tests is $4{\cdot}35 \times 10^7$, and for the last four $3{\cdot}35 \times 10^7$, so the evidence here favours the view that nt is approximately independent of the concentration. This result was amply confirmed by other series of observations, though the mean value of nt was generally higher, probably owing to the specimens being less dry, so that some of the iodide was insensitive. A set of tests at distances varying from 1 to 3 cms. indicated an increase in the value of nt with distance, i.e. a decrease in the detonating efficiency of α particles with decrease of velocity. The results are rendered somewhat indefinite by unavoidable differences in sensitivity among the test specimens, and by the large variations in number of particles per explosion, inherent in the effect. The detonating efficiency of an α particle at 1 cm. is apparently about twice as great as that at 3 cms.

We see, then, that all the evidence favours the theory that a single α particle is responsible for the detonation. It is natural to ascribe this to a nuclear collision, probably with either a nitrogen or a hydrogen atom. Such a collision would almost inevitably disrupt the molecule to which the atom belonged, and may even, as Sir Ernest Rutherford has shown, disrupt the nitrogen atom itself. The probabilities involved are quite of the order we should expect. The decrease in the detonating efficiency of an α particle with its velocity also accords well with this view.

As regards the second question, it is evident that any compound containing a light element would occasionally have one of its molecules disrupted in a stream of α particles. This would appear to be certain in the case of the disruption of a nitrogen atom, and almost certain in any case of nuclear collision. In the case of iodide of nitrogen the disruption of one molecule appears frequently to entail the detonation of the whole. We would expect that the probability of detonation of any body sufficiently unstable to be exploded at all by a single molecule would be of the same order of magnitude as that for iodide of nitrogen.

The following is a brief record of the various substances tested. In no case did detonation occur, though some of the bodies are notoriously sensitive. The

tests varied in duration from five minutes to about twenty hours, but the decay of the deposit rendered the period after the first two hours of negligible importance. The number of α particles in each case is calculated from the initial activity of the source, as measured by a γ-ray electroscope, the curve of decay of the active deposit, and the geometrical conditions:—

Substance.	Area of Specimen.	Distance of Source.	Number of α Particles.
Fulminate of Mercury, . . .	36 sq. mm.	10 mm.	7×10^9
,, ,, . . .	36 ,,	2 ,,	6×10^{10}
,, ,, . . .	36 ,,	1 ,,	10^{11}
,, ,, another specimen,	20 ,,	2 ,,	5×10^{10}
Silver Azide, ...	80 ,,	5 ,,	3×10^{10}
,, ,, another specimen, .	80 ,,	2 ,,	10^{11}
Dynamite, ...	20 ,,	2 ,,	4×10^{10}
Nitro-Glycerine, ...	12 ,,	2 ,,	10^{11}
,, ,, ...	12 ,,	2 ,,	3×10^{10}
Potassium Picrate, ...	30 ,,	1 ,,	2×10^{11}
,, ,, ...	30 ,,	1 ,,	2×10^{11}

The potassium picrate was considerably darkened in colour by the exposure to α rays.

The total number of α particles involved in all these tests amounted to about 10^{12}, which would have caused about 30,000 detonations in iodide of nitrogen, so that the chance of detonation for the other bodies tested is very small indeed compared with that for the iodide, and probably zero. It must be remembered, however, that unless the chance is absolutely non-existent, the danger remains, as the fact that 10^{12} α particles did not produce an explosion does not in itself ensure safety. This number of α particles would be emitted in about three weeks in one ton of average rock, and so would be exceeded in a comparatively short time in a large mass of explosive if the radioactivity of the latter were at all comparable with that of ordinary materials.

In conclusion, I wish to express my indebtedness to Professor Werner and Mr. J. V. Collins, of Trinity College, Dublin, for their kindness in preparing the majority of the specimens used.

No. 12.

THE VARIATIONS OF MILK YIELD WITH THE COW'S AGE AND THE LENGTH OF THE LACTATION PERIOD.

By JAMES WILSON, M.A., B.Sc.

[Read November 28. Printed December 12, 1922.]

Ten or twelve years ago it was necessary to have scales by which individual milk yields could be "corrected" for the age of the cow and the length of her lactation. As it was known by that time that a cow's capacity is indicated by her yield when it is at the maximum, a few weeks after calving, a scale was constructed from the average daily yields of the cows exhibited at the London Dairy Show during the ten or twelve years prior to 1909. Most of these Dairy Show cows are among the best of their kind and, therefore, age for age, of nearly like capacity. They are also shown when their yields are near their best; and it was expected that, if they were classified by age, their average yields, taken class by class, would indicate the variation of yield with age. Unfortunately, the data were not many, and, as there was no separate competition for young cows till a year or two before 1909, very few for the younger ages. The number of cows of different ages and their average daily yields were as follows :—

Age : years, . .	3	4	5	6	7	8	9	10	11	and over
Number of cows, . .	14	7	35	60	50	30	12	7	5	
Pounds of milk a day, .	39·3	47	51·6	55·4	56·9	58	54·2	56·9	60·1	

A few years later another scale was constructed from better materials by Mr. W. Gavin, who was then making a statistical examination of the records which had been kept in Lord Rayleigh's dairy herds in Essex. He was able to bring together the records of over three hundred cows through their first five, and gradually declining numbers of the same cows through three more, lactations. Having also seen that a cow's capacity is indicated by her yield at the flush, Mr. Gavin found that the daily fluctuations at this time could be smoothed out and the normal yield determined by reading "the maximum daily yield maintained or exceeded for not less than three entries in the record book." He called this the "Revised Maximum," and thus described how it is determined: "The three highest daily yields (whether entered weekly or daily) are first noted. Four cows, for example, might give 16, 16, 16—16, 16, 17—16, 18, 18—16, 17, 18 quarts. The revised maximum is then taken as the highest yield common to the three entries. Thus, in all four cases quoted, it would be sixteen quarts."[1] The following table gives the number of cows whose records, through eight

[1] "Journal of Agricultural Science" for October, 1913, p. 379.

successive lactations, were brought together by Mr. Gavin and the average revised maximum yields in quarts a day[1]:—

Lactation	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
Number of cows, .	320	313	326	328	323	221	148	83
Quarts of milk a day, .	9·3	12·8	14·2	14·9	15·4	15·85	15·51	15·48

Had Mr. Gavin been able to classify his cows by age rather than lactations, his scale would probably have stood for good, for cows' yields vary with the years they have lived rather than with the lactations they have passed. As it stands, however, it needs to be only slightly revised by the figure for three-year-olds being raised in proportion as it was lowered originally through some of his "first lactation" cows being only two-year-olds.

By reducing them to a common base we shall see how far the two scales agree; and, since both are at their highest when the cows are eight years old, they can be reduced by multiplying the figures in each scale by the number which brings their highest figure to 100. Assuming Mr. Gavin's first lactation cows to have been three-year-olds and the rest to have been a year older with each succeeding lactation, the result is:—

Age, ...	3	4	5	6	7	8	9	10 years
Dairy Show cows' yields multiplied by $\frac{100}{58}$,	67·8	81	89	95·5	98·1	100	93·5	98·1
Essex cows' yields multiplied by $\frac{100}{15·85}$,	58·7	80·8	89·6	94	97·3	100	97·7	97·5

When allowance had been made for the probability that some of the Essex first lactation cows were two-year-olds, these two scales agreed so closely that it was assumed they indicated very fairly how the cow's yield varies with her age. But confidence in them was shaken by papers published in the "Journal of Agricultural Research" for September, 1919, by Dr. Raymond Pearl and Mr. Miner, and in the "Transactions" of the Highland and Agricultural Society of Scotland for the same year by Dr. J. F. Tocher. These workers made use of the Ayrshire cow records published by the Scottish Milk Records Committee. Systematic milk-testing was begun among breeders of Ayrshire cattle in 1903 by the late John Speir, who, for the first five years, published annual reports in the "Transactions" of the Highland Society, while subsequent reports have been published separately by the Milk Records Committee. In these reports the cows were not always classified upon the same plan. Till that for 1910, the dates the cows were due to calve again were not given, nor were the yields of in-calf cows separated from those which were not in calf again. Consequently, Dr. Pearl and Mr. Miner's results are based upon the average weekly yields of in-calf and not-in-calf cows during the time they were actually in milk. In the reports for 1910, 1911, and 1912 the yields are divided into two classes, according as the cows had "complete" or "incomplete" lactations, which are "a lactation which has concluded in (the recording year) and has been succeeded by the birth of a calf in that year," and another "which, whether the cow was milking

[1] "Journal of Agricultural Science" for October, 1913, pp. 378 and 379.

or not at the time of the last test in (the recording year), had not been succeeded by the birth of a calf in that year."

Dr. Tocher, who kept the figures for the two years 1911 and 1912 separate and made estimates or scales for each of these years,[1] made use of both "complete" and "incomplete" lactation yields, and thus used data nearly parallel with those used by Dr. Pearl and Mr. Miner; but his scales are expressed in gallons per lactation instead of gallons per week. If these three scales are multiplied by the numbers which bring the figures at eight years old to 100, they can be compared, not only with each other, but with the two earlier scales. None of these three scales reached maximum at eight years old, but this does not spoil the comparison. Besides, it is doubtful, as we shall see later, if any of the scales indicates clearly the year at which the maximum is reached.

	Dairy Show Cows.	Essex Cows.	Ayrshires, 1908–9.	Ayrshires, 1911.	Ayrshires, 1912.	
2 years	—	—	74·6	73·5	74	2 years
3 ,,	67·8	58·7	75·8	80	80	3 ,,
4 ,,	81	80·8	83·4	85·7	85·3	4 ,,
5 ,,	89	89·6	90·2	90·5	90	5 ,,
6 ,,	95·5	94	95·7	94·5	94	6 ,,
7 ,,	98·1	97·3	98·8	97·7	97·3	7 ,,
8 ,,	100	100	100	100	100	8 ,,
9 ,,	93·5	97.7	101·6	101·5	102	9 ,,
10 ,,	98·1	97·5	102	102·2	103·3	10 ,,
11 ,,			99·2	102	103·9	11 ,,
12 ,,			101·1	101	103·9	12 ,,
13 ,,			102·1	99·2	103·2	13 ,,
14 ,,			97·3	96·5	101·9	14 ,,
15 ,,			99·2	88·6	99·7	15 ,,
16 ,,			97·9	83·4	97	16 ,,

These columns, in which the five scales are reduced to a comparable basis, suggest that, since they are inconsistent with each other, the three Ayrshire scales may not indicate correctly the variation of milk-yield with age. It will be noticed that all five scales are closely agreed as to five-, six-, seven-, and eight-year-old cows, but the two earlier scales disagree with the Ayrshire ones about younger cows. In view of the excellence of Mr. Gavin's method and of the probability that his figure for three-year-olds would have approached that for the Dairy Show cows had his cows been classified by age, it does not seem likely that scales can be accurate which make four-year-old Ayrshires proportionately higher yielders than four-year-olds of other breeds, three-year-old Ayrshires equal to other four-year-olds, and two-year-old Ayrshires much better than other three-year-olds. Still less is

[1] "Transactions" of the Highland and Agricultural Society of Scotland for 1919, p. 246.

it likely that the gaps between two-year-old and three-year-old, three-year-old and four-year-old, and even four-year-old and five-year-old yields should be as small as these scales indicate. Indeed, something was happening among Ayrshire cattle at the time the records which have been relied on were being taken which made them inappropriate for determining how milk yield varies with age.

As already stated, the late John Speir got a small number of Ayrshire breeders to begin keeping milk records in 1903. One of the first results was that, when the good cows came to be distinguished from the bad by an accurate method, some which would have been retained before were now discarded, while others which would have been discarded were now retained; and the yields in milk-recording herds began to rise. It would be difficult to say whether the breeders were readier to discard younger or older cows which had been shown to be of poor capacity, and thus whether the average yields of older and younger cows were affected equally.

Another result was that breeders began to select sires by their dams' yields rather than by the old and generally worthless tests. This did not affect the yields at once, however, for, since sires cannot be used till they are yearlings and must be four years old when their eldest daughters are two, it could scarcely have affected the breed yields at all before 1906 or 1907. And, when it did have effect, it must have affected only two-year-olds the first year, two- and three-year-olds the second year, two-, three-, and four-year-olds the third year, and so on. Thus, though the average yields in milk-recording herds were increased by both the new way of discarding cows and the new way of selecting sires, the increase was not distributed equally over all ages, and the records after 1907 or so cannot be used to say how milk yield varies with age. All they can say is how yields varied with age in the year or years in which they were taken.

The shifting of the relative positions of the average yields at different ages is indicated in the following diagram, in which the averages for two- to eight-year-old cows at four different periods are plotted out and traced together. The averages for each period have been multiplied by the figures which bring their eight-year-old averages to 100. The periods chosen are 1903 to 1907, 1908 and 1909, 1913 and 1920. The basal figures for the first period were extracted by Mr. Speir himself,[1] who used them to find how yield varied with age; those for the second are Dr. Pearl and Mr. Miner's; those for the third and fourth were extracted shortly after the reports for 1913 and 1920 were published.

Thus, since scales based upon the Ayrshire records cannot represent the normal rise in milk yield fairly—even Mr. Speir's was constructed too late—the scales based upon the London Dairy Show and Lord Rayleigh's cows, or a combination of these, must stand in the meantime. Since Mr. Gavin's scale is probably too low for three-year-olds, the combined scale would indicate that the yields of three-, four-, five-, six-, and seven-year-old cows should stand to their yields at eight years old as approximately 67, 80, 90, 95, and 98 to 100. As yet, little can be said about two-year-old yields. The early Ayrshire records give no clear help, because, while the average ages are approximately $3\frac{1}{2}$ years for three-year-olds, $4\frac{1}{2}$ for four-year-olds, and so on, the average age of two-year-olds is undoubtedly more than $2\frac{1}{2}$ years. If the combined figures got from the London Dairy Show and Lord Rayleigh's cows were plotted to scale and produced to the left, the produced line would indicate that the yields of two-year-old cows whose ages average about $2\frac{1}{2}$ years should be to their yields at eight years old as about 50 to 100.

[1] Report on Milk Records for Season 1908, p. 23.

Nor can much more be said as to the yields of cows over eight years old. Dr. Pearl and Mr. Miner's figures indicate that the cow comes to her maximum when she is somewhere between ten and thirteen years old, Dr. Tocher's when she is about ten or eleven, Mr. Speir's when she is ten or eleven; while the figures for 1913 indicate ten, those for 1919 nine to eleven, and those for 1920 eight years.

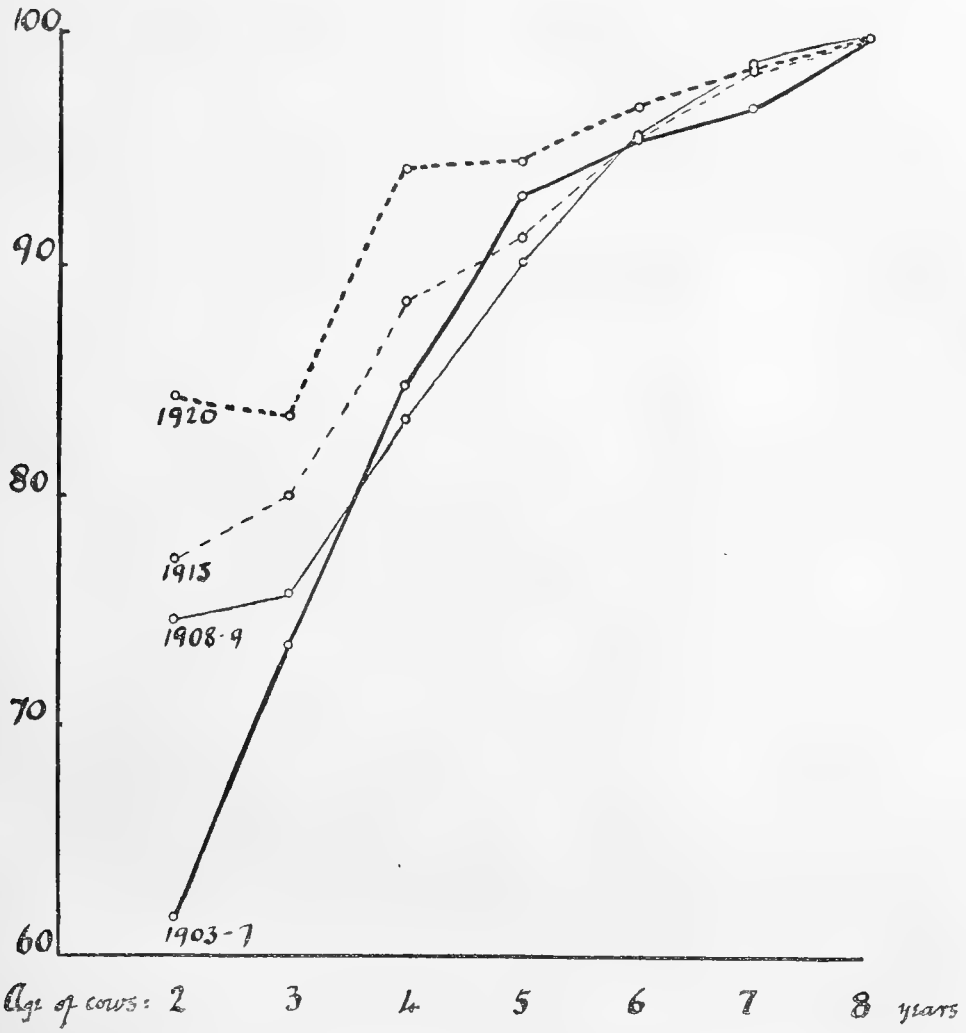


FIG. 1.—Relative yields of cows of different ages at different periods.

It is very doubtful, however, whether the Ayrshire records can be used to say when the cow comes to her maximum yield, for, when cows no longer young have to be discarded for age, the poor ones are likely to be discarded first, the best ones last; and the older age records become loaded with those of cows which are above the average. Mr. Gavin, though his materials were excellent and his eight-year-old cows actually gave more milk than his seven-year-olds, did not insist that the maximum is reached at eight years rather than at seven. He had fewer cows after

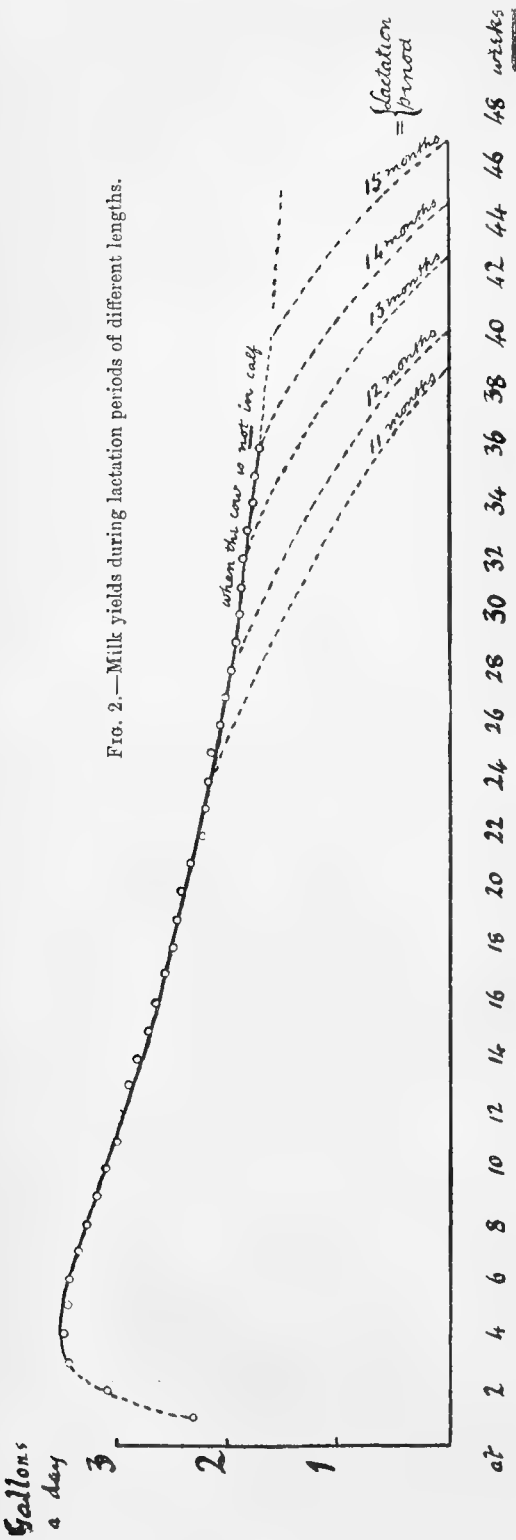


FIG. 2.—Milk yields during lactation periods of different lengths.

their ages passed seven years, and he remarked that their yields "cannot be strictly comparable with the others, since as soon as one ceases to deal with the same number of cows throughout, the influence of selection must come in. It is probable that only the best or the healthiest of the 336 cows would tend to remain in the herd after their fifth calf."

A scale to show how milk yield varies with length of lactation was even more necessary, for though the cow's capacity can be told from her yield at the flush, the yield for the lactation period is what is nearly always published; and as the intervals between successive calvings are not always the same, a scale had to be found which would indicate how much should be added to short time and how much subtracted from long time yields to bring them to the normal. A normal yield is one in which the calf which induced it is followed by another at about twelve months.

In the absence of disturbing causes, the cow's yield rises quickly for a week or two, remains near the maximum for two or three more, and then slowly but gradually falls. If the cow is not in calf again, the yield may continue, falling slowly all the time, for fifteen or eighteen months; if she is in calf, it begins, at a certain time, to fall more quickly, and ends sooner. This certain time Mr. Gavin found to be when the cow had been in calf about sixteen weeks. Till about this time the cow's yield is not affected by her being in calf. Thus the average yield of a number of cows till they are about sixteen weeks in calf gives their average yield, over the same time, as if they were not in calf. The thick line in the following diagram, which is constructed from the average of Mr. Gavin's figures,[1] shows the rise and fall in yield for cows which are not in calf. The line is continued at both ends by dotted lines: at the beginning, because some of the cows were suckling calves during the two first weeks and the recorded yields are not the true yields; at the end, because Mr. Gavin's figures stop at the thirty-sixth week.

[1] "Journal of Agricultural Science," vol. v, p. 314.

Had Mr. Gavin continued his observations over a longer time, he would probably have been able to indicate how yield varies with length of lactation. This can be done, however, with the Ayrshire reports. The records in those for 1913, 1919, and 1920—the war period reports contained less material data—have first been divided into separate groups according to the cows' ages; then each of these age groups has been subdivided into groups according to the lengths of the lactation periods in calendar months. Groups have been formed for all ages of cows between two years and twelve and for all lengths of lactations between nine months and eighteen. But, as the numbers in the remaining groups are few, we shall use only those containing the yields of three- to eight-year-old cows in eleven-, twelve-, thirteen-, fourteen-, and fifteen-month lactation periods.

In some groups there are yields which are abnormally large and others which are abnormally small. They may be correct, but they are still abnormal and distort the averages. A two-year-old heifer may have given 1050 gallons of milk in a thirteen months' lactation period; but if her yield be added to those of thirty other heifers whose average is nearer 600 gallons, the average for the group is increased by fifteen. Such yields must be neglected, and this has been done by eliminating all which were more than twenty-five per cent. above or below the average of their group as it stood when the extraction from the reports was completed. This method does not give absolute accuracy—no method does—for groups which were loaded originally with an unusual number of high or low figures may still have an unfairly high or low average when the elimination has been completed, but the unfairness is not great.

The following table, which is constructed from the data in the Ayrshire Report for 1920, shows the average number of weeks cows of different ages were in milk during lactation periods of different lengths. The numbers of cows are enclosed within brackets :—

Ages of Cows.	Lengths of Lactation Periods.				
	11 months.	12 months.	13 months.	14 months.	15 months.
3 years	(174) 38·8	(235) 40·6	(206) 44·1	(145) 45	(69) 47·2
4 ,,	(73) 38·8	(207) 40·6	(125) 42·2	(49) 43·9	(17) 46·7
5 ,,	(103) 38·1	(196) 40	(93) 42·7	(48) 44·6	(16) 46·6
6 ,,	(72) 37·7	(182) 39·9	(77) 42	(26) 44·4	(18) 47
7 ,,	(67) 37·9	(155) 39·5	(72) 42·1	(18) 44·7	(14) 46·9
8 ,,	(55) 37·2	(103) 39·4	(51) 41·7	(24) 44·3	[(7) 52·6][1]
	General average 38·8	40·1	42·84	44·7	47·1

Mr. Gavin found that the yields of cows which are in calf begin to fall below those of cows which are not in calf about twenty-four weeks before the next calves are born (40 weeks in gestation, minus 16, during which the yield is unaffected, equal 24). The foregoing table indicates how long cows continue in milk in lactation periods of different lengths. If, then, as in the diagram, the points on the thick line where the yields of cows having lactation periods of different length begin to fall below those which are not in calf be joined with the points on the

[1] This figure is omitted in the average.

base line at which the yields cease, the spaces between any two joining lines will indicate by how much the yields in lactations of different lengths should differ. According to the diagram, from which the calculation can be made, the yields for an eleven-month lactation should be about 20 gallons below, and those for thirteen-, fourteen-, and fifteen-month lactations about 35, 65, and 90 gallons above, that for a normal lactation.

The next and last table, constructed from the Ayrshire Reports of 1913, 1919, and 1920, gives the average yields of three- to eight-year-old cows in eleven-, twelve-, thirteen-, fourteen-, and fifteen-month lactations. Groups containing yields of less than thirty cows are omitted. The first column in every set of these columns gives the number of cows, the second their average yield in gallons, and the third the decrease (–) or increase (+) over the average yields in the normal lactation period of 12 months.

Length of Lactation Period.

	11 months.			12 months.			13 months.			14 months.			15 months.			
3 year olds	82	614	– 36	131	650	0	68	685	+ 35	32	730	+ 80				1913
	83	599	+ 2	196	597	0	203	631	+ 34	141	652	+ 55	75	656	+ 59	1919
	108	606	– 18	214	624	0	196	652	+ 28	128	699	+ 75	57	705	+ 81	1920
4 year olds	45	731	+ 2	70	729	0	45	763	+ 34							1913
	39	708	– 8	141	717	0	95	721	+ 4	51	735	+ 18				1919
	70	740	+ 2	198	738	0	120	751	+ 13	40	775	+ 37				1920
5 year olds	32	752	– 15	57	767	0										1913
	53	735	– 2	132	737	0	64	733	– 4	33	737	0				1919
	99	724	– 19	186	743	0	88	760	+ 17	47	778	+ 25				1920
6 year olds	33	746	– 18	46	764	0	30	834	+ 70							1913
	54	709	– 41	144	750	0	76	773	+ 23							1919
	65	744	– 18	176	762	0	71	769	+ 7							1920
7 year olds	38	721	– 38	75	759	0	55	783	+ 24							1919
	64	735	– 41	144	776	0	68	797	+ 21							1920
8 year olds				56	742	0	56	764	+ 22							1919
	50	749	– 43	100	782	0	47	805	+ 23							1920
	Average of averages		– 22			0			+ 24			+ 41			+ 70	

These results are in fair agreement with those inferred from Mr. Gavin's data, and the truth may be taken to be somewhat near them.

No. 13.

A NOTE ON GROWTH AND THE TRANSPORT OF ORGANIC SUBSTANCES IN BITTER CASSAVA (*MANIHOT UTILISSIMA*).

By T. G. MASON, M.A., B.Sc.

[Read November 28. Printed December 28, 1922.]

Introduction.

A considerable amount of interest has in recent years been evinced in the quantitative aspects of plant growth. Possibly the most significant outcome of this work has been the correspondence revealed between the course of a monomolecular reaction and the rate of growth of a number of plants of a widely divergent habit (cf. Reed (5)). As a result of this correspondence, Reed (6) has suggested that growth is some sort of a catalytic process, and that consequently the organism may be regarded as the end-product of a process in which a catalyst acts upon a substrate. By others (West, Briggs, and Kidd (9)) the similarity to a monomolecular reaction has been referred to an increasing differentiation into productive and non-productive tissues rather than to mass action.

Some observations recently made by the present writer (3) on the growth of the cotton plant seem to accord with the latter rather than the former view. It was concluded as a result of this work that the correspondence with a monomolecular reaction was probably quite illusory, and that the falling-off in the rate of growth was, on the contrary, due to a correlation factor, which found expression in a deflection of the substances needed for growth from the growing-point to fruit developing on the basal fruiting-branches. After the primary axis has ceased to elongate it was found possible, for instance, to activate the dormant apical meristem by isolating it from the growth-inhibiting influence of this factor by removing it, and budding it on to a young cotton plant. The present inquiry was undertaken in order to ascertain whether there was any evidence of the presence of such a factor correlating the activity of the cells of the apical meristem of Bitter Cassava and the expansion of its tuberous roots.

Experimental.

Though Bitter Cassava is generally referred to as a shrubby plant, with long, thick, fleshy, starch-filled, cylindrical roots, this description is scarcely applicable to the plant as it grows in St. Vincent, West Indies, where the present observations were undertaken; for here the growing-point of the primary axis seldom relinquishes its dominance over the lateral buds, which remain dormant. Consequently the plant normally remains unbranched.

Twenty plants were grown in a row from cuttings in the ordinary way; the cuttings consisted of pieces of the stem of twelve to eighteen internodes. Only one plant was permitted to grow from each cutting. The height of the stem was determined weekly over a period of eighteen weeks. The measurements, which

commenced about five weeks after planting, were made from the apical bud to an ink-mark situated a couple of centimetres above the ground. A further period of ten weeks elapsed before the final measurements, when the plants were dug up and the tuberous portions of the roots and the stems weighed. A ring, approximately half an inch wide, of the extra-xyliary tissues immediately above the ink-marks was removed from the stems of alternate plants. The ringing operation was done between the eleventh and twelfth weeks after the commencement of the measurements. The results reported in Table 1 show the rate at which the stem elongated in the two groups. The weights of the tuberous portions of the roots will be found in Table 2. In making the weight determinations of the stem, which are also recorded in Table 2, all the leaves were removed, and the stem cut into three equal segments, and then weighed. The reproductive phase of development was not initiated during the course of the experiment.

TABLE 1.

Showing rate at which stem elongated in ringed and unringed plants.

The figures in the body of the table show the height in centimetres at various dates.

Class.	Ringed.						Unringed.					
No. of Plant.	11	16	7	5	3	Mean.	10	17	6	4	2	Mean.
Nov. 6	26·9	17·5	22·0	31·8	26·8	25·0	20·9	9·1	21·1	29·0	27·6	21·5
,, 13	34·6	23·3	29·4	40·4	35·0	32·5	27·8	13·6	30·0	37·1	36·0	28·9
,, 20	44·8	30·4	38·7	52·5	45·9	42·5	37·2	19·2	40·8	47·9	47·7	38·6
,, 27	56·2	38·3	48·9	64·3	57·5	53·0	46·9	25·5	50·6	58·8	59·1	48·2
Dec. 4	68·7	48·2	61·3	78·0	71·0	65·4	59·0	34·3	62·9	71·7	73·1	60·2
,, 11	81·0	59·5	76·1	92·1	85·4	78·8	72·3	44·9	76·4	85·2	88·2	73·4
,, 18	91·5	70·3	87·8	103·9	98·5	90·4	85·4	55·6	86·2	96·7	101·9	85·2
,, 25	102·9	80·9	100·3	114·6	110·9	101·9	97·2	64·6	96·7	107·6	113·4	95·9
Jan. 1	117·1	94·1	125·3	133·6	129·3	119·9	112·1	76·5	108·6	121·9	133·3	110·5
,, 8	128·8	104·3	138·7	145·2	139·8	131·4	122·1	85·7	118·5	131·7	144·4	120·5
,, 15	137·9	112·4	146·8	153·8	149·2	140·0	130·5	93·8	126·4	139·0	153·2	128·6
,, 22	144·9	119·2	152·1	160·7	155·5	146·5	137·5	99·5	132·9	145·5	155·9	134·5
,, 29	153·8	126·8	159·5	169·1	164·1	154·7	145·4	106·8	141·0	153·4	168·7	143·1
Feb. 5	164·2	135·7	167·5	179·5	173·8	164·1	154·5	114·8	150·3	162·8	178·6	152·2
,, 12	170·7	142·0	174·0	186·5	180·1	170·7	161·1	119·9	157·8	169·6	186·3	158·9
,, 19	177·4	148·6	180·5	193·0	188·1	177·5	168·5	126·9	166·2	176·4	194·6	166·5
,, 26	182·5	155·0	185·7	199·1	193·9	183·2	174·4	132·6	173·7	183·4	202·8	173·4
Mar. 5	187·6	160·5	189·6	205·1	199·9	188·5	180·9	138·5	180·8	191·4	211·6	180·6
May 16	232·0	202·0	235·6	257·6	249·2	235·3	253·7	193·3	255·1	269·0	283·7	251·0

TABLE 2.

Weight of tuberous roots and stem, and height of stem in ringed and unringed plants at termination of experiment.

Class.	No. of Plant.	No. of Tuberous Roots per Plant.	Wt. of Tuberous Roots in kilograms.	Height of stem in cms.	Weight of stem in kilograms.			
					Apical Segment.	Middle Segment.	Basal Segment.	Total Weight.
Unringed,	2	14	2·34	283·7	0·24	0·67	1·07	1·97
	4	10	2·40	269·0	0·24	0·64	1·09	1·97
	6	13	2·59	255·1	0·24	0·57	0·98	1·79
	17	7	1·58	193·3	0·16	0·30	0·50	0·95
	10	10	2·30	253·7	0·21	0·58	0·95	1·75
	Mean,	10·8	2·24	250·96	0·22	0·55	0·92	1·69
Ringed,	3	10	0·61	249·2	0·24	0·75	1·21	2·20
	5	12	0·84	257·6	0·24	0·82	1·29	2·36
	7	10	0·67	235·6	0·19	0·61	1·04	1·83
	16	7	0·26	202·0	0·19	0·53	0·91	1·72
	11	12	0·38	232·0	0·21	0·65	1·12	1·99
	Mean,	10·2	0·55	235·28	0·21	0·67	1·11	2·02

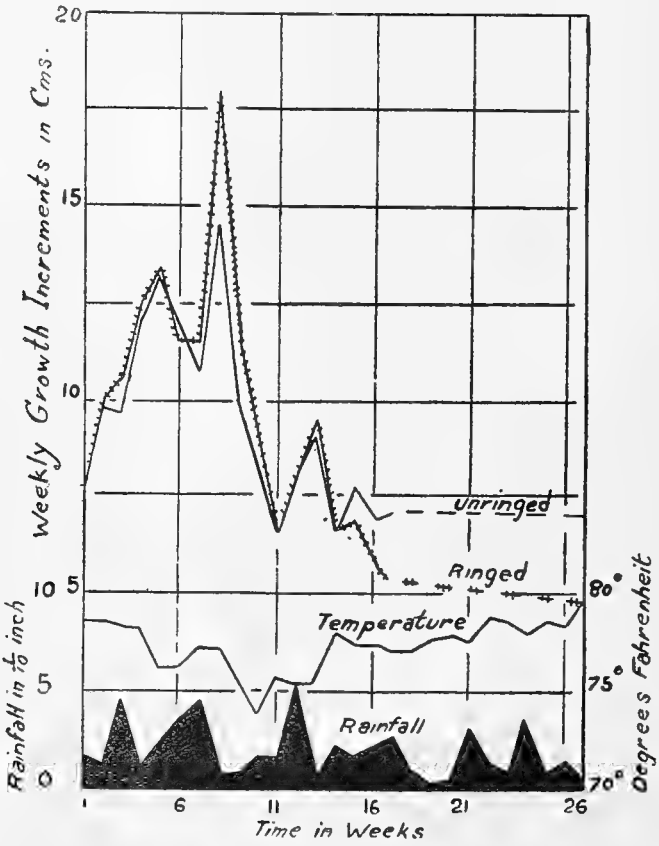
The Weekly Growth Increments.

The weekly growth increments, which are exhibited graphically in the figure, demonstrate that the velocity at which the axis elongated was in both groups slow initially, became more rapid, and then declined. At the end of the eleventh week the rate again increased, and then declined once more. As weekly measurements were not made between the seventeenth and the twenty-seventh weeks, owing to the temporary absence of the writer from the colony, information is not available as to how the rate of growth varied throughout this period. The total growth made by the ringed plants in this ten-week period was, however, only 33·6 per cent. less than in the unringed.

In comparing the growth made by the two groups, it will be observed that growth ran almost parallel up to the eleventh week, between which and the twelfth week the ringing operation was performed. It would seem that any divergences shown subsequently may confidently be referred to the removal of the ring of extra-xyliary tissues. For the three following weeks, that is to say, during the twelfth, thirteenth, and fourteenth, the rate of growth still continued to run almost parallel. After this the ringed plants commenced to lag somewhat behind the others. It would seem legitimate to refer this lag to the absence of growth in the xylem, occasioned by the cessation of, or a marked retardation in, the activity of the cambium below the ring.

Inasmuch as growth was not sensibly retarded for some three weeks following the operation, and then not very markedly, it is to be presumed that both water and solutes continued to ascend the stem, and that consequently no pronounced blocking of the tracheae occurred as a result of morbid changes spreading inwards through the wood-parenchyma and rays from the injured region. The antiseptic properties of the poisonous latex are doubtless in some measure responsible for this.

Inspection of the graphs in the figure will make it clear that the fluctuations in temperature and rainfall provide no adequate basis for interpreting the changes observed in the rate of growth.



Records were also kept of wind-velocity and evaporation, but are not presented, as no significant relationship can be traced between these factors and the changes in the rate of growth. The period the sun was above the horizon varied by just one hour and a quarter during the period occupied by the experiment.

The subterranean environment was kept as uniform as possible; a dust mulch was maintained, and water added whenever it was suspected that growth might be limited by a reduction in the water-supplying power of the soil.

The Transport of Organic Substances.

Inspection of Table 2, in which are recorded the weights of the tuberous portions of the roots, and of the three segments of the stem, will show that the subterranean storage organs were more than four times as heavy in the unringed as in the ringed plants. From this, and the greater weight of the basal portions of the stem in the latter group (cf. Table 2), it would seem that the transport of organic substances, especially carbohydrates, must have ceased, either completely or almost so, from the time of ringing; many of the tuberous roots of the ringed plants were, in fact, obviously shrunken. It cannot, of course, be inferred from this that the translocation of carbohydrates occurs in the phloem rather than in xylem.[1] When, however, one considers the results in the light of the evidence adduced by Dixon and Ball (2) in support of the view that the xylem is the channel for the longitudinal movement of carbohydrates, it would seem that though the actual translocation may take place in the xylem, yet the phloem must take an active part in this transmission; from the view-point of correlation such a hypothesis assumes a not improbable aspect.

It will be patent that the movement of organic substances in the plant, now in one direction and now in another, according to the phase of development, must in some way be dependent on the mechanism which correlates the varied activities of the organism (cf. Smith (8)). It is a matter of common knowledge that the removal of the growing apex of a shoot in some way releases the buds immediately below from their condition of dormancy. The state of dominance and subordination is sometimes (cf. Reed and Halma (4)) ascribed to the transport of specific substances (hormones), and sometimes (cf. Child (1)) to the transmission of an excitation through the living protoplasm. In the course of the work reported here, it was observed that, shortly after the removal of the ring of extra-xyliary tissues, the bud immediately below the ring commenced to grow.[2]

It would seem that the removal of the ring of extra-xyliary tissue not only interrupted the transport of carbohydrates, but also blocked the transmission of the correlating agency, whatever its nature, which is responsible for the condition of dormancy in the lateral buds. Now, if it be admitted that the movement of organic substances in the plant is in some way dependent on the existence of this correlation factor, and if it be further granted that the presence of the phloem is necessary for the transmission of this factor, it ought to follow that, though the actual channel for the movement of carbohydrates may be the xylem, yet the removal of a ring of phloem would interrupt their translocation.

The Internal Factors Controlling Growth.

It is now possible to consider what are the internal factors which determine the changes in the activity of the cells of the apical meristem. In what follows it will be presumed that the changes observed in the rate at which the stem elongated were determined by similar changes in the activity of these cells. If it be assumed that their activity was controlled by the supply of organic substances available,

[1] It will be evident that the downward flow of carbohydrates in the ringed plants cannot have ceased as a result of the blocking of the tracheae; for, had this occurred, the ascent of water and the inorganic solutes necessary for growth would have been similarly checked. Nor, it may be remarked, does an examination of the xylem in the region of the ring afford any grounds for such a view.

[2] These shoots were removed after they had attained a length of a couple of centimetres.

then the effect of the ringing ought to have resulted in an acceleration in the rate of growth. This, however, was not the case, for the banking up of organic substances, which was indicated by the relatively greater weight of the basal segments of the stem of the ringed plants (cf. Table 2), did not increase the rate of growth. From this it seems legitimate to conclude that the rate of growth was not influenced by the supply of organic nutrients available. It will be therefore evident that no correlation can exist between the rate at which the subterranean storage organs increased in weight and the activity of the cells of the apical meristem.

It would seem that for a given complex of external conditions the rate of growth in Bitter Cassava is determined by autogenous changes within the cells of the apical meristem ; possibly, as suggested by Reed, by the catalytic activity of these cells. The experimental results are, in fact, in harmony with this view, provided it be assumed that growth proceeded in two cycles, each of which followed the course of an autocatalytic reaction, and provided also it be assumed that these two cycles ran concurrently over a considerable portion of the latter period of growth. The differential equation

$$\frac{dx}{dt} = kx\,(a - x),$$

which is characteristic of an autocatalysed reaction (cf. Robertson (7)), has been employed in making the computations shown on Table 3. In this equation x represents the height of the stem, a the final height which would have been attained in each cycle, and k is a constant. When integrated the above equation becomes

$$\log_e \frac{x}{a - x} = K(t - t'),$$

where $K = ak$, and t' is the time at which the stem has grown to half its final height for each cycle, that is to say, when $x = \frac{a}{2}$. By means of tables prepared by Robertson, the constants K and the theoretical values of x are obtained for each cycle from the observed values. The values taken for a and t' and the values of K derived from the observations made on the unringed group of plants will be seen in Table 3, the logarithms being reduced to the base ten and K modified accordingly. It will be observed that the calculated and the experimentally obtained values of x are on the whole in good accord.

TABLE 3.

Showing observed and computed values of rate at which stem elongated.

x observed in cms.	PRIMARY CYCLE.		SECONDARY CYCLE.		x from both cycles.
	t in weeks.	x from $\log \frac{x}{160-x} = \cdot 14\,(t - 5 \cdot 5)$.	t in weeks.	x from $\log \frac{x}{110-x} = \cdot 13\,(t - 10)$.	
21·5	0	23·2			23·2
28·9	1	30·4			30·4
38·6	2	39·0			39·0
48·2	3	49·4			49·4
60·2	4	61·0			60·8
73·4	5	73·6			73·6
85·2	6	86·4			86·4
95·9	7	98·9			98·9
110·5	8	110·6			110·5
120·5	9	120·8			120·8
128·6	10	129·6			129·6
134·5	11	136·8			136·8
143·1	12	142·4	0	5·3	147·7
152·2	13	146·9	1	6·9	153·8
158·9	14	150·2	2	9·2	159·3
166·5	15	152·8	3	12·1	164·9
173·4	16	154·7	4	15·6	170·3
180·6	17	156·2	5	20·1	176·3
251·0	27	160	15	89·9	249·9

Summary.

1. The work reported in this paper was undertaken in order to ascertain whether there was any evidence for the presence of a factor correlating the activity of the cells of the apical meristem and the growth of the tuberous roots of Bitter Cassava.

2. Measurements of stem height were made weekly over a period of eighteen weeks, and also at the end of the twenty-seventh week. Half the plants were ringed fifteen weeks before the termination of the experiment.

3. It was found that the rate of growth of the ringed plants was not affected by the operation for a period of about three weeks; it then commenced to lag behind the unringed plants.

4. The weight of the tuberous roots of the ringed plants was approximately a quarter that of the unringed ; the weight of the stem, on the other hand, was more than 1·2 times as heavy.

5. It was concluded that the activity of the cells of the apical meristem was not controlled by the supply of organic substances available, but was, on the contrary, determined by autogenous changes within the growing point. No evidence was obtained of the presence of a factor correlating the activity of the apical meristem and the growth of the tuberous roots.

6. It was pointed out that the experimental results were in accord with the view that the rate of growth of the stem was conditioned by the catalytic activity of the cells of the apical meristem.

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No. 14.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON DIPHENYLURETHANE.

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AND

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[Read December 19, 1922. Printed February 22, 1923.]

Introduction.

It has been shown by one of us, in conjunction with Miss P. Ryan (Proc. R.I.A., xxxiv, B, pp. 194 and 212), that diphenylnitrosamine can be converted by nitric acid into nitro derivatives of diphenylamine and of diphenylnitrosamine more easily and more smoothly than diphenylamine. Thus diphenylnitrosamine reacted with nitric acid at the ordinary temperature and at low concentrations of the acids with formation of mononitro- and dinitro-diphenylamines with their nitrosamines, a trinitro- and a tetranitro-diphenylame. Under the same conditions diphenylamine reacted less smoothly with nitric acid with formation of similar nitro derivatives, together with relatively large amounts of brown resinous products, obviously arising from a secondary oxidation process. The difference in the behaviour of the two substances is probably due to the protective influence of the nitroso group during the nitration, and in this connexion the results which we obtained during the nitration of diphenylurethane may be of interest.

By the action of concentrated nitric acid on diphenylurethane Hager (Ber. Dtsch. Chem. Ges., xviii (1885), p. 2574) obtained 4·10-dinitro-diphenylurethane and a syrupy substance, which proved to be 2·10-dinitro-diphenylurethane. So far as we are aware, no other nitro derivatives of the substance have been obtained, nor has its behaviour towards nitric acid at low concentrations of the interacting substances been hitherto investigated.

In our experiments we found that cold concentrated nitric acid converted diphenylurethane into 4-nitro-diphenylurethane ; and we established the constitution of the latter substance by converting it, by means of alcoholic potash, into 4-nitro-diphenylamine melting at 133°–134° C.

With fuming nitric acid, on the other hand, we obtained the 4·10- and 2·10-dinitro derivatives, already described by Hager (*loc. cit.*).

Tetranitro-diphenylurethane, hitherto unknown, was formed by the action of a mixture of sulphuric and nitric acids on diphenylurethane, and also on 4-nitro-diphenylurethane. Since the tetranitro compound was converted by concentrated hydrochloric acid into 2·4·8·10-tetranitro-diphenylurethane, it must be regarded as 2·4·8·10-tetranitro-diphenylurethane. By the further nitration of the tetranitro-urethane symmetrical hexanitro-diphenylamine was formed.

At low temperatures and concentrations, in acetic acid solution, apparently no reaction occurred between the acid and the urethane, even on prolonged standing. In carbon tetrachloride solution, however, the acid and the urethane reacted with difficulty. When the amount of the acid was equivalent to three molecular proportions and the mixture was allowed to remain at the room temperature for six weeks, 4-nitro-diphenylurethane was formed, while with four and with six molecular amounts of the acid the main products were 4·10- and 2·10-dinitro-diphenylurethanes. On the other hand, nitrogen peroxide, in carbon tetrachloride solution, converted it more readily into an oily mixture, from which, by decomposition with alcoholic potash, 4·10- and 2·10-dinitro-diphenylamines were obtained.

Experimental.

A.—*Action of Nitric Acid on Diphenylurethane.*

I.—*Action of Cold Concentrated Nitric Acid.*

Five grams of diphenylurethane were added slowly to seven and a half cubic centimetres of nitric acid (sp. g. 1·42), which was kept well cooled during the addition of the urethane. The mixture was allowed to remain for five days in a stoppered bottle at the temperature of the laboratory. During this time a dark-coloured oil separated. The contents of the flask were poured into a considerable excess of water, and the aqueous layer was decanted from the underlying heavy brown oil. The latter was dissolved in warm alcohol, from which a yellowish-white solid separated on cooling. The solid was removed by filtration. It crystallised from alcohol in clusters of colourless prismatic crystals, which melted at 68° C. A mixture of this solid with the original urethane, which melted at 72° C., melted at about 35° C.

It gave on analysis the following results :—

0·2051 g. of the substance gave 17·8 c.c. of moist nitrogen at 17·8° C. and 768 mm., corresponding to N 10·0.

$C_{15}H_{14}O_4N_2$ requires N 9·8.

A small quantity of the solid was heated with alcoholic potash for a few hours on a water-bath ; the solution was diluted with water, and the solid which separated was filtered off. When the solid was crystallised from a mixture of chloroform and alcohol, yellow leafy crystals melting at 133°–134° C. were obtained. These crystals gave a violet coloration with a mixture of concentrated sulphuric acid and a trace of sodium nitrite, and their melting point was not affected by addition to them of 4-nitro-diphenylamine, with which the substance was identical. It follows, therefore, that the mononitro compound, melting at 68° C., must be 4-nitro-diphenylurethane.

4-Nitro-diphenylurethane consists of colourless prisms which are sparingly soluble in petroleum ether, and very soluble in ether, alcohol, chloroform, benzene, or acetone.

II.—*Action of Cold Fuming Nitric Acid.*

Five grams of diphenylurethane were added slowly, with frequent shaking of the mixture, to ten cubic centimetres of fuming nitric acid. The dark-coloured solution was allowed to remain at the laboratory temperature for two days, and was then diluted with several times its volume of cold water. The flocculent precipitate which separated was washed with water, and dissolved in hot alcohol, from which a yellow solid and a brown oil separated on cooling.

The solid, when recrystallised from alcohol, melted at 133°–134° C., and gave on analysis the following results :—

0·2363 g. of the substance gave 26·3 c.c. of moist nitrogen at 13·5° C. and 764 mm., corresponding to N 13·2.
$C_{15}H_{13}O_6N_3$ requires N 12·7.

Its solution in cold alcoholic potash was at first colourless, but on standing gradually developed the violet coloration characteristic of 4·10-dinitro-diphenylamine. The crystals dissolved in concentrated sulphuric acid, forming a yellow solution, the colour of which was not affected by the addition of sodium nitrite.

When the dinitro-urethane was heated with alcoholic potash and the solution was diluted with water, a yellow solid was precipitated, which, when filtered, washed, and recrystallised from alcohol, consisted of yellow crystals melting at 214°–216° C. The melting point was not affected by addition to the substance of 4·10-dinitro-diphenylamine, with which it was therefore identical.

The dinitro-diphenylurethane melting at 133°–134° C. was therefore the 4·10-dinitro-diphenylurethane already obtained by Hager (*loc. cit.*).

4·10-*Dinitro-diphenylurethane* consists of acicular faint yellow prisms, which are scarcely soluble in petroleum ether, soluble with difficulty in alcohol, soluble in ether or chloroform, and very soluble in acetone.

The brown oil, which was formed together with 4·10-dinitro-diphenylurethane, and which has been mentioned above, did not crystallise. It was therefore decomposed by heating with alcoholic potash, and the resulting diphenylamine derivative, when recrystallised from xylene, was obtained mainly in the form of red crystals, which melted at 219° C., and proved to be 2·10-dinitro-diphenylamine.

III.—*Action of a Mixture of Concentrated Sulphuric and Nitric Acids.*

In this experiment five grams of diphenylurethane were added to a cold mixture of $7\frac{1}{2}$ c.c. of concentrated nitric acid and 15 c.c. of concentrated sulphuric acid. The mixture became hot, with separation of a dark-coloured oily substance, and was then heated on the water-bath for five or six hours. The brown solid which separated on standing overnight, was freed from the acids by diluting the mixture with cold water and filtering. The solid was washed, dried, and recrystallised from chloroform and alcohol. The yellowish-white crystals, thus obtained, melted at 184°–185° C., and gave on analysis the following results :—

0·1785 g. of the substance gave 25·8 c.c. of moist nitrogen at 19° C. and 766·5 mm., corresponding to N 16·76.
$C_{13}H_{11}O_{10}N_5$ requires N 16·63.

When this substance was heated with alcoholic potash a black, tarry mass was obtained. It was, however, decomposed without resinification by heating it with concentrated hydrochloric acid in a sealed tube to 160°–180° C. for eight hours. The contents of the tube were neutralized with sodium carbonate, and the solid reaction product was recrystallised from benzene. It consisted of light yellow crystals, which melted at 198°–200° C., and proved to be identical with 2·4·8·10-tetranitro-diphenylamine.

The tetranitro derivative, which melted at 184°–185° C., was therefore 2·4·8·10-tetranitro-diphenylurethane.

2·4·8·10-*Tetranitro-diphenylurethane* consists of yellowish-white platy prisms, which are sparingly soluble in petroleum ether, cold alcohol, or ether; soluble in hot alcohol, cold chloroform, or benzene; and readily soluble in acetone.

IV.—*Action of Nitric Acid at low Concentrations.*

(a) *In Acetic Acid Solutions.*—(1) Two grams of diphenylurethane were dissolved in fifty grams of glacial acetic acid, and four molecular proportions of fuming nitric acid were added. The solution, which had a light greenish colour, was allowed to remain for ten weeks at the laboratory temperature, and was then poured into water. The solid which separated was filtered and recrystallised from alcohol. The colourless crystals thus obtained melted at 72° C., and proved to be diphenylurethane. As the weight of the solid recovered was almost two grams, little, if any, reaction can have occurred between the nitric acid and the diphenylurethane.

(2) Three similar solutions containing respectively one, two, and three molecular amounts of nitric acid to two grams of diphenylurethane in glacial acetic acid were prepared and allowed to remain at the room temperature for ten weeks. At the end of that time the diphenylurethane was in each case recovered unchanged.

It is evident, therefore, that at the ordinary temperature any nitration of diphenylurethane by means of nitric acid in an acetic acid solution which may occur must be a very slow one.

(b) *In Carbon Tetrachloride Solutions.*—(1) Two grams of diphenylurethane were dissolved in fifty grams of carbon tetrachloride, and 0·7 c.c. of fuming nitric acid (two molecular amounts) was added. After a few days the solution had a lemon-yellow colour, and the slight upper layer was brown. At the end of five weeks the carbon tetrachloride was evaporated, and the residue, which consisted of a colourless solid intermixed with a small quantity of oil, was dissolved in hot alcohol. The solid which separated from the alcohol on cooling proved to be unchanged diphenylurethane, melting at 72° C. The small amount of oil which was simultaneously formed was insufficient for further examination.

(2) In a second experiment three molecular quantities of nitric acid were added to a solution, similar to the last, of two grams of diphenylurethane in carbon tetrachloride. As in the last experiment, the solution was yellow, and the upper layer was brown, in colour. After three weeks crystals began to separate, and at the end of another week the carbon tetrachloride was evaporated. The oily, crystalline solid which remained was washed with ether and alcohol. It consisted of colourless crystals which melted at 68° C., and proved to be identical with 4-nitro-diphenylurethane.

(3) In another experiment four molecular quantities of nitric acid were added to the solution, which in this case rapidly acquired a deep yellow colour, and on standing overnight a brown oil separated. After the first four weeks yellowish-white crystals formed. When the mixture had remained ten weeks in all at the laboratory temperature, the solid was filtered, washed with a little carbon tetrachloride, dried, and recrystallised from alcohol, from which it separated in the form of yellow crystals, which melted at 133°–134° C., and proved to be identical with 4·10-dinitro-diphenylurethane.

When the carbon tetrachloride was evaporated from the filtrate from the crystals just mentioned, a brown oil remained. As this oil did not crystallise, it was decomposed by heating on the water-bath with alcoholic potash for about an hour. The mixture was diluted with water, and the solid which separated was filtered and dried. When heated with chloroform part of the solid dissolved; the solution was filtered while hot, and from it 4·10-dinitro-diphenylamine, melting at 214° C., was deposited on cooling. The portion which remained undissolved was crystallised from benzene, from which it separated in the form of red crystals, melting at 219° C., and which were identical with 2·10-dinitro-diphenylamine.

The oil formed by the action of nitric acid on the urethane was a solution of 4·10-dinitro-diphenylurethane in 2·10-dinitro-diphenylurethane.

(4) The behaviour of a solution of diphenylurethane in carbon tetrachloride, to which a quantity of nitric acid corresponding to six molecular proportions of the acid had been added, was quite similar to the last—the products formed in this case being also 4·10- and 2·10-dinitro-diphenylurethanes.

B.—*ACTION OF NITRIC ACID ON 4-DINITRO-DIPHENYLURETHANE.*

(1) 4-Nitro-diphenylurethane (1 gram) was added slowly with constant shaking to 4 c.c. of concentrated nitric acid (sp. g. 1·42). The solid dissolved, forming a yellow solution. On examining it after two days it was found that the nitric acid had not reacted with the nitro-urethane, which was recovered unchanged.

(2) One gram of 4-nitro-diphenylurethane was added to a cold mixture of 4 c.c. of concentrated nitric acid and 8 c.c. of concentrated sulphuric acid. The solution was allowed to stand at the temperature of the room for two days. On pouring it into water a white solid separated, which, when recrystallised from chloroform, melted at 184°–185° C., and proved to be 2·4·8·10-tetranitro-diphenylurethane.

Hence 4-nitro-diphenylurethane on nitration by "mixed acids" in the cold goes into 2·4·8·10-tetranitro-diphenylurethane.

C.—*ACTION OF NITRIC ACID ON 2·4·8·10-TETRANITRO-DIPHENYLURETHANE.*

One gram of tetranitro-diphenylurethane was added slowly to a cold mixture of 4 c.c. of concentrated nitric acid and 8 c.c. of concentrated sulphuric acid, which was then heated on the water-bath for about seven hours. On standing overnight a yellow solid separated. The mixture was diluted with water, and the solid was crystallised from chloroform, in which it was only sparingly soluble. It consisted of yellow prisms, which melted at 246° C., and were found to be identical with 2·4·6·8·10·12-hexanitro-diphenylamine.

D.—*ACTION OF NITROGEN PEROXIDE ON DIPHENYLURETHANE.*

A stream of nitrogen peroxide (generated by heating lead nitrate) was passed into a solution of two grams of diphenylurethane in fifty grams of carbon tetrachloride. The yellow solution became wine-coloured on standing at the temperature of the laboratory, and at the end of a week a brown oil separated. The solution was again saturated with nitrogen peroxide. When the solution remained at the laboratory temperature for a fortnight, the carbon tetrachloride was distilled off, and a brown oil was left behind. As the oil did not crystallise, it was boiled with alcoholic potash for an hour; the mixture was diluted with water, and the solid which separated was filtered and dried. When heated with chloroform part of the solid dissolved; the solution was filtered while hot, and from it 4·10-dinitro-diphenylamine, melting at 214° C., was deposited on cooling. The portion which remained undissolved was crystallised from benzene, from which it separated in the form of red crystals, melting at 219° C., and which were identical with 2·10-dinitro-diphenylamine.

E.—*ATTEMPTS TO SYNTHESISE THE NITRO-DIPHENYLURETHANES.*

(1) A small quantity of 4-nitro-diphenylurethane (0·5 gram) was dissolved in xylene, and the solution heated with 0·5 gram of chloroformic ester in a sealed tube at 160° C. for eight hours. The excess of chloroformic ester and the xylene were then distilled off, and the residue crystallised from alcohol, from which it separated in the form of yellow leaves, melting at 133°–134° C., and which proved to be the original solid—4-nitro-diphenylamine.

(2) Equal quantities of 4-nitro-diphenylamine (1 gram) and chloroformic ester were heated with a little alcohol in a sealed tube at 160° C. for ten hours. In this experiment also the 4-nitro-diphenylamine was recovered unchanged.

(3) In another experiment some 2·10-dinitro-diphenylamine was dissolved in xylene, and excess of chloroformic ester was added to the solution, which was then heated to boiling under a reflux condenser for ten hours. In this case also the solid recovered proved to be the original 2·10-dinitro-diphenylamine.

(4) Equal quantities of symmetrical tetranitro-diphenylamine and chloroformic ester were heated in a sealed tube at 180° C. for eight hours. Large yellow crystals were formed, which on recrystallisation from xylene melted at 199° C., and were therefore the original solid recovered unchanged.

Summary.

1. Nitric acid at the ordinary temperature and at low concentrations in acetic acid solution had apparently no action on diphenylurethane.

Under similar conditions in carbon tetrachloride solution, nitric acid converted the urethane into its 4-nitro, 4·10-dinitro, and 2·10-dinitro derivatives.

2. Cold concentrated nitric acid (sp. g. 1·42) converted the urethane into its 4-nitro derivative, while fuming nitric acid under similar conditions formed a mixture of the 4·10- and the 2·10-dinitro-diphenylurethanes.

3. The urethane, or its 4-nitro derivative, was nitrated by a mixture of concentrated nitric and sulphuric acids into 2·4·8·10-tetranitro-diphenylurethane, and the latter reacted further with the hot mixed nitrating acids forming 2·4·6·8·10·12-hexanitro-diphenylamine.

4. Nitrogen peroxide reacted with diphenylurethane in carbon tetrachloride solution with the formation of the 4·10- and the 2·10-dinitro derivatives of the urethane.

5. Notwithstanding the similarity in structure between diphenylurethane and diphenylnitrosamine, the former substance reacts with nitric acid much less readily than the latter, the nitration at low temperatures and concentrations stopping at the dinitro stage.

In conclusion we wish to state that the above research was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for a grant in aid of the investigation.

No. 15.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON ETHYL-O-TOLYLURETHANE.

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Introduction.

In previous communications it was shown that a substituted diphenylamine, such as diphenylnitrosamine [H. and P. Ryan, Proc. R.I.A., xxxiv, B, pp. 194, 212], nitrates more easily and more smoothly than diphenylamine. It was, however, found later [H. Ryan and A. Donnellan, p. 113] that the urethane formed by the substitution of a carbethoxy group for the imino hydrogen atom of diphenylamine was less easily nitrated than the parent amine.

It appeared of interest, therefore, to study the behaviour of other aromatic urethanes towards the oxides and the oxyacids of nitrogen at low temperatures and concentrations of the interacting substances. This study, although at first necessarily a qualitative one, might be expected to throw some light on the influence of a urethane group on the ease of nitration of an aromatic nucleus.

Ethyl-o-tolylurethane was selected for our experiments. Since the imino hydrogen atom of the parent substance, o-tolylurethane, was replaced by the ethyl radical, the possibility of the formation of an easily nitratable nitroso compound was excluded.

o-Tolylurethane has been obtained by Lachmann [Ber. Dtsch. Chem. Ges., xii (1879), p. 1349; cf. Merz, *ibid.*, vi (1873), p. 444; Cosack, *ibid.*, xii (1879), p. 1450; Nevile and Winther, *ibid.*, xii (1879), p. 2324] by the condensation of o-toluidine with ethyl chloroformate, and by the action of alcoholic potash on the dichloride of o-tolylisonitrile.

Ethyl-o-tolylurethane, for which we are indebted to Nobel's Explosives Company, has not, however, been hitherto described in the literature. It is a colourless, oily liquid, which boils at 255° C.

In every case where the oxides or the oxyacids of nitrogen interacted with ethyl-o-tolylurethane at the ordinary temperature, or at more or less high temperatures, directly, or in the presence of solvents, the ethyl radical attached to the imino nitrogen atom was invariably eliminated, the products being in all cases nitro derivatives of o-tolylurethane. The nitro-o-tolylurethanes previously described have all been prepared from the corresponding nitro-o-toluidines, and in no case was any of them got by nitration of the urethane.

The action of nitrogen peroxide in the vapour phase on ethyl-o-tolylurethane resulted in the formation of a compound which, when decomposed by hydrochloric acid, gave 4-nitro-2-methyl-phenylamine,[1] and was therefore 4-nitro-2-methyl-phenylurethane.

In addition to the 4-nitro derivative, a small quantity of oxalic acid was isolated. Other than the formation of a very small amount of oxalic acid, nitrogen peroxide had very little action on the urethane in solution. A similar inactivity was observed in the behaviour of nitrous fumes towards solutions of the urethane in acetic acid or carbon tetrachloride.

Concentrated nitric acid (sp. g. 1·4) had very little action on the urethane, but the fuming acid (sp. g. 1·5) reacted readily to form a compound melting at 159°–160° C., from which by decomposition with hydrochloric acid 4·6-dinitro-2-methyl-phenylamine was obtained. Moreover, as the substance was formed by the nitration of both 4-nitro- and 6-nitro-2-methyl-phenylurethane, it must have been 4·6-dinitro-2-methyl-phenylurethane. The same dinitro derivative also resulted from the prolonged action in the cold of one, two, three, four, or six molecular proportions of fuming nitric acid with solutions of the urethane in carbon tetrachloride, as well as by the action of excess of fuming nitric acid on a solution of the urethane in glacial acetic acid.

Apparently the nature of the solvent influences the reaction. Under similar conditions of temperature and concentration, nitration takes place much more easily in carbon tetrachloride than it does in glacial acetic acid solution.

For the purpose of establishing the constitutions of the substances obtained by nitration it was necessary to synthesise some nitro-o-tolylurethanes. 5-nitro-2-methyl-phenylurethane has been prepared by Vittenet [Bull. Soc. Chim. (3), xxi (1899), p. 591] by the action of alcohol on the nitro-methyl-phenyl-carbimide produced by the interaction of phosgene and 5-nitro-2-methyl-phenylamine; and by Schiff and Vanni [Liebig's Ann. d. Chem., cclxviii (1891), p. 323] by the condensation of ethyl chloroformate with 5-nitro-2-methyl-phenylamine. Vittenet gives the melting point as 129° C., and Schiff and Vanni as 137° C.; our preparation melted at 137° C. It was converted by fuming nitric acid into a crystalline dinitro-compound, which melted with decomposition at 193° C.

In a similar manner we obtained 4-nitro-2-methyl-phenylurethane from the corresponding amine. According to Vittenet [Bull. Soc. Chim. (3), xxi (1889), p. 591], who obtained the substance by the action of alcohol on nitro-methyl-phenyl-carbimide, the compound melts at 127° C. Our preparation, consisting of almost colourless prisms, melted at 135° C., and was identical with the product resulting from the direct action of nitrogen peroxide on ethyl-o-tolylurethane.

The constitution of the compound obtained by the action of fuming nitric acid on ethyl-o-tolylurethane was established in the following manner:—

6-Nitro-2-methyl-phenylamine, on condensation with ethyl chloroformate, gave 6-nitro-2-methyl-phenylurethane. The latter compound was converted by the action of fuming nitric acid into a substance identical with the nitration product

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[1] According to the usual method of nomenclature—
4-Nitro-2-methyl-phenylamine is known as 5-nitro-o-toluidine.
5-Nitro-2-methyl-phenylamine is known as 4-nitro-o-toluidine.
6-Nitro-2-methyl-phenylamine is known as 3-nitro-o-toluidine.
4·6-Dinitro-2-methyl-phenylamine is known as 3·5-dinitro-o-toluidine.

The nomenclature adopted in this paper shows the relation of these substances to the corresponding urethanes.

melting at 159°–160° C. In like manner, 4-nitro-2-methyl-phenylurethane on interaction with fuming nitric acid produced the same substance. The dinitro compound was therefore 4·6-dinitro-2-methyl-phenylurethane.

EXPERIMENTAL.

A.—*ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON ETHYL-O-TOLYLURETHANE.*

I.—*Nitrogen Peroxide.*

(a) *Action in the Vapour Phase.*—Ten grams of ethyl-o-tolylurethane and dry, liquid nitrogen peroxide were placed in shallow dishes side by side under a bell jar. Fresh quantities of nitrogen peroxide were added when necessary from time to time. In a few days crystals were deposited from the red liquid, and, after five weeks standing, the mother liquid, which had acquired a reddish-brown colouration, and was strongly acid in reaction, was poured off, and the crystals were washed with petroleum ether. On recrystallisation from alcohol almost colourless crystals were deposited in the form of large prisms, melting at 135° C. A further quantity was obtained by adding alcohol to the acid mother liquid, and allowing the mixture to stand for a few days. On analysis the following results were obtained :—

0·1741 g. of the substance gave 18·5 c.c. of moist nitrogen at 14° C. and 760 mm., corresponding to N 12·5.

$C_{10}H_{12}O_4N_2$ requires N 12·5.

4-*Nitro*-2-*methyl-phenylurethane* is soluble in most organic solvents, somewhat soluble in boiling water, and slightly soluble in petroleum ether.

When one gram of the substance was heated with 100 c.c. of concentrated hydrochloric acid under a reflux condenser for four hours, the nitro compound was decomposed with the formation of 4-nitro-2-methyl-phenylamine. A few crystals of oxalic acid were also isolated.

(b) *Action in the Presence of Solvents.*—A solution of 5 g. of ethyl-o-tolylurethane in 20 g. of carbon tetrachloride, saturated with nitrogen peroxide, was allowed to remain at the room temperature for four months. At the end of that period the solution had an orange-red colour and contained some crystals of oxalic acid. Most of the urethane was, however, recovered unchanged.

Similar experiments were performed with solutions of the urethane in ether and glacial acetic acid, and with similar negative results.

II.—*Nitrous Fumes.*

Experiments resembling those just described were performed with solutions of the urethane in carbon tetrachloride and glacial acetic acid, and the nitrous fumes generated by the action of arsenious oxide on nitric acid. Again, although a small amount of oxalic acid was formed, most of the urethane was recovered unchanged.

B.—*ACTION OF NITRIC ACID ON ETHYL-O-TOLYLURETHANE.*

I.—*Concentrated Nitric Acid.*

On the addition of concentrated nitric acid (sp. g. 1·4) to ethyl-o-tolylurethane there was no separation of solid matter, and, even after heating on the water-bath for several hours, two layers remained. When the mixture was poured into water the only product obtained consisted of unchanged ethyl-o-tolylurethane,

II.—*Fuming Nitric Acid.*

Ten grams of fuming nitric acid (sp. g. 1·5) were added slowly, with shaking, to half its weight of ethyl-o-tolylurethane, the mixture being kept cold, as the reaction was inclined to become violent. After allowing it to stand for a short time, the reaction subsided, and the liquid was then heated for some hours on the water-bath. Brown fumes were evolved, and the action was allowed to proceed gently, a few c.c. of acid being added from time to time, as the evolution of fumes decreased. In addition to a small amount of oxalic acid, a solid cake was deposited.

The cake was washed with water, and recrystallised a few times from alcohol. The pure compound consisted of fine white needles, which melted at 159°–160° C. On analysis the following results were obtained :—

0·1215 g. of the substance gave 16·7 c.c. of moist nitrogen at 20° C. and 744 mm., corresponding to N 15·8.

$C_{10}H_{11}O_6N_3$ requires N 15·6.

4.6-*Dinitro*-2-*methyl-phenylurethane* is easily soluble in chloroform, hot alcohol, or benzene, somewhat soluble in cold alcohol and ether, and slightly soluble in petroleum ether.

C.—*ACTION OF NITRIC ACID ON ETHYL-O-TOLYLURETHANE IN THE PRESENCE OF SOLVENTS.*

I.—*In Cold Solvents.*

Carbon tetrachloride.—One, two, three, four, and six molecular proportions of fuming nitric acid (sp. g. 1·5) were added to five solutions, each containing five grams of ethyl-o-tolylurethane in 50 c.c. of carbon tetrachloride. As two layers were formed in each case, the liquids were shaken continuously for a month, at the end of which time they were yellow in colour, the intensity varying with the concentration of the acid. The solution to which six molecular proportions of nitric acid had been added contained a few crystals of oxalic acid. There was no formation of solid in the other bottles, but on allowing the two layers to evaporate separately in each case, crystals melting at 159°–160° C. identical with the substance described in B—II were obtained from the contents of the flasks, to which one, two, three, four, and six molecular proportions of nitric acid had been added respectively.

In similar experiments in which glacial acetic acid was the solvent the urethane was recovered unchanged.

II.—*In Hot Solvents.*

(a) *Glacial acetic acid.*—About 10 c.c. of fuming nitric acid (sp. g. 1·5) were added gradually to a solution of ten grams of ethyl-o-tolylurethane in 50 c.c. of glacial acetic acid. An immediate reaction ensued, with evolution of brown fumes, the liquid becoming orange-red in colour. After prolonged heating on the water-bath, with addition of small quantities of the acid from time to time, the liquid was poured into water. The oil obtained was washed free from acid, and dissolved in alcohol, from which it crystallised in a few days. The crystals melted at 159°–160° C., and were identical with the compound described in B—II.

D.—*SYNTHESES AND CONSTITUTIONS OF SOME NITRO-O-TOLYLURETHANES.*

I.—6-*Nitro*-2-*Methyl-Phenylurethane.*

A mixture of 4-nitro- and 6-nitro-methyl-acetanilides was obtained by the action of nitric acid on o-aceto-toluidide. On steam distilling the mixed nitro-methyl-anilines obtained by the hydrolysis of the acetanilides with hydrochloric acid, 6-nitro-methyl-aniline, which is volatile in steam, was separated (Lellmann and Würthner, Liebig's Annal. d. Chem., ccxxviii (1884), p. 240). The latter compound was condensed with ethyl chloroformate as follows:—

A concentrated benzene solution of 6-nitro-methyl-phenylamine was heated on the water-bath under a reflux condenser with one molecular proportion of chloroformic ester, which was added in small quantities. When the action had proceeded for an hour, two grams of powdered calcium carbonate were added to decompose the amine hydrochloride. After an hour's further heating, one molecular proportion of the ester was again added as before. The heating was continued for another hour, after which the solution was filtered while hot, and the benzene was removed by distillation. On cooling, almost colourless prismatic crystals were deposited, which, after washing with petroleum and recrystallising from alcohol, melted at 131° C. On analysis the following results were obtained:—

0·1020 g. of the substance gave 11·22 c.c. of moist nitrogen at 20° C. and 768 mm., corresponding to N 12·7.
$C_{10}H_{12}O_4N_2$ requires N 12·5.

6-*Nitro*-2-*methyl-phenylurethane* is readily soluble in benzene, alcohol, or chloroform, somewhat soluble in ether, and slightly soluble in petroleum ether.

II.—5-*Nitro*-2-*Methyl-Phenylurethane.*

This compound has been obtained by Vittenet [Bull. Soc. Chim. (3), xxi (1889), p. 592] from alcohol and the corresponding nitro-methyl-phenyl-carbimide; and by Schiff and Vanni [Liebig's Annal. d. Chem., cclxviii (1891), p. 323] by the condensation of 5-nitro-2-methyl-phenylamine with ethyl chloroformate. We obtained an almost quantitative yield of it by employing a method similar to that used in the preparation of the 6-nitro compound (above). On recrystallisation of the product from 50 p.c. alcohol, fine white needles were obtained. The melting point (137° C.) was somewhat higher than that obtained by Vittenet (129° C.), but agreed with that got by Schiff and Vanni.

III.—4-*Nitro*-2-*Methyl-Phenylurethane.*

This compound, which had previously been obtained by Vittenet (Bull. Soc. Chim. (3), xxi (1889), p. 591), from alcohol and nitro-methyl-phenyl-carbimide, was prepared by us by the combination of 4-nitro-2-methyl-phenylamine and ethyl-chloroformate, the method employed being similar to that used in the case of the 6-nitro compound. After removal of the benzene by distillation, the recrystallisation of the resulting solid from alcohol, large, almost colourless, prisms, melting at 135° C. (Vittenet's product melted at 127° C.), were obtained in good yield. This compound was found to be identical with the substance of similar melting point described in A—I (a).

IV.—4.6-*Dinitro-2-Methyl-Phenylurethane.*

A solution of one gram of 4-nitro-2-methyl-phenylurethane in 5 c.c. of fuming nitric acid was heated on the water-bath, with addition of further quantities of the acid from time to time. After prolonged heating, the mixture was cooled, and the white cake which was deposited was washed with water and recrystallised from alcohol. The pure substance melted at 159°–160° C., and was identical with the compound of similar melting point described in B—II.

The nitration of 6-nitro-2-methyl-phenylurethane was performed in a similar manner. After heating the mixture of fuming nitric acid and the nitro compound for three days, the solution was poured into water. The purified product melted at 159°–160° C., and was identical with the compound described in B—II. The latter substance was therefore 4·6-dinitro-2-methyl-phenylurethane.

Attempts to convert the 4·6-dinitro compound into a higher nitro derivative were unsuccessful, the original substance being recovered in each case.

V.—*Action of Fuming Nitric Acid on 5-Nitro-2-Methyl-Phenylurethane.*

Two grams of 5-nitro-2-methyl-phenylurethane were heated on the water-bath with 5 c.c. of fuming nitric acid, the reaction being allowed to proceed in the usual manner. After two days the solid on cooling, on washing with water, and recrystallising from alcohol, melted with decomposition at 193°–194° C. This compound, which crystallised in the form of fine white needles, was readily soluble in alcohol and ether, somewhat soluble in hot benzene and hot chloroform, almost insoluble in petroleum ether, and gave on analysis the following results :—

0·1604 g. of the substance gave 22 c.c. of moist nitrogen at 19° C. and 760 mm., corresponding to N 15·8.

$C_{10}H_{11}O_6N_3$ requires N 15·6.

The compound is therefore a dinitro-2-methyl-phenylurethane in which one nitro radical is in the position 5, and the other is probably in the position 4.

Summary.

1. Ethyl-o-tolylurethane reacts with the oxides and the oxyacids of nitrogen much less readily than diphenylamine.

2. By the action of the oxides and the oxyacids of nitrogen on the urethane the ethyl radical is slowly eliminated, and the nitro products were in all cases derived from o-tolylurethane.

3. Nitrogen peroxide converted the urethane into oxalic acid and 4-nitro-2-methyl-phenylurethane, melting at 137° C., and identical with the product formed by the action of ethyl chloroformate on 4-nitro-2-methyl-phenylurethane.

4. In carbon tetrachloride solution fuming nitric acid converted ethyl-o-tolylurethane into 4·6-dinitro-2-methyl-phenylurethane. The constitution of the latter compound was determined by its formation by the further nitration of either 4-nitro- or 6-nitro-2-methyl-phenylurethane.

In conclusion we wish to state that the above research was undertaken on the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for a grant in aid of the investigation.

No. 16.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON ETHYL-PHENYLURETHANE.

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AND

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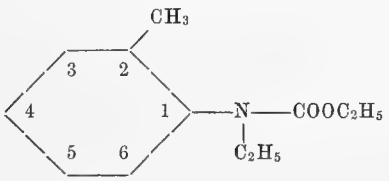
Introduction.

It has been found by one of us, in conjunction with N. Cullinane (p. 119), that ethyl-o-tolylurethane undergoes little, if any, direct nitration. The nitrating agent appears to remove the ethyl radical by oxidation to oxalic acid, and then gives nitro derivatives of o-tolylurethane only.

Nitration and oxidation of the urethane could occur simultaneously. It is probable, however, that the directive influence of the methyl radical in the position 2 impeded the formation of a 4-nitro derivative of the original urethane



A retardation from such a cause in the speed of nitration of a tertiary urethane would make the oxidation reaction preponderant, and in this way an explanation would be got for the apparent formation of derivatives of only the secondary urethane. Indications that this explanation may be correct have been obtained from a study of the behaviour of ethyl-phenylurethane.

The latter substance, which is a dense, colourless oil, has, so far as we are aware, been hitherto studied only by O. Schmidt [Ber. Dtsch. Chem. Ges., xxxvi (1903), p. 2477], who examined some of its physical properties.

Nitrogen peroxide converted ethyl-phenylurethane in carbon tetrachloride solution into ethyl-4-nitro-phenylurethane, the constitution of which was established by its conversion into p.-nitro-ethylaniline.

In the same reaction a very small quantity of a less soluble, crystalline solid, melting at 88° C., was obtained. This proved to be a dinitro-ethyl-phenylurethane.

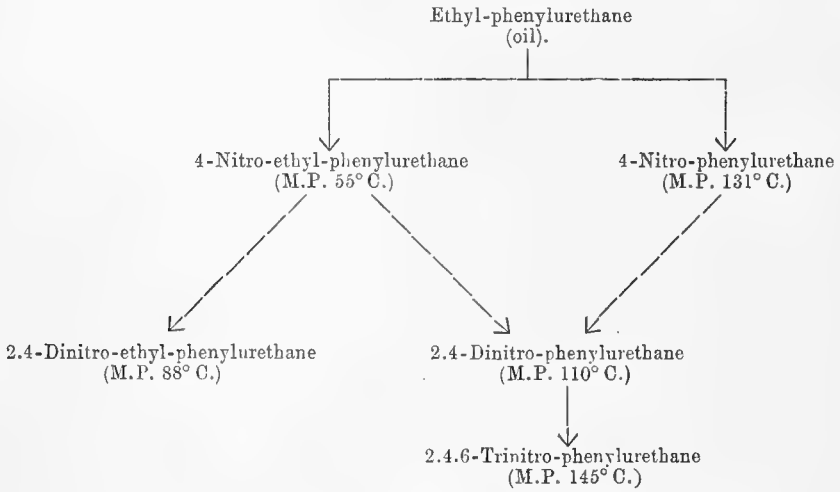
The same two compounds, 4-nitro-ethyl-phenylurethane and dinitro-ethyl-phenylurethane, were obtained by the action of fuming nitric acid on ethyl-phenylurethane.

On the other hand, a mixture of concentrated sulphuric and nitric acids converted 4-nitro-ethyl-phenylurethane into the same dinitro- and trinitro-phenyl-

urethane as were obtained under somewhat similar conditions from 2- or 4-nitro-phenylurethane.

The mononitro- and dinitro-ethyl-phenylurethanes were also obtained—the latter in very small quantity—by the action of concentrated nitric acid on ethyl-phenylurethane in a gently heated acetic acid solution. At the ordinary temperature and at low concentrations, nitric acid reacted very slowly and incompletely with ethyl-phenylurethane in acetic acid solutions to which one or two equivalents of the nitric acid had been added, and the product consisted, even after five months standing, mainly, if not wholly, of the unchanged urethane. On the other hand, from a solution to which three molecular quantities of nitric acid had been added we were able to separate some 4-nitro-ethyl-phenylurethane, while from solutions to which four or five molecular amounts of nitric acid had been added we separated in each case dinitro-ethyl-phenylurethane. The quantities obtained, however, in all cases were small, the reactions in the cold acetic acid solution being very incomplete, even when the reactions were allowed to go on for a long period.

The course of the reactions may be represented by the following scheme:—



Experimental.

A.—*Action of Nitrogen Peroxide on Ethyl-phenylurethane.*

A rapid current of nitrogen peroxide fumes, generated by heating lead nitrate, was passed for three hours through a solution of ethyl-phenylurethane in carbon tetrachloride. The clear red-coloured solution thus obtained was allowed to stand for twenty-four hours at the temperature of the laboratory. The solvent was removed and the oily residue was washed, first with water, then with ether, and was finally dissolved in boiling alcohol. In this way the reaction product was separated into a less soluble crystalline body, melting at 88°–89° C., and a more soluble crystalline substance, which melted at 55°–56° C.

The crystalline substance, which melted at 55°–56° C., gave on analysis the following results:—

0·2076 g. of the substance gave 21·5 c.c. of moist nitrogen at 18° C. and 750 mm., corresponding to N 11·9.

$C_{11}H_{14}N_2O_4$ requires N 11·85.

The substance was therefore a mononitro-ethyl-phenylurethane, and was, as is shown later, the 4-nitro derivative of the urethane.

4-*Nitro-ethyl-phenylurethane* crystallises from alcohol in large, colourless, rhombohedral, platy crystals, which are insoluble in water, dilute acids or alkalies, sparingly soluble in petroleum ether or benzene, soluble in ligroin, chloroform, or alcohol, and very soluble in hot alcohol and ether.

A mixture of this substance with 2-nitro-phenylurethane, which melts at 58° C., melted about 35°–38° C.

The mononitro-ethyl-phenylurethane was decomposed by heating it for four or five hours with alcoholic potash (4 parts) under a reflux condenser. When the reaction product was poured into water, a bright yellow crystalline substance separated. The solid was filtered, washed, and recrystallised from hot alcohol, from which it separated in the form of sulphur-yellow prismatic crystals, which melted at 96° C., and gave on analysis the following results:—

0·1242 g. of the substance gave 17·7 c.c. of moist nitrogen at 17° C. and 776 mm.,
corresponding to N 17·1.
$C_8H_{10}N_2O_2$ requires N 16·9.

The substance was therefore identical with nitro-ethyl-aniline, for which A. Weller gave the melting point 95°–96° C. [Ber. d. Dtsch. Chem. Ges., xvi (1883), p. 32; cf. E. Nölting and Collin, *ibid.*, xvii (1884), p. 267].

By addition of an aqueous solution of sodium nitrite to a cold solution of the amine in dilute hydrochloric acid the nitrosamine separated in the form of light yellow crystals, which, when filtered, washed, and recrystallised from alcohol, were obtained as cream-coloured, almost white, needles. Its melting point, 119°–120° C., was identical with that found by R. Meldola and S. Streatfield [Jour. Chem. Soc., 45, p. 61] for p.-nitro-ethyl-phenyl-nitrosamine.

The substance, which melted at 88°–89° C., consisted of colourless rhombic prisms, which were less soluble in alcohol than the mononitro compound described above. It was insoluble in water, dilute alkalis, or acids, sparingly soluble in carbon tetrachloride or ligroin, and very readily soluble in chloroform, ether, or acetone.

On analysis it gave the following results:—

0·1007 g. of the substance gave 12·8 c.c. of moist nitrogen at 15° C. and 722 mm.,
corresponding to N 14·9.
$C_{11}H_{13}N_3O_6$ requires N 14·8.

The substance was therefore a dinitro-ethyl-phenylurethane, but attempts to convert it into 2.4-dinitro-ethyl-aniline by the interaction of it with alcoholic potash have not hitherto been successful.

B.—*ACTION OF NITRIC ACID ON ETHYL-PHENYLURETHANE.*

I.—*Without a Solvent.*

Fuming nitric acid was added slowly with continuous shaking to 10 c.c. of ethyl-phenylurethane, the temperature being kept as low as possible during the reaction. The mixture, which was allowed to remain at the laboratory temperature for a few days, acquired a greenish colour and an oily consistency. The product, which separated when the contents of the flask were poured into water, was filtered, washed, and dissolved in boiling alcohol. The relatively small amount of crystals which separated when the solution was cooled consisted of dinitro-ethyl-phenyl-urethane, melting at 88°–89°. The main product of the reaction, which was separated by slow evaporation of the alcohol, consisted of 4-nitro-ethyl-phenylurethane, melting at 55°–56° C.

In another experiment fifty cubic centimetres of fuming nitric acid (sp. g. 1·52) were added in small quantities to twenty-five cubic centimetres of ethyl-phenyl-urethane. During the addition of the nitric acid the mixture, which tended to become hot, was shaken and cooled from time to time. The dark red, turbid mixture was allowed to remain overnight in a vessel surrounded by cold water; it was then left in a stoppered flask at the room temperature for three weeks. During this time a considerable amount of long, felted, acicular crystals separated from the orange-red solution. The crystals were drained on a porcelain sieve, and the filtrate was reserved for further examination. The solid was washed with water and recrystallised from alcohol, from which it separated in the form of colourless, needle-shaped crystals, which melted at 131°–132° C. Its melting point was not affected by addition to it of 4-nitro-phenylurethane, with which it was therefore identical. About seven grams of the pure solid were obtained, the mother liquid containing a further quantity, which was, however, impure owing to contamination by an oil.

The acid solution referred to above, which had been separated from the crystals, was diluted with water. The yellowish oil which was precipitated measured about 10 c.c. It was dissolved in alcohol, and the solution was allowed to evaporate at the room temperature. During the evaporation nearly colourless crystals separated; these were filtered, and after recrystallisation from alcohol they melted at 110°–111° C., and proved to be identical with 2.4-dinitro-phenylurethane. At this period, however, the alcohol still contained a considerable amount of oily matter, which was not further examined.

II.—*In Glacial Acetic Acid Solution.*

(a) *In hot acetic acid solution.*—Ten c.c. of ethyl-phenylurethane was dissolved in 50 c.c. of glacial acetic acid, and four equivalent proportions of nitric acid (sp. g. 1·42) were added. When the solution was heated on the water-bath, the mixture, which was at first yellow in colour, changed through greenish-black to yellow, and at the same time brown fumes were evolved. After three or four hours the evolution of the brown fumes ceased; the mixture was then poured into water and let stand overnight. When the yellow crystals which had separated were filtered, washed, and recrystallised from hot alcohol, they were found to consist mainly of 4-nitro-ethyl-phenylurethane, melting at 55°–56° C., with a small amount of dinitro-ethyl-phenylurethane melting at 88°–89° C.

(b) *In cold acetic acid solution.*—Five solutions were prepared by dissolving 5 grams of ethyl-phenylurethane in 100 grams of glacial acetic acid. To these solutions quantities of concentrated nitric acid, corresponding to 1, 2, 3, 4, and 5 molecular amounts of the acid to 1 molecular amount of the base, were added respectively. The mixtures were allowed to remain in stoppered flasks at the room temperature for five months in each case.

The solutions were at first light yellow in colour, but gradually acquired a greenish tint, which in turn became darker in colour, and finally, on prolonged standing, again became light yellow. The colour changes were the more rapid and the greenish tint the deeper, the greater the amount of the nitric acid in the mixture, but otherwise there was little difference in the behaviour of the contents of the various bottles.

At the end of five months the contents of each bottle were poured into about three volumes of water, and the oily mixture was in each case shaken for some time in a shaking machine.

The oils which separated from the solutions containing one and two molecular proportions of nitric acid respectively did not crystallise, and apparently consisted largely, if not entirely, of unchanged ethyl-phenylurethane.

When the yellow-coloured solution, obtained by allowing a solution of 5 grams of ethyl-phenylurethane with three molecular amounts of nitric acid in 100 grams of acetic acid to remain for five months at the temperature of the laboratory, was poured into water, an oil separated. The oil was extracted with ether, and the oil, which again separated on evaporation of the ether, was dissolved in alcohol from which 4-nitro-ethyl-phenylurethane, melting at 55°–56° C., separated.

Similarly from the solutions to which four and five molecular proportions of nitric acid had been added to 5 grams of ethyl-phenylurethane an oil separated when the solutions, after standing, were poured into water. The oil was extracted with ether, the solvent was allowed to evaporate, and the residue was dissolved in alcohol. Crystals of dinitro-ethyl-phenylurethane, melting at 88°–89° C., were in each case obtained.

III.—*In Carbon Tetrachloride Solution.*

Fourteen cubic centimetres (approx. four molecular proportions) of fuming nitric acid were added slowly to a solution of fifteen cubic centimetres of ethyl-phenylurethane in carbon tetrachloride. The mixture consisted of a small upper aqueous and a large lower carbon tetrachloride layer. After remaining a couple of days at the temperature of the room, a small amount of large, colourless, platy crystals separated, the quantity of which did not, however, appreciably increase during the remainder of the time reaction was allowed to continue. At the end of three weeks the crystals were separated from the liquid. They melted at about 101° C., but their amount was not sufficient for a further examination. The carbon tetrachloride layer was washed with dilute sodium carbonate, filtered through a dry filter paper, and distilled. The oily residue which was left in the distilling flask was dissolved in alcohol. During the spontaneous evaporation of the solution colourless crystals of 4-nitro-ethyl-phenylurethane were obtained.

C.—*ACTION OF NITRIC ACID ON 4-NITRO-ETHYL-PHENYLURETHANE.*

(*a*) Two grams of 4-nitro-ethyl-phenylurethane were dissolved in concentrated sulphuric acid, and to the mixture, which was kept cold, 10 c.c. of fuming nitric acid were added with constant stirring. The mixture was allowed to remain overnight at the temperature of the room. It was then poured into water, and the oil which separated was extracted with ether. The residue left after evaporation of the ether was dissolved in boiling alcohol, from which colourless acicular crystals separated. The crystals thus obtained melted at 109°–111° C., and this melting point was not altered by the addition to the substance of 2.4-dinitro-phenylurethane, which was prepared by the method described below. The substance was, therefore, 2.4-dinitro-phenylurethane.

(*b*) More or less prolonged action (three days) of the nitric acid on the 2·4-dinitro-phenylurethane obtained in the last experiment converted it into colourless felted needles, which, when recrystallised from alcohol, melted at 145°–146° C., and proved to be identical with the 2.4.6-trinitro-phenylurethane obtained as described below.

D.—*ACTION OF NITRIC ACID ON NITRO-PHENYLURETHANE.*

2-Nitro-phenylurethane was prepared according to the method of Rudolph [Ber. d. Dtsch. Chem. Ges., xii (1879), p. 1295] by boiling a benzene solution of nitraniline and chloroformic ester under a reflux condenser for four hours.

The benzene solution was washed with water and the benzene distilled; the residue was washed with water and recrystallised from alcohol, from which it separated in the form of sulphur-yellow prisms, which melted at 56°–58° C. A mixture of it with the nitro compound, melting at 55°–56° C., formed by the action of nitrogen peroxide or nitric acid on ethyl-phenylurethane, melted about 35°–38° C.

2.4-*Dinitro-phenylurethane.*—Following the method of Hager [Ber. d. Dtsch. Chem. Ges., xxvi (1893), p. 29], 2-nitro-phenylurethane was converted, by dissolving it in fuming nitric acid and keeping the mixture cool, into 2.4-dinitro-phenylurethane, which melted at 109°–111° C.

A mixture of the latter compound with the dinitro substance formed by the action of "mixed acid" on 4-nitro-ethyl-phenylurethane melted at 108°–110° C. It is evident, therefore, that, although the mononitro compound was nitrated by nitrogen peroxide or nitric acid in the cold to 2.4-dinitro-ethyl-phenylurethane without the separation of the ethyl radical, the latter radical was separated during the further nitration of the mononitro body by means of "mixed acid." This view was further substantiated by the conversion of the two dinitro compounds, which were obviously identical, into the same trinitro derivative, which melted at 144°–146° C.

Hager's statement (*loc. cit.*) that the dinitro-phenylurethane can be obtained by nitrating either o. or p. nitro-phenylurethane, and must therefore be 2.4-dinitro-phenylurethane, was confirmed.

4-*Nitro-phenylurethane*, which was obtained by heating a benzene solution of p.-nitraniline and chloroformic ester under a reflux condenser for three hours, after recrystallisation from alcohol melted at 132° C. It was converted by treatment with fuming nitric acid into the same dinitro-phenylurethane as was obtained by the action of nitric acid on 2-nitro-phenylurethane.

Summary.

1. Like other urethanes, ethyl-phenylurethane nitrated with difficulty. Unlike ethyl-o-tolylurethane, it nitrated to derivatives of the tertiary urethane as well as to derivatives of the parent secondary urethane. The former reaction preponderated at low, the latter reaction at moderately high, temperatures.

2. Nitrogen peroxide in carbon tetrachloride solution converted the urethane into its 4-nitro derivative and into another crystalline substance, which was probably 2.4-dinitro-phenyl-ethylurethane.

3. The same two nitro derivatives were obtained by the action of fuming nitric acid on ethyl-phenylurethane at a low temperature, but, when the temperature was allowed to rise spontaneously during the reaction, the chief products were 4-nitro- and 2.4-dinitro-phenylurethane.

4. A mixture of concentrated nitric and sulphuric acids converted ethyl-phenylurethane into 2.4-dinitro- and 2.4.6-trinitro-phenylurethanes.

5. At low concentrations in acetic acid solution and at the ordinary temperature the urethane was converted very slowly and incompletely by nitric acid into its mononitro and dinitro derivatives.

In carbon tetrachloride solution the substances reacted more easily with formation of 4-nitro-phenyl-ethyl-urethane.

The above research was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for a grant in aid of the investigation.

No. 17.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON PHENYL-BENZYLURETHANE.

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Introduction.

In previous communications the action of the oxides and the oxyacids of nitrogen, more especially at low temperatures and concentrations, on derivatives of some aromatic amines has been described. H. and P. Ryan showed [Proc. R.I.A., xxxiv, B, pp. 194 and 212] that diphenylnitrosamine nitrated more easily and more smoothly than diphenylamine. When, however, the imino hydrogen atom of diphenylamine was replaced by a carbethoxy radical, the urethane thus formed nitrated [H. Ryan and A. Donnellan, p. 113, above] with difficulty at the ordinary temperature and at low concentrations of the nitrating acid.

Similar decreases in the ease of nitration due to the substitution of an ethyl radical for an imino hydrogen atom were found by H. Ryan and N. Cullinane (p. 119) with ethyl-o-tolylurethane, and by H. Ryan and A. Connolly with ethyl-phenylurethane. Nitro derivatives of ethyl-phenylurethane were formed by nitration at the ordinary temperature, but only nitro derivatives of o-tolylurethane were got in similar experiments with ethyl-o-tolylurethane.

Of the three urethanes examined, only diphenylurethane contained two nitratable radicals, and these were both purely aromatic. For purposes of comparison it appeared of interest to examine the nitratability of a urethane, such as phenyl-benzylurethane, which would contain two nitratable residues, only one of which would be a purely aromatic radical.

Neither phenyl-benzylurethane itself, which is a colourless oil, and for which we are indebted to Nobel's Explosives Company, nor any of its nitro derivatives has hitherto been described in the literature.

By the action of nitrogen peroxide and of nitric acid on phenyl-benzylurethane, Dr. Nolan [private communication from Nobel's Explosives Company] obtained benzoic acid, paranitro-phenylurethane, benzaldehyde, p-p′-phenyl-dinitro-diphenylurea, o-nitro-phenylurethane, and an oil which must mainly have consisted

of 4-nitro-phenyl-benzylurethane,[1] since on hydrolysis it yielded 4-nitro-phenyl-benzylamine.

In our experiments nitrogen peroxide converted the urethane into an oily mixture, from which 4-nitro-phenyl-benzylurethane, melting at 70° C., a trinitro-phenyl-benzylurethane, melting at 110° C., and oxalic acid were isolated.

The urethane was converted by nitric acid at low temperatures or low concentrations into oxalic acid and 4-nitro-phenyl-benzylurethane, the constitution of the latter substance being established by its conversion on hydrolysis into 4-nitro-phenyl-benzylamine. With a higher concentration of the acid we obtained a trinitro-phenyl-benzylurethane melting at 110° C., which, from its formation from 4-nitro-phenyl-benzylurethane and from analogy, is probably either 2·4·10 or 4·8·10-trinitro-phenyl-benzylurethane. Further nitration of this compound, or the action of a mixture of sulphuric and nitric acids on the original urethane at a low temperature, resulted in the formation of a tetranitro compound, probably 2·4·8·10-tetranitro-phenyl-benzylurethane melting at 126° C.

When the temperature at which "mixed acid" was allowed to act on the urethane was not kept continuously low, a yellow viscid mass was obtained, from which we isolated a crystalline substance melting at 274° C., which was probably 2·4·6·8·10-pentanitro-phenyl-benzylamine, and a crystalline substance melting at 238° C., the properties of which indicated its identity with 4-nitro-benzoic acid, and in addition a much reduced yield of the tetranitro-phenyl-benzylurethane previously mentioned.

When the urethane was nitrated by heating with "mixed acids" for several days on the water-bath, with addition of nitric acid from time to time, and by subsequent treatment with potash, a fair yield of 2·4-dinitro-phenylurethane was obtained, and also 4-nitro-benzoic acid and a compound melting at 264° C., which was probably either a trinitro-phenyl-benzylamine or pentanitro-phenyl-benzyl-urethane, were obtained.

Owing to the facts that no dinitro derivatives were isolated, and that the positive evidence available is insufficient, the course of the reactions is somewhat difficult to trace. The urethane yields first the 4-nitro-phenyl-benzylurethane, and then, probably through dinitro derivatives, a trinitro-phenyl-benzylurethane, only the 4-position of which is known to be occupied by a nitro group. However, since further nitration is attended by the simultaneous formation of 2·4-dinitro-phenyl-urethane and 4-nitro-benzoic acid (the latter indicating a nitro group in the 10-position), it is very probable that the constitution of this trinitro compound is 2·4·10-trinitro-phenyl-benzylurethane. The next stage in the reaction is the formation of a tetranitro derivative and the probable formation of a pentanitro-phenyl-benzylurethane, with simultaneous decomposition of the urethane group, yielding pentanitro-phenyl-benzylamine.

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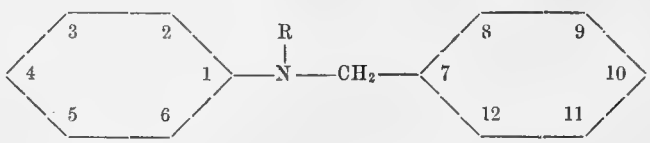
[1] The nomenclature adopted in this communication is based on the following formula :—



where R is either H or $COOC_2H_5$.

Secondary oxidation reactions similar to those met in the case of ethyl-o-tolylurethane or ethyl-phenylurethane could, and did, occur during the nitration. It is very likely that 2·4-dinitro-phenylurethane and 4-nitro-benzoic acid were formed by the oxidation and subsequent hydrolysis of 2·4·10-trinitro-phenyl-benzylurethane.

EXPERIMENTAL.

A.—*ACTION OF NITROGEN PEROXIDE ON PHENYL-BENZYLURETHANE.*

I.—*In the Vapour Phase.*

Phenyl-benzylurethane (10 grams) and liquid nitrogen peroxide (obtained by the decomposition of lead nitrate) were placed in shallow vessels, side by side, under a bell-jar. The urethane rapidly absorbed the nitrogen peroxide, which was renewed from time to time; its colour changed through reddish-brown to green, and after about ten days some long, colourless prisms were observed in the acid oil. The crystals, which were left in the dish when the oil was removed by means of chloroform, melted in the hydrated condition at 99°–101° C., and in the anhydrous state at 189° C. They reduced potassium permanganate and consisted of oxalic acid, which was probably formed by oxidation of the alcohol set free by a partial hydrolysis of the urethane.

The oil left after the evaporation of the chloroform partially crystallised on prolonged standing. By means of petroleum ether a crystalline solid was extracted from the mixture, and this, when boiled with animal charcoal and alcohol, filtered, and cooled, gave about 2 g. of long, rhombic crystals, having two truncated angles, and melting at 110°–111° C. It proved to be a *trinitro-phenyl-benzylurethane*; a mixture of it with 2.4-dinitro-phenylurethane, which also melts at 110°–111° C., melted at 75°–80° C. It gave on analysis the following results:—

0·1348 g. of the substance gave 16·4 c.c. of moist nitrogen at 15·5° C. and 765 mm.,
corresponding to N 14·4.
$C_{16}H_{14}O_8N_4$ requires N 14·4.

Trinitro-phenyl-benzylurethane is readily soluble in ether, chloroform, nitro-benzene, hot alcohol, and hot petroleum ether. On hydrolysis with alcoholic potash it gave a very small amount of a brown solid, melting between 180° and 190° C., from which we were unable to isolate any pure substance.

The reddish oil from which the trinitro compound had been mechanically removed was dissolved in boiling alcohol, from which it again separated as an oil. This was dissolved in ether, and by spontaneous evaporation of the solvent about 3 g. of the 4-nitro-phenyl-benzylurethane, described later on, were obtained.

II.—*In Solution.*

(a) *In glacial acetic acid.*—Nitrogen peroxide vapour was passed through a solution of 5 g. of phenyl-benzylurethane in 50 g. of glacial acetic acid. The mixture was allowed to remain for some months in a stoppered flask, the supply of nitrogen peroxide being renewed twice as the reaction progressed. The yellow oil which separated when the mixture was poured into water was dissolved in hot alcohol. By cooling the solution and inoculating it with a couple of crystals of 4-nitro-phenyl-benzylurethane and 10-nitro-phenyl-benzylurethane, about one gram of the former substance was obtained in a pure condition.

(b) *In carbon tetrachloride.*—In an experiment similar to the last, but in which carbon tetrachloride was employed as the solvent, oxalic acid separated on the walls of the flask, after a few days, in the form of long, colourless prisms. The carbon tetrachloride solution was washed with water and distilled under reduced pressure. The yellowish-red oily mixture which remained was dissolved in hot alcohol, from which 4-nitro-phenyl-benzylurethane and a small amount of somewhat impure trinitro-phenyl-benzylurethane, melting at 107°–109° C., separated on standing. The main portion obtained by concentration of the alcohol remained, however, in the form of an oil.

B.—*ACTION OF NITRIC ACID ON PHENYL-BENZYLURETHANE.*

I.—*In the Absence of Solvents.*

(1) Ten grams of phenyl-benzylurethane were placed in a flask immersed in a freezing mixture, and 7 c.c. of fuming nitric acid (sp. g. 1·52) were added slowly to the urethane, the temperature of which was kept at – 5° C. The mixture was then allowed to remain at the temperature of the room for two days. Some large crystals of oxalic acid which had formed were isolated and identified. The brown oily mixture was poured into a large volume of water, and the yellow oil which was thus separated was redissolved in boiling alcohol. The oil which separated from the alcohol on cooling became largely crystalline in the course of a couple of months. After a few recrystallisations from alcohol, the substance was obtained in the form of almost colourless platy prisms, which probably belonged to the rhombic system, but had truncated angles. It melted at 70°–71° C., and gave on analysis the following results:—

0·2008 g. of the substance gave 16·5 c.c. of moist nitrogen at 12·5° C. and 757 mm., corresponding to N 9·7.
$C_{16}H_{16}O_4N_2$ requires N 9·33.

By boiling this substance with alcoholic potash it was converted into 4-nitro-phenyl-benzylamine, which separated in the form of golden-yellow leafy crystals, melting at 147°–148° C. The substance, which melted at 70°–71° C., was therefore 4-nitro-phenyl-benzylurethane.

4-Nitro-phenyl-benzylurethane is readily soluble in ether, chloroform, carbon tetrachloride, or nitrobenzene, is soluble in hot alcohol, and is moderately soluble in petroleum ether.

(2) Ten c.c. of phenyl-benzylurethane were added slowly to a mixture of 15 c.c. of fuming nitric acid (sp. g. 1·5) and 15 c.c. of fuming sulphuric acid (20 p.c. SO_3), which was kept at – 10° C. by immersion in a freezing mixture. After remaining overnight, the wine-red, syrupy liquid was poured into a large volume of water, and the light yellow, curdy solid thus obtained was recrystallised from glacial acetic acid, from which about 3 g. of colourless platy crystals separated. These crystals of a tetranitro-phenyl-benzylurethane, melted at 126°–137° C., and gave on analysis the following results:—

0·1343 g. of the substance gave 18·6 c.c. of moist nitrogen at 15° C. and 759 mm., corresponding to N 16·2.
$C_{16}H_{13}O_{10}N_5$ requires N 16·3.

Tetranitro-phenyl-benzylurethane is readily soluble in chloroform, is soluble with more difficulty in hot alcohol or acetic acid, and is only slightly soluble in ether.

(3) In a similar experiment at a somewhat higher temperature the nitrating mixture became a yellow viscid mass, which was then divided into two portions.

One part was freed from volatile matter by distillation in a current of steam, and from the more soluble matter by extraction with hot alcohol. By recrystallising the residue a few times from glacial acetic acid the tetranitro-phenyl-benzylurethane just described was obtained. The substance which was obtained by concentrating the parent liquids separated from hot acetone in the form of yellowish-white silky needles, which melted with decomposition at 274° C., and proved to be a pentanitro-phenyl-benzylamine, as the following analysis indicates:—

0·0978 g. of the substance gave 16·8 c.c. of moist nitrogen at 14° C. and 768 mm., corresponding to N 20·43.
$C_{13}H_8O_{10}N_6$ requires N 20·6.

Pentanitro-phenyl-benzylamine is soluble in hot alcohol, glacial acetic acid, and acetone. It is almost insoluble in ether or chloroform.

The more soluble matter contained in the alcoholic extracts mentioned above was dissolved in aqueous potash, and from the filtered solution, by acidification and recrystallisation from alcohol, almost colourless crystals, melting at 238° C. and containing 8·7 p.c. of nitrogen, were obtained. This substance was probably 4-nitro-benzoic acid, which melts at 238° C. and contains 8·53 p.c. of nitrogen.

The second part of the yellow viscid mass was heated with fuming nitric acid for some days on the water-bath, and was then poured into water. From the hard, yellow, solid product crystals, melting at 238° C. (probably 4-nitro-benzoic acid), were extracted by means of alkali, and the residue was separated by alcohol into 2·4-dinitro-phenylurethane and a mixture which, when heated with potash, gave a small amount of a reddish crystalline solid melting at 228°–330° C., together with yellow felted needles, which melted with decomposition at 264° C. The latter substance gave the following results on analysis :—

0·1104 g. of the substance gave 16·6 c.c. of moist nitrogen at 17° C. and 767 mm., corresponding to N 17·6.
$C_{16}H_{12}O_{12}N_6$ requires N 17·5.
$C_{13}H_{10}O_6N_4$ requires N 17·6.

It is doubtful whether this substance is pentanitro-phenyl-benzylurethane or a trinitro-phenyl-benzylamine.

II.—*In the Presence of Solvents.*

(a) *In glacial acetic acid.*—Quantities of fuming nitric acid (sp. g. 1·52), corresponding to one, two, three, four, and six molecular amounts of the solid, were added respectively to five solutions, containing in each case 5 grams of phenyl-benzylurethane, dissolved in 50 grams of glacial acetic acid. The solutions, which varied in colour from orange-red to yellow, were allowed to remain at the temperature of the room for four months.

From the solutions to which four and six molecular amounts of the acid had been added we obtained in each case about half a gram of 4-nitro-phenyl-benzyl-urethane in a pure state. The oil obtained from the solution to which two molecular amounts of the acid had been added did not crystallise, but from it by hydrolysis with potash we obtained 4-nitro-phenyl-benzylamine. The oil from the solution to which one molecular amount of the acid had been added also refused to crystallise.

(b) *In carbon tetrachloride.*—To a solution of 5 grams of phenyl-benzylurethane in 50 grams of carbon tetrachloride was added a quantity of fuming nitric acid (sp. g. 1·52) corresponding to four molecular amounts of the acid.

After a few days crystals separated from the two-layered liquid formed, and on removal were identified as oxalic acid, melting at 99°–101° C. The remainder was shaken for a week and allowed to stand for two months, when it was freed from carbon tetrachloride by distillation under reduced pressure. The yellow oil obtained became largely crystalline on standing for three weeks, giving a good yield of 4-nitro-phenyl-benzylurethane.

C.—*ACTION OF NITRIC ACID ON NITRO-PHENYL-BENZYLURETHANE.*

I.—4-*Nitro-phenyl-benzylurethane.*

One gram of 4-nitro-phenyl-benzylurethane was added slowly to 4 c.c. of fuming nitric acid (sp. g. 1·5), which was kept at 0° C. during the addition of the urethane. The oil which was precipitated on diluting the mixture with water was dissolved in hot alcohol, from which the rhombic prisms of the trinitro-phenyl-benzyl-urethane, melting at 108°–110° C., separated.

II.—*Trinitro-phenyl-benzylurethane.*

To an ice-cold mixture of 2 c.c. of fuming nitric acid (sp. g. 1·52) and 2 c.c. of fuming sulphuric acid (20 p.c., SO_3) 0·4 gram of trinitro-phenyl-benzylurethane was added slowly, keeping the temperature of the mixture low. The flask was then removed from the ice-cold water and allowed to remain overnight at the temperature of the room. The product was precipitated by diluting the mixture with water, and was then recrystallised from alcohol. It proved to be the tetranitro-phenyl-benzylurethane already described, in a slightly impure condition (melting at 123°–125° C.).

D.—*PREPARATION OF* 10-*NITRO-PHENYL-BENZYLURETHANE.*

10-Nitro-phenyl-benzylurethane, which was prepared from 4-nitro-benzyl-chloride and aniline by the method of Paal and Sprenger [Ber. d. Dtsch. Chem. Ges., xxx (1897), p. 69], was heated with a benzene solution of ethyl chloroformate for six hours under a reflux condenser. The greenish-yellow oil left after distillation of the benzene was dissolved in hot alcohol, from which a somewhat oily crystalline solid separated on standing. After several recrystallisations the substance was obtained in the form of almost colourless rhombic plates, which melted at 68°–69° C., were identical neither with the original amine nor with the 4-nitro-phenyl-benzylurethane described above, and gave on analysis the following results:—

0·1642 g. of the substance gave 13·8 c.c. of moist nitrogen at 13° C. and 755 mm., corresponding to N 9·87.
$C_{16}H_{16}O_4N_2$ requires N 9·33.

10-*Nitro-phenyl-benzylurethane* is soluble in warm petroleum, ether, or alcohol, and is readily soluble in ether, chloroform, or carbon tetrachloride.

SUMMARY.

1. Like other tertiarily constituted aromatic urethanes, phenyl-benzylurethane is difficult to nitrate. The benzyl radical can, however, be nitrated, as well as the phenyl radical, without the decomposition of the urethane.

2. Nitrogen peroxide converted the urethane into 4-nitro-phenyl-benzyl-urethane, melting at 70° C., and a trinitro-phenyl-benzylurethane melting at 110° C. A small amount of oxalic acid was also formed.

3. At low temperatures and at low concentrations nitric acid acted on the urethane, forming again 4-nitro-phenyl-benzylurethane and oxalic acid.

At higher concentrations of the nitrating acid the urethane was converted into the trinitro derivative, melting at 110° C.

4. When the temperature was more or less high and the concentrations were also high, a tetranitro-phenyl-benzylurethane, melting at 126° C., and a crystalline substance, melting at 274° C. (probably a pentanitro-phenyl-benzylurethane), were formed.

Secondary reactions also occurred. These were attended by the formation of 4-nitro-benzoic acid, 2·4-dinitro-phenylurethane, and pentanitro-phenyl-benzyl-urethane.

In conclusion we wish to state that the above research was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for a grant in aid of the investigation.

No. 18.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON THE PHENYLUREAS.

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Introduction.

In experiments already described [H. Ryan and A. Donnellan, p. 113] it was found that the replacement of the imino hydrogen atom of diphenylamine by a carbethoxy radical decreased the readiness with which the substance could be nitrated. As diphenylurethane possessed, however, no basic properties, it appeared of interest to study the nitration of substances, such as diphenylurea, in which the COOEt radical of the urethane would be replaced by an amido group, $CONH_2$. In this communication we describe therefore the results of experiments which we have carried out with phenylureas in a manner similar to those described for the urethanes.

If all the hydrogen atoms of the amino groups of urea are not replaced by alkyl radicals, or if one or more of the latter is capable of reacting with the oxides or the oxyacids of nitrogen, a substituted urea may function like diphenylamine towards these oxides and oxyacids. We have examined all the phenylureas except tetraphenylurea, and in all those examined there was at least one hydrogen atom attached directly to nitrogen.

Our experiments were mainly directed towards the investigation of the action of the oxides and the oxyacids of nitrogen on the phenylureas at the ordinary temperature and at low concentrations of the interacting substances.

Phenylurea and as-diphenylurea, as would be expected, reacted similarly with the oxides of nitrogen, each easily splitting off the free amino group. The former yielded nitro-phenol, whilst the latter gave diphenylamine derivatives. s-Diphenylurea and triphenylurea, on the contrary, reacted readily without decomposition, the former giving a dinitro derivative and the latter a trinitro derivative. Only s-diphenylurea yielded a nitroso compound with oxides of nitrogen.

Phenylurea and s-diphenylurea reacted with nitrous acid, giving nitroso derivatives, whereas nitrous acid had no action on either as-diphenylurea or triphenylurea.

In acetic acid solution at low concentrations with nitric acid, only triphenylurea was nitrated. It yielded a di- and a trinitro derivative. Phenylurea, however, formed a nitrate.

In alcoholic solution neither phenylurea nor s-diphenylurea was nitrated.

All the ureas reacted with nitric acid at low concentrations in carbon tetrachloride suspension. s-Diphenylurea and triphenylurea nitrated easily. The former gave mono-, di-, and tetranitro derivatives, and the latter di-, tri-, and pentanitro derivatives. Phenylurea gave phenylurea nitrate as well as p-nitro-phenylurea and 2·4-dinitro-phenyl-nitro-urea. as-Diphenylurea, however, did not react with one or two molecular amounts of nitric acid, and with four and six gave products from which no pure compound was obtained. Phenylurea was unique in being nitrated in the amino group.

The direct action of nitric acid on these ureas was also examined. Phenylurea and s-diphenylurea yielded a tri- and a tetranitro derivative respectively, whereas as-diphenylurea and triphenylurea gave as a decomposition product 2·4·2′·4′-tetranitro-diphenylamine.

The nitric acid used in this investigation contained oxides of nitrogen, and its specific gravity was 1·51.

1.—Phenylurea.

The action of the oxyacids of nitrogen on phenylurea has been incidentally examined in some previous work.

Walther and Wlodkowaki [Journ. f. pr. ch. (2), 59, p. 282], by the action of nitrous acid on phenylurea in glacial acetic acid solution, obtained nitroso-phenylurea as light yellow needles, melting with decomposition at 95° C., and in a similar experiment we obtained the same result.

J. L. F. Reudler [Rec. trav. chim. Pays-Bas, 33 (1913), pp. 35–84] examined the direct action of nitric acid, both alone and in the presence of sulphuric acid, on phenylurea and ortho- and paranitro-phenylurea. He obtained in every case a compound which decomposed at 142° C., with formation of a red sublimate, and melted at 154°-157° C. with decomposition. When heated with water it was decomposed, giving 2·4 dinitraniline. With alcohol it formed 2·4-dinitro-phenyl-urethane, and when treated with ammonia in ethereal suspension at 0° C. for three and a half hours it was converted into 2·4-dinitro-phenylurea. He regarded the substance therefore as 2·4-dinitro-phenyl-nitrourea.

Reudler nitrated the phenylurea at low temperature with twelve molecular amounts of nitric acid, and obtained the above-mentioned compound by precipitation with water and subsequent crystallisation from acetone, using 99·7 per cent. nitric acid free from nitrous acid.

Working under the same conditions, but using nitric acid (sp. gr. 1·51) containing some nitrous acid, we got a separation of a colourless substance in prisms melting at 164° C. (uncorr.) with decomposition. By boiling with acetone it assumed a yellow colouration, and crystallised as needles, the melting point being lowered. It contained a percentage of nitrogen corresponding to a trinitro derivative of phenylurea. Boiled with water it gave 2·4-dinitraniline, and, treated in hot ethereal suspension with ammonia for a quarter of an hour, it gave 2·4-dinitro-phenylurea. The original substance was therefore 2·4-dinitro-phenyl-nitro-urea. The yellow colour and somewhat low melting point of this substance as prepared by Reudler was probably due to a slight decomposition of the body by the hot acetone.

The action of nitric acid on phenylurea in the presence of solvents has not been previously examined. When dissolved in acetic acid or alcohol, and on allowing to remain at room temperature for two months, phenylurea at 5 per cent.

concentration gave, with one, two, four, and six molecular amounts of nitric acid, only phenylurea nitrate, which melted with decomposition at 131° C.

This compound is described by Pickard and Kenyon [Trans. Chem. Soc., 1907, p. 902], who obtained it by cooling a warm concentrated nitric acid solution of phenylurea. It crystallised as colourless leaflets, melting with decomposition at 134°–135° C.

In carbon tetrachloride suspension phenylurea nitrate was obtained with one, two, and four molecular amounts of nitric acid. In the latter case traces of p-nitro-phenylurea [Reudler, *loc. cit.*] were obtained. With six molecular amounts of nitric acid it gave 2·4-dinitro-phenyl-nitro-urea.

Phenylurea in carbon tetrachloride suspension interacted with both nitrous fumes and nitrogen peroxide at room temperature, giving in each case ortho- and paranitro phenol, with an evolution of gas.

A.—*ACTION OF NITROUS FUMES ON PHENYLUREA.*

Phenylurea (10 g.) was suspended in 200 g. of carbon tetrachloride, and the mixture was saturated at the room temperature with the nitrous fumes evolved from arsenious oxide and nitric acid. After some time a brisk evolution of gas was observed, and the substance assumed a greenish colour; but later all the solid was converted into a brownish-black oil with a further evolution of gas and some heat. After allowing to remain at room temperature overnight the carbon tetrachloride was distilled off under reduced pressure from the yellowish-brown solution, and the black oil which remained was distilled with steam. In the distillate was found a substance consisting of yellow needles melting at 40° C. It proved to be ortho-nitrophenol, a mixture of this substance with it also melting at the same temperature.

The residue after the steam distillation consisted of a black oil. This was extracted several times with boiling water, and the united extracts boiled down to small bulk. It was then made alkaline with sodium hydroxide, boiled with animal charcoal, and evaporated still further. By acidifying with hydrochloric acid a small quantity of a substance was obtained. This, after crystallisation from a small quantity of alcohol, consisted of colourless needles melting at 114° C., and, as a mixture of it with p-nitrophenol also melted at the same temperature, it must have been identical with the latter substance.

B.—*ACTION OF NITROGEN PEROXIDE ON PHENYLUREA.*

Phenylurea (5 g.) was suspended in 100 g. of carbon tetrachloride, and nitrogen peroxide was passed into the mixture until it was saturated at room temperature. The phenylurea was converted into a black oil with the evolution of gas and heat.

After allowing the mixture to remain overnight at the temperature of the room, ortho- and paranitro phenols were isolated from it by methods similar to those described above.

C.—*ACTION OF NITRIC ACID ON PHENYLUREA.*

I.—*In the Presence of Solvents.*

(a) *Glacial acetic acid.*—One, two, four, and six molecular amounts of nitric acid were added respectively to four bottles each containing 5 g. of phenylurea dissolved in 100 g. of glacial acetic acid. The mixtures were allowed to remain at the temperature of the room for two months in well-stoppered bottles.

In every case, on the addition of the nitric acid, heat was evolved, increasing as the amount of nitric acid added increased, and after a short time a white substance began to separate in leaflets. The amount of substance separating, moreover, increased with the amount of acid added. The only change effected on allowing the bottles to remain at the temperature of the room for two months was the appearance of a brown colouration in the solution, and a green or brown one in the substance.

At the end of two months the solution in each bottle was filtered from the solid, which latter was found to be the same in every case. On drying in air the substance melted with decomposition at 131° C. The addition of phenylurea to it lowered the melting point considerably. When treated with cold water it melted at 146° C., and an addition of phenylurea to this did not affect the melting point. The water used was found to contain nitric acid. The original substance gave, therefore, on treatment with water, phenylurea and nitric acid.

To a weighed quantity of the colourless, freshly prepared substance water was added and then titrated with standard alkali. As a mean of several consistent determinations it was found that 1 g. of the substance required for complete neutralization 75·3 c.c. of 0·0877 N alkali,

Corresponding to, therefore, 31·66 p.c. of nitric acid.

$C_6H_5 . NH . CO . NH_2 . HNO_3$ required 31·66 p.c. of nitric acid.

The substance was, therefore, the mononitrate of phenylurea.

Phenylurea nitrate is a white crystalline substance, separating from glacial acetic acid as leaves, and melting with decomposition at 131° C. It is soluble in acetone and alcohol, less so in ether and acetic acid, and almost insoluble in chloroform and carbon tetrachloride. It has previously been described by Pickard and Kenyon (*loc. cit.*).

The solutions from each of the bottles were diluted with water, when no separation occurred, and almost neutralized with sodium carbonate. In the case of the bottles with one, two, and four molecular amounts of nitric acid no separation occurred, but in the case of that with six molecular parts of nitric acid about half a gramme of a dark brown substance was got, but from which no crystalline compound was obtained. From the almost neutralized solutions unchanged phenylurea was recovered in all cases by evaporation to dryness, and subsequent extraction with alcohol.

(b) *Alcohol.*—Five grammes of phenylurea were treated with one, two, four, and six molecular amounts of nitric acid, and allowed to remain at the temperature of the room for two months. In every case the solution became yellow, and gave the nitrate of phenylurea after shaking with barium carbonate, filtering, and allowing to evaporate.

(c) *Carbon tetrachloride.*—Phenylurea (5 g.) was suspended in carbon tetrachloride and treated with one, two, four, and six molecular parts of nitric acid. The solutions were examined after remaining two and a half months at the temperature of the room.

(1) The bottle containing one molecular amount of nitric acid had a yellow solid in suspension and a yellow solution. The solid was phenylurea nitrate, and from the solution a trace of a red oil, which gave no crystalline compound, was obtained.

(2) The bottle containing two molecular amounts of nitric acid gave a yellow solid, melting with decomposition from 80°–90° C., and a yellow solution, which contained only a little red oil. The solid was extracted with hot benzene, which on cooling deposited a small quantity of colourless needles of phenylurea nitrate.

After extracting the nitrate completely in this manner a small quantity of a red oil remained, from which no crystalline substance was obtained.

(3) The bottle containing four molecular amounts of nitric acid had a yellow solid in suspension and a yellow solution. The solution contained only a little red oil. The solid, which melted with decomposition from 70°–80° C., on treatment with cold water melted from 120°–160° C. The washing water contained nitric acid. Some phenylurea was obtained from the residue. The original substance, therefore, probably contained phenylurea nitrate. From the residue was also obtained a red crystalline substance, melting at 220° C. (uncorr.). This was probably p-nitro-phenylurea.

(4) The bottle containing six molecular amounts of nitric acid gave a yellow, oily solid. The solution contained only a small amount of a red oil. The solid was extracted with ether. The ethereal extract gave on evaporation a red oil, from which no crystalline substance was obtained. The residue was dissolved in acetone, and from the solution, by addition of petroleum ether, yellow needles separated. These melted with decomposition at 155° C., and, when boiled with water, dissolved with evolution of gas. The solution on cooling deposited red needles, melting at 183° C., and consisting, probably, of 2·4-dinitraniline.

An analysis of the original substance gave the following result:—

0·0965 g. of the substance gave 21·8 c.c. of moist nitrogen at 19° C. and 758 mm.,
corresponding to N 25·91.
$C_7H_5O_7N_5$ required N 25·83.

This substance was probably therefore 2·4-dinitro-phenyl-nitro-urea.

II.—*In the Absence of Solvents.*

Phenylurea (5 g.) was added in small quantities to twelve molecular amounts of nitric acid, cooled in ice and salt. The phenylurea dissolved, and the nitric acid became first yellow and afterwards red. Before all the phenylurea had been added a solid began to separate from the acid in the form of colourless prisms. On the addition of the remainder of the phenylurea more solid separated, and the mixture was allowed to remain at room temperature overnight. It was then filtered through glass wool, and the solid was washed with acetic acid and ether. The product was colourless, and melted with decomposition at 164° C. (uncorr.). It was soluble in acetone and alcohol, from which it crystallised as needles. Continued boiling with acetone gave it a permanent yellow colour, and caused the melting point to fall. When it was dissolved in hot acetone and then quickly cooled, colourless needles were obtained, decomposing at 16° C. (uncorr.). When boiled with water it dissolved, with the evolution of a gas, giving a yellow solution, which, on cooling, deposited red needles, melting at 183° C. (probably 2·4-dinitraniline). It was suspended in hot ether, and ammonia was passed through the mixture for a quarter of an hour. The colour of the suspended substance became bright yellow. It consisted of yellow needles, melting at 200° C. (uncorr.) with decomposition, and was identical with 2·4-dinitro-phenylurea described by Reudler.

An analysis of the original substance gave the following result:—

·0638 of the substance gave 14 c.c. of moist nitrogen at 17° C. and 768 mm.,
corresponding to N 25·70.
$C_7H_5O_7N_5$ required N 25·83.

The substance was therefore 2·4-dinitro-phenyl-nitro-urea.

By precipitation with water a further quantity of this substance was obtained from the nitric acid, but it could not be obtained in a colourless condition.

2.—Symmetrical Diphenylurea.

Hantzch [Liebeg's Annal. d. Chem., 325, 244, 1903], by the action of nitrous fumes on s-diphenylurea in glacial acetic acid solution, obtained nitroso-diphenylurea as yellow needles melting with decomposition at 82° C.

We did not obtain this substance, but got in glacial acetic acid solution dinitroso-s-diphenylurea as cream-coloured prismatic needles decomposing at 103° C.

By acting on s-diphenylurea in acetic acid solution with nitrogen peroxide we obtained successively dinitroso-s-diphenylurea, and 4·4′-dinitro-s-diphenylurea melting with decomposition at 320° C. [Struve, Radenhauser, J. f. pr. chem (2), 52, 233]. This latter compound we also obtained by the condensation of two molecules of p-nitraniline with one of urea.

The direct nitration of s-diphenylurea has been examined by several investigators with varying results.

Losanitch [Ber. d. Dtsch. Chem. Ges., x, p. 690] dissolved it in cold nitric acid, and warmed the solution to complete the reaction. He obtained a product melting about 200° C., which, according to analysis, was a tetranitro-s-diphenylurea. An alcoholic potash solution of it gave a dipotassium derivative which on boiling with water gave 2·4-dinitraniline. The compound was therefore 2·4·2′·4′-tetranitro-s-diphenylurea.

Fleischer and Nemes [Ber. d. Dtsch. Chem. Ges., x, p. 1295] have also described this reaction, but their results do not appear to have been reliable.

A. G. Perkin [J. Chem. Soc., 63 (1893), p. 1068] treated the tetranitro derivative obtained by Losanitch with a mixture of equal parts of nitric acid (sp. gr. 1·5) and sulphuric acid, which did not dissolve it, but transformed it, on heating, into a substance melting at 203° C. with decomposition. This compound, on treatment with ammonia, gave 2·4·6-trinitraniline melting at 186° C., and was therefore 2·4·6·2′·4′·6′-hexanitro-s-diphenylurea.

Curtius [J. f. pr. chem. (2), 52, p. 513], working at a lower temperature, obtained a result different from that of Losanitch. He nitrated s-diphenylurea in the cold, and obtained a yellow substance melting at 247–270° C., evidently impure, as his analysis indicated. By heating it in a sealed tube with hydrochloric acid he got m-nitraniline.

J. L. F. Reudler [Rec. trav. chim. Pays-Bas, 33 (1913), pp. 35–84] added s-diphenylurea to nitric acid (99·7 p.c.) at a low temperature, and obtained a compound as fine yellow needles, in which decomposition began to take place at 150° C., and which melted at 218° C. with decomposition. This substance was a tetranitro-s-diphenylurea, and when boiled with potash gave 2·4-dinitrophenol, and with aqueous ammonia gave 2·4-dinitraniline. It was therefore 2·4·2′·4′-tetranitro s-diphenylurea.

Working under similar conditions, with twenty molecular parts of acid to one of the urea, we obtained 2·4·2′·4′-tetranitro-s-diphenylurea using acid (sp. gr. 1·51) containing some oxides of nitrogen. It was also observed that 4·4′-dinitro-s-diphenylurea was formed as an intermediate product in this reaction.

The action of nitric acid on s-diphenylurea in the presence of solvents has not been previously examined.

In glacial acetic acid and in alcoholic solution at low concentration, and with one,

two, four, and six molecular amounts of nitric acid, the urea was unattacked even on allowing to remain at the room temperature for prolonged periods.

In carbon tetrachloride suspension, however, the substances reacted. With one two, and four molecular parts of nitric acid 4·4′-dinitro-s-diphenylurea was formed, as well as traces of 4-nitro-s-diphenylurea, melting at 212° C. [Leuckart J. f. pr. chem. (2), 41, 322]. With six molecular amounts of nitric acid, 2·4·2′·4′-tetranitro-s-diphenylurea was obtained as well as 4·4′-dinitro-s-diphenylurea.

By the action of nitrous acid (from aqueous hydrochloric acid and sodium nitrite) on s-diphenylurea, dissolved in a mixture of acetone and ether, a substance was formed at room temperature, which on partial evaporation of the solvents separated as cream-coloured prismatic needles decomposing at 103° C. The amounts of the urea and nitrous acid or the proportion of acetone to ether were not observed in this experiment, and various attempts to repeat it under observed conditions in acetone-ether solution failed. The compound was not formed in ether or acetone. It was finally obtained in glacial acetic acid solution with two or more molecular parts of nitrous acid. The compound was dinitroso-s-diphenylurea, which was also formed by the action of nitrous fumes and nitrogen peroxide on s-diphenylurea in glacial acetic acid solution.

The direct nitration of dinitroso-s-diphenylurea was also carried out, and 2·4·2′·4′-tetranitro-s-diphenylurea was obtained in this way. Nitrated in the presence of acetic acid at low concentrations and at ordinary room temperatures it yielded, on long standing, 4·4′-dinitro-s-diphenylurea as a main product.

A—*ACTION OF NITROUS FUMES ON SYMMETRICAL DIPHENYLUREA.*

s-Diphenylurea (2·5 g.) was well shaken with 100 g. of glacial acetic acid, and nitrous fumes were passed into the mixture for three quarters of an hour. The diphenylurea dissolved with simultaneous separation of another substance in the form of cream-coloured needles. The mixture was allowed to remain in a stoppered flask at the temperature of the room for thirty-six hours. It was then filtered, and the residue was washed with water and dried. It decomposed about 100° C.

On addition of water to the filtrate a further quantity of the same compound was obtained. By dissolving it in hot ether, and allowing most of the ether to evaporate, it was obtained as prismatic needles, which decomposed with evolution of gas at 103° C.

Analysis of this substance gave the following results:—

(*a*) 0·1327 g. of the substance gave 23·5 c.c. of moist nitrogen at 18° C. and 762 mm.,
which corresponded to N 20·51.

(*b*) 0·1103 g. of the substance gave 19·3 c.c. of moist nitrogen at 17° C. and 766 mm.,
which corresponded to N 20·53.

$C_{13}H_{10}O_3N_4$ required N 20·74.

On boiling with alcohol for some time it gave s-diphenylurea. It was therefore dinitroso-s-diphenylurea.

Dinitro-s-diphenylurea is a nearly colourless substance, which crystallises in prismatic needles. It is decomposed by hot water, and dissolves in most organic solvents.

B—*ACTION OF NITROGEN PEROXIDE ON SYMMETRICAL DIPHENYLUREA.*

(*a*) s-Diphenylurea (2·5 g.) was well shaken with 100 g. of glacial acetic acid, and nitrogen peroxide was passed into the mixture. The urea dissolved, whilst another substance was seen to take its place. After some time the mixture was filtered. The residue was washed with water, and on drying decomposed at 102°–103° C. The addition of dinitroso-s-diphenylurea to this substance did not affect its decomposition point. Hence it was dinitroso-s-diphenylurea. From the filtrate a further quantity of this substance was obtained.

(*b*) As before, 2·5 g. of s-diphenylurea in 100 g. of glacial acetic acid was treated with nitrogen peroxide, when dinitroso-s-diphenylurea was seen to be formed. On further treatment with nitrogen peroxide this dissolved slowly, giving a red solution, and on allowing the latter to remain in a stoppered flask at the temperature of the room for five days a yellow solid slowly separated.

The mixture was filtered, and the residue was well washed with alcohol, when a yellow substance remained, melting with decomposition at 305°–315° C. This, on crystallisation from nitro-benzene, gave minute needles, decomposing at 320° C. From the filtrate no pure substance was obtained.

An analysis of the above-mentioned substance gave the following result:—

0·1032 g. of the substance gave 16·45 c.c. of moist nitrogen at 18° C. and 767 mm.,

corresponding to N 18·58.

$C_{13}H_{10}O_5N_4$ required N 18·54.

This compound was also obtained by heating two molecular weights of p-nitraniline with one of urea for five hours at 190° C., and was therefore 4·4′-dinitro-s-diphenylurea.

4·4′-dinitro-s-diphenylurea is a bright yellow compound, sparingly soluble in most organic solvents, but soluble in hot nitro-benzene, from which it separates on cooling as minute yellow needles.

C.—*ACTION OF NITRIC ACID ON SYMMETRICAL DIPHENYLUREA.*

I.—*In the Presence of Solvents.*

(a) *Glacial acetic acid.*—s-Diphenylurea (2·5 g.) was treated in 200 g. of glacial acetic acid with one, two, four, and six molecular parts of nitric acid in the cold, and allowed to remain at the temperature of the room for two months. In the case of one, two, and four parts of the acid no reaction was found to have taken place, but in that with six parts of nitric acid a compound melting with decomposition at 320° C. (probably 4·4′-dinitro-s-diphenlyurea) and one melting with decomposition at 218° C. (probably 4-nitro-s-diphenylurea) were found. In this case, however, it had been found necessary, during the two months, to warm the bottle to melt the glacial acetic acid, which solidified several times.

This nitration was repeated without heating, and no reaction was found to take place.

(b) *Alcohol.*—s-Diphenylurea (2·5 g.) with 150 c.c. of alcohol were treated in the cold with one, two, four, and six molecular parts of nitric acid and allowed to remain at room temperature for two months, when in every case all the urea was recovered unchanged.

(c) *Carbon tetrachloride.*—s-Diphenylurea (2·5 g.) was suspended in 200 c.c. of carbon tetrachloride, and treated in the cold with one, two, four, and six molecular

parts of nitric acid. After allowing the bottles to remain at the temperature of the room for two months their contents were examined:—

(1) The bottle to which one molecular part of nitric acid had been added contained a red solution, with a yellow solid in suspension. From the solution a trace of red oil was obtained, and from the solid, by extraction with hot alcohol, some of the unchanged urea was obtained. After repeated extraction with hot alcohol a yellow substance remained. This after crystallisation from nitro-benzene decomposed at 320° C., and was 4·4′-dinitro-s-diphenylurea, a mixture of it with this substance melting at the same temperature.

The alcoholic extract gave a reddish-brown substance melting at 140°–170° C., from which no pure substance was isolated.

(2) The bottle containing two molecular amounts of nitric acid gave a red solution and a greenish-yellow solid. The former yielded only a little red oil. As in the previous case, 4·4′-dinitro-s-diphenylurea was obtained from the solid by thoroughly extracting it with alcohol. From the alcoholic extract a yellowish-brown substance was obtained, from which no crystallising substance could be got.

(3) The bottle which contained four molecular parts of nitric acid gave a red solution and a greenish-yellow solid. The solution was filtered from the solid, and gave, on shaking with barium carbonate, filtering, and evaporating to dryness, a small quantity of red oil. The solid was well extracted with alcohol, and a substance, which was found to be 4·4′-dinitro-s-diphenylurea, remained. From the red oil obtained from the carbon tetrachloride solution, and also from the brown substance contained in the alcoholic extracts, a compound, crystallising in yellow needles, and melting with decomposition at 212° C., was obtained by fractional crystallisation from alcohol. This substance was probably 4-nitro-s-diphenylurea.

(4) The bottle to which six molecular amounts of nitric acid had been added contained a red solution and a brown solid. The former, on filtering from the solid, was shaken with barium carbonate, filtered, and evaporated to dryness. It yielded only a trace of a red oil, from which no crystalline product was obtained.

By extracting the solid with alcohol as before, 4·4′-dinitro-s-diphenylurea was left. From the alcohol extract, using acetone as solvent, a small quantity of 2·4·2′·4′-tetranitro-s-diphenylurea was obtained. From this also a small quantity of a substance was obtained, using a mixture of carbon tetrachloride and chloroform as solvent. This substance melted at 212° C., and was probably 4-nitro-s-diphenylurea.

II.—*In the Absence of Solvents.*

s-Diphenylurea (5 g.) was added slowly to twenty molecular parts of nitric acid cooled in salt and ice. At first the urea dissolved easily, giving a red solution. Later on, however, a precipitate formed after each addition of the urea, and this dissolved with increasing difficulty towards the end of the operation. When all the urea had been added, a considerable quantity of the precipitate was present. Some of it was removed, and was found to possess all the properties of 4·4′-dinitro-s-diphenylurea. On standing overnight all the precipitate dissolved, giving a deep red solution, from which, by precipitation with water and crystallisation from acetone, yellow needles were obtained. These began to decompose about 150° C., and melted with decomposition at 218° C.

An analysis of this substance gave the following result :—

0·1680 g. of the substance gave 31·3 of moist nitrogen at 17° C. and 756 mm.,
corresponding to N 21·50.
$C_{13}H_8O_9N_6$ required N 21·43.

The compound was, therefore, 2·4·2′·4′-tetranitro-s-diphenylurea, as described by Reudler.

D.—*ACTION OF NITROUS ACID ON SYMMETRICAL DIPHENYLUREA.*

(*a*) One molecular part of sodium nitrite dissolved in the least quantity of water was added, a little at a time, and with constant shaking, to 2 g. of 3-diphenylurea in 100 g. of glacial acetic acid. The mixture was allowed to remain at the temperature of the room for three days, when the liquid had assumed a deep green tint. All the urea was, however, recovered unchanged.

(*b*) Two molecular parts of sodium nitrite dissolved in the least quantity of water were added, a little at a time, and with constant shaking, to 2 g. of glacial acetic acid. All the urea dissolved, and a cream-coloured substance separated and remained in suspension throughout the solution. After continued shaking for an hour it was added to water, when more of the substance separated as needles. It decomposed at 100°–103° C. Crystallised from ether it decomposed at 103° C., and a mixture of it with dinitroso-s-diphenylurea also melted at the same temperature. The substance was, therefore, dinitroso-s-diphenylurea.

(*c*) Dinitroso-s-diphenylurea was also obtained by the action of four and six molecular parts of nitrous acid on s-diphenylurea in acetic acid solution.

E.—*ACTION OF NITRIC ACID ON DINITROSO-S-DIPHENYLUREA.*

(*a*) To ten molecular parts of nitric acid, cooled in salt and ice, 1 g. of dinitroso-s-diphenylurea was added in small quantities. It dissolved easily, giving a red solution. This was allowed to remain overnight at the temperature of the room. By adding to much water, and crystallising from acetone the precipitate thus obtained, 2·2·2′·4′-tetranitro-s-diphenylurea was got. The yield was theoretical.

(*b*) One, two, four, and six molecular parts of nitric acid were added respectively to four solutions, each containing 2·5 g. of dinitroso-s-diphenylurea in 100 g. of glacial acetic acid. On the addition of the nitric acid the undissolved nitroso compound went into solution in every case, and later a precipitate began to separate.

The bottles were allowed to remain at the room temperature for two months. A large quantity of substance had separated in every case. This was found to consist mainly of 4·4′-dinitro-s-diphenylurea.

3.—Asymmetrical Diphenylurea.

Michler [Ber. d. Dtsch. Chem. Ges., ix, 715] obtained asymmetrical diphenylurea by heating diphenylurea chloride in a sealed tube with alcoholic ammonia at 100° C.

We found, however, that the reaction

$$(C_6H_5)_2N \cdot CO \cdot Cl + 2NH_3 = (C_6H_5)_2 \cdot N \cdot CO \cdot NH_2 + NH_4Cl$$

proceeded in the cold, though somewhat slowly. In boiling spirit solution the reaction went easily, and by passing ammonia into a hot solution of the chloride the urea was readily formed.

as-Diphenylurea reacted readily with nitrous fumes and nitrogen peroxide in acetic acid solution with evolution of a gas. It yielded in each case a yellow compound, which melted with decomposition about 146° C. Boiled with acetic acid it yielded a red substance, from which 4·4′-dinitro-diphenylamine was separated. The original substance was probably, therefore, 4·4′-dinitro-diphenyl-nitrosamine, mixed with some of its isomers.

4·4′-Dinitro-diphenyl-nitrosamine has been described by P. Juillard [Bull. Soc. Chim. Paris (3), xxxii (1905), pp. 1172–1190]. He obtained it as orange yellow needles, melting with decomposition at 150° C.

J. L. F. Reudler (*loc. cit.*) nitrated as-diphenylurea with ten molecular parts of 99·7 per cent. nitric acid, and obtained a reaction product from which no pure substance was isolated. It gave no nitramine reactions, neither did it yield a gas when boiled with water. It contained, therefore, no nitramine group. By using a smaller excess of nitric acid he obtained 4·4′-dinitro-as-diphenylurea. At a somewhat elevated temperature and with much nitric acid in the presence of sulphuric acid he obtained 2·4·-2′·4′-tetranitro-as-diphenylurea, and probably 2·4·6·2′·4′·6′-hexanitro-diphenylamine.

By the action of nitric acid (sp. gr. 1·51) on this urea in the cold we obtained 2·4·2′·4′-tetranitro-diphenylamine.

as-Diphenylurea in acetic acid at low concentrations was not nitrated by one, two, four, or six molecular parts of nitric acid in the cold. Yellow colourations were, however, developed in all cases. It did not nitrate in carbon tetrachloride suspension with one or two molecular parts of nitric acid, but with four and six parts of acid it gave a yellow substance, from which no pure compound was separated.

In the cold, nitrous acid (up to six molecular parts) was without action on as-diphenylurea in glacial acetic acid solution.

A.—*PREPARATION OF AS-DIPHENYLUREA.*

(*a*) 5 g. of diphenylurea chloride were shaken with 100 c.c. of methylated spirit containing 1·4 g. of ammonia (four molecular parts to one of the chloride). The mixture was allowed to remain at the temperature of the room for twenty-four hours. The undissolved diphenylurea chloride had then gone into solution, and its place was taken by large prismatic needles, which, on washing with water, melted at 189° C. The substance was as-diphenylurea.

(*b*) It was more expeditious, however, to obtain the urea by allowing the reaction to take place at the temperature of boiling spirit.

25 g. of diphenylurea chloride were heated to boiling with 100 c.c. of methylated spirit, and a stream of ammonia was passed into the mixture for a quarter of an hour. The undissolved diphenylurea chloride went into solution, and long colourless needles of as-diphenylurea separated. On allowing the mixture to cool a further quantity of the urea separated. The solution was filtered from the separated solid. This, when well washed with water to remove ammonium chloride, melted at 189° C. Recrystallisation from alcohol did not raise the melting point. The as-diphenylurea obtained was therefore pure. By evaporating the spirit solution down to small bulk a further quantity of substance was obtained, which, when washed with water, left the as-diphenylurea.

The yield was theoretical.

B.—*ACTION OF NITROUS FUMES ON AS-DIPHENYLUREA.*

as-Diphenylurea (5 g.) was dissolved in 100 g. of glacial acetic acid, and nitrous fumes were passed in at room temperature. The solution became red, then green, with the evolution of a gas throughout the liquid. On allowing to remain at room

temperature overnight a light yellow solid separated as needles. After two days the solution was filtered from the solid. The latter, after washing with alcohol, melted with decomposition at 145°–146° C. An attempt to recrystallise this substance from acetone seemed to decompose it, yielding a red substance melting with decomposition between 145°–162° C. The original substance gave a violet colouration with alcoholic potash.

Some of this substance was boiled for an hour with glacial acetic acid, in order to complete the decomposition effected by the acetone, during which time brown fumes were observed in the neck of the flask. This process yielded a red solution, from which, by precipitation with water, a red substance was obtained melting between 138°–155° C. By fractional crystallisation of this substance from acetic acid, a compound was obtained as prisms melting at 213° C. It was 4·4′-dinitro-diphenylamine, as the addition of the latter substance to it did not affect its melting point.

The original compound was therefore probably 4·4′-dinitro-diphenyl-nitrosamine (M.P. 150° C), which is very difficult to obtain pure. This would account for the low melting point of it as obtained above. Further, 4·4′-dinitro-diphenyl-nitrosamine gives, like this compound, a violet colouration with alcoholic potash, and yields, on elimination of the nitroso radical by boilings with acetic acid, 4·4′-dinitro-diphenylamine.

No pure substance was obtained from the original acetic acid solution.

C.—*ACTION OF NITROGEN PEROXIDE ON AS-DIPHENYLUREA.*

as-Diphenylurea (5 g.) was dissolved in 100 g. of glacial acetic acid and saturated with nitrogen peroxide in the cold. A gas was evolved throughout the solution. After allowing to remain at room temperature for two days a yellow substance separated out. This was identical with the substance obtained by the action of nitrous fumes on the urea, and was therefore 4·4′-dinitro-diphenyl-nitrosamine, mixed probably with some of its isomers.

No pure substance was obtained from the red glacial acetic acid fibrate.

D.—*ACTION OF NITRIC ACID ON AS-DIPHENYLUREA.*

I.—*In the Presence of Solvents.*

(a) *Glacial acetic acid.*—as-Diphenylurea (5 g.) was dissolved in 100 g. of glacial acetic acid and treated with one, two, four, and six molecular parts of nitric acid in the cold. On allowing to remain at the temperature of the room for two months, yellow colourations were developed in every case. This colouration persisted in the solids obtained by precipitation with water. The solid so obtained consisted, however, in every case of the unchanged as-diphenylurea.

(a) *Carbon tetrachloride.*—One, two, four, and six molecular parts of nitric acid were added to 5 g. of as-diphenylurea suspended in carbon tetrachloride. The mixtures were allowed to remain at room temperature for two months. A yellow solution and a black oil were obtained in every case. In the bottles to which four and six molecular parts of nitric acid had been added a red substance appeared towards the end of the two months.

On addition of ether to the bottles with one and two parts of nitric acid the oil was dissolved, with the separation of a white crystalline substance. This was the unchanged urea. By neutralizing with barium carbonate, filtering, and evaporating to dryness the ether-carbon-tetrachloride solution yielded a small quantity of black oil, from which, however, no pure solid was obtained.

The solutions in the bottles to which four and six parts of nitric acid had been added were filtered from the red solids. The latter, after removal of the oily matter with cold glacial acetic acid, yielded in each case a red substance (M.P. 170°–190° C.), from which no crystalline substance was obtained. The carbon tetrachloride solutions were neutralized with barium carbonate, filtered, and evaporated. In each case a black oil was obtained. In both cases there was much free nitric acid present.

II.—*In the Absence of Solvents.*

5 g. of as-diphenylurea were added in small quantities to twelve molecular parts of nitric acid cooled in salt and ice. The urea dissolved, giving a red solution. On allowing the mixture to remain at room temperature overnight, a gas was slowly evolved, and a yellow solid separated as prisms. This, when recrystallised from glacial acetic acid, yielded yellow leaves melting at 200° C. It was 2·4·2′·4′-tetranitro-diphenylamine, as the addition of the latter substance to it did not affect its melting point. A further quantity of this substance was obtained from the nitric acid solution.

E.—*ACTION OF NITROUS ACID ON AS-DIPHENYLUREA.*

One, two, four, and six molecular parts of sodium nitrite dissolved in a small amount of water were added to 5 g. of as-diphenylurea in 100 g. of glacial acetic acid in the cold. The mixtures were allowed to remain at room temperature for fourteen days, when all the urea was recovered unchanged.

4.—Triphenylurea.

The action of the oxides of nitrogen on triphenylurea has not been previously examined. Triphenylurea in glacial acetic acid solution reacted easily with both nitrous fumes and nitrogen peroxide, giving in each case the same trinitro-triphenylurea. The constitution of this substance was not determined.

Reudler (*loc. cit.*) nitrated triphenylurea in the cold, and obtained a product which gave no nitramine reactions. He obtained from it, after boiling with alcohol, 2·4·2′·4′-tetranitro-diphenylamine and 2·4-dinitro-phenylurethane. By using larger excess of nitric acid he obtained 2·4·2′·4′-tetranitro-diphenylamine and 2·4·6-trinitro-phenylurethane. In a similar experiment we only isolated 2·4·2′·4′-tetranitro-diphenylamine.

Triphenylurea was nitrated for long periods at room temperature and at low concentrations in acetic acid solution and in carbon tetrachloride suspension.

In acetic acid solution with one and three molecular amounts of nitric acid the urea was recovered unchanged; with six and nine molecular parts, however, a dinitro- and the trinitro-triphenylurea were formed in both cases.

In carbon tetrachloride suspension, with one molecular amount of nitric acid, the dinitro-triphenylurea was formed; with three and six amounts the dinitro- as well as the trinitro-triphenylurea was obtained; and with nine amounts a pentanitro-triphenylurea was formed.

Nitrous acid was without action on triphenylurea in acetic acid solution even on prolonged standing.

A.—*ACTION OF NITROUS FUMES ON TRIPHENYLUREA.*

5 g. of triphenylurea were dissolved in 100 g. of glacial acetic acid, and nitrous fumes (from arsenious acid and nitric acid) passed into saturation at room temperature. The mixture was allowed to stand for a week at ordinary room temperature,

more nitrous fumes having been passed in once during the week. The solution was at first green, and became brown on standing. A yellow solid also separated.

The yellow solid was separated from the liquid, and when crystallised from acetic acid or acetone it gave slightly yellow-coloured leaves, melting at 205°–206° C.

An analysis of this compound gave the following result :—

0·1045 g. of the substance gave 15 c.c. of moist nitrogen at 18·5° C. and 762 mm., corresponding to N 16·67.

$C_{19}H_{16}O_7N_5$ required N 16·55.

The compound was therefore a trinitro-triphenylurea.

It is a slightly coloured substance, fairly soluble in alcohol, acetone, and acetic acid; slightly soluble in ether, chloroform, and carbon tetrachloride.

A further quantity of this substance was obtained from the acetic acid solution.

B.—*ACTION OF NITROGEN PEROXIDE ON TRIPHENYLUREA.*

5 g. of triphenylurea were dissolved in 100 g. of glacial acetic acid, and nitrogen peroxide passed into saturation at room temperature. The mixture was allowed to stand for a week at room temperature, more nitrogen peroxide having been passed in during the week. The solution, which was at first yellow, became deep brown, and a yellow solid separated.

The solution was filtered from the solid, which was found to be identical with the trinitro-triphenylurea described above. From the solution, by precipitation with water, a further quantity of this substance was obtained.

C.—*ACTION OF NITRIC ACID ON TRIPHENYLUREA.*

I.—*In the Presence of Solvents.*

(a) *Glacial acetic acid.*—Triphenylurea (5 gr.) was dissolved in 100 g. of glacial acetic acid, and treated in the cold with one, three, six, and nine molecular amounts of acetic acid. The mixtures were allowed to remain at room temperature for two months.

(1) The solutions in the bottles with one and three molecular amounts of nitric acid developed a purple colouration, but all the triphenylurea was recovered unchanged.

(2) In the bottle to which six molecular amounts of nitric acid had been added a purple colouration was also developed in the solution. This after some time changed to deep brown. Towards the end of the two months a yellow substance began to separate.

The solution was filtered from the small amount of solid. The latter was found to consist of two substances, one easily soluble and the other difficultly soluble in acetic acid. The difficultly soluble substance crystallised from glacial acetic acid in leaves melting at 205°–206° C. It was identical with the trinitro-triphenylurea obtained previously, as the addition of the latter substance to it did not affect the melting point. The more easily soluble substance was soluble in alcohol, acetone, and glacial acetic acid, from which it crystallised as yellow prisms, melting at 190°–191° C. (uncorr.). This substance, as was afterwards found, was a dinitro-triphenylurea.

From the original acetic acid filtrate some of the unchanged urea was obtained, together with further quantities of the dinitro-triphenylurea.

(3) In the bottle to which nine molecular amounts of nitric acid were added a purple colouration was developed in the solution. Later on this changed to a deep brown, with the separation of a yellow substance. At the end of two months a fairly large quantity of this substance had separated.

As in the previous case, the solid was found to consist of a mixture of two compounds, one easily soluble and the other difficultly soluble in glacial acetic acid. The former was the yellow dinitro-triphenylurea previously obtained, as the addition of this substance to it did not affect its melting point. An analysis of it gave the following result:—

0·1308 g. of the substance gave 16·8 c.c. of nitrogen (moist) at 16° C. and 766 mm., corresponding to N 15·02.

$C_{19}H_{14}O_5N_4$ required N 14·81.

The substance was therefore a dinitro-triphenylurea.

The difficultly soluble substance was identical with the above-mentioned trinitro-triphenylurea.

From the acetic acid solution a further quantity of these compounds was obtained, as well as some of the unchanged urea.

(b) *Carbon tetrachloride.*—Five g. of triphenylurea were suspended in 100 g. of carbon tetrachloride and treated in the cold with one, three, six, and nine molecular parts of nitric acid. The mixtures were allowed to remain at the temperature of the room for six weeks.

(1) The bottle containing one molecular part of nitric acid gave a yellow solution, with a black oil floating on the surface. The latter was separated from the solution and boiled with ether. A small quantity of a yellow substance remained undissolved. This was soluble in acetone, from which it crystallised as yellow prisms, melting at 190°–191° C. It was dinitro-triphenylurea, as a mixture of it with this substance melted at the same temperature. The ethereal extract gave on evaporation a black oil, from which no pure substance was obtained.

By neutralizing with barium carbonate, filtering, and evaporating the carbon tetrachloride solution yielded a black oil, from which a further small quantity of dinitro-triphenylurea was obtained by boiling with ether.

(2) The bottle to which three molecular parts of nitric acid had been added contained a yellow solid and a red solution. The solution was filtered from the solid, which, after washing with ether and crystallising from acetic acid, gave yellow leaves melting at 203°–206° C. It was identical with trinitro-triphenylurea, as a mixture of the two substances melted at the same temperature as the individual substances.

From the carbon tetrachloride solution, on neutralization, filtration, and evaporation, a black oil was obtained. This, when boiled with ether, left dinitro-triphenylurea.

The black oil obtained by evaporating the ethereal extract yielded no crystalline substance.

(3) The bottle containing six molecular parts of nitric acid gave a yellow solid and a red solution. By methods similar to those employed in (2) the yellow solid was found to consist mainly of trinitro-triphenylurea, whilst the solution was made to yield dinitro-triphenylurea and a black oil which did not crystallise.

(4) In the case of the bottle to which nine parts of the acid were added, the temperature rose somewhat during the addition of the acid. It yielded a yellow solution, with a black tarry mass in suspension. By boiling the latter with glacial acetic acid a yellow crystalline substance remained undissolved. This was well washed with glacial acetic acid. When heated it began to decompose about 180° C.

with evolution of brown fumes, and melted with decomposition at 235°–236° C. (uncorr.). An analysis of this substance gave the following result :—

0·1237 g. of the substance gave 20 c.c. of moist nitrogen at 16° C. and 763 mm., corresponding to N 18·92.
$C_{19}H_{11}O_{11}N_7$ required N 19·11.

The substance was therefore a pentanitro-triphenylurea.

Pentanitro-triphenylurea is a light yellow substance, and as obtained above crystallises in prisms. It is sparingly soluble in most organic solvents, but soluble in hot nitro-benzene.

No solid was obtained either from the carbon tetrachloride or from the acetic acid solution.

I.—*In the Absence of Solvents.*

5 g. of triphenylurea was added slowly to twelve molecular parts of nitric acid cooled in salt and ice. The interaction was at first very violent, but towards the end became more moderated. The triphenylurea dissolved, giving a red solution. The mixture was allowed to remain overnight at the temperature of the room. On pouring into much water an oily, yellow substance was obtained. This was filtered, and the solid was well extracted with hot acetene and alcohol. A small quantity of a yellow substance remained undissolved. This crystallised from glacial acetic acid as leaves, melting at 200° C. (uncorr.). This substance was therefore similar to 2·4·2′·4′-tetranitro-diphenylamine in melting point and crystalline form. The addition of 2·4·2′·4′ tetranitro-diphenylamine to it had no effect upon its melting point. Is was therefore 2·4·2′·4′-tetranitro-diphenylamine.

No other pure substance was isolated from the nitration product.

D.—*ACTION OF NITROUS ACID ON TRIPHENYLUREA.*

Triphenylurea (5 g.) was dissolved in 100 g. of glacial acetic acid and treated in the cold with one, two, four, and six molecular parts of sodium nitrite, dissolved in the smallest amount of water. The mixtures were allowed to remain at room temperature for a fortnight. Yellow colourations were developed in all cases, but the triphenylurea was recovered unchanged.

Summary.

1. The action of nitric acid on substituted ureas at the ordinary temperature and at low concentrations was examined.

Phenylurea formed phenylurea nitrate; *sym-* and *as*-diphenylureas were unacted upon; and triphenylurea yielded a di- and a trinitro-triphenylurea.

Under similar conditions, but in carbon tetrachloride suspension, phenylurea formed its nitrate, its p-nitro, and its 2·4-dinitro derivatives. *Sym*-diphenylurea was nitrated to its 4-nitro, its 4·4′-dinitro, and its 2·4·2′·4′-tetranitro derivatives, but *as*-diphenylurea yielded substances from which no pure compound was obtained. Triphenylurea formed a mono-, a tri-, and a pentanitro-triphenylurea.

2. Cold fuming nitric acid acted upon all four of the substituted ureas. Phenylurea gave 2·4-dinitro-phenyl-nitro-urea; *sym*-diphenylurea formed its 4·4′-dinitro and its 2·4·2′·4′-tetranitro derivatives; and from *as*-diphenylurea as well as from triphenylurea 2·4·2′·4′-tetranitro-diphenylamine was obtained.

3. In cold glacial acetic acid solution nitrous acid reacted with phenylurea to form nitroso-phenylurea, and with *sym*-diphenylurea to form dinitroso-diphenylurea. The other two substituted ureas were apparently unacted upon under the same conditions.

4. Phenylurea, suspended in carbon tetrachloride, was converted by nitrous fumes into ortho- and para-nitrophenol. *Sym*-diphenylurea in acetic acid solution was converted by nitrous fumes into dinitroso-diphenylurea. By the prolonged action of nitrogen peroxide upon it 4·4′-dinitro-diphenylurea was, however, obtained.

As-diphenylurea was decomposed by nitrogen peroxide at the ordinary temperature, forming diphenylamine derivatives, e.g., 4·4-dinitro-diphenyl-nitrosamine and triphenylurea under similar conditions yielded a trinitro-triphenylurea.

5. In regard to ease of nitration the phenylureas resemble closely the corresponding phenylurethanes. They are much less easily nitrated than the corresponding phenyl-nitrosamines.

In conclusion we wish to state that the above research was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for a grant in aid of the investigation.

No. 19.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON PHENYL-METHYLUREA.

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Introduction.

It has already been shown that urethanes are more difficultly nitratable [H. Ryan and A. Donnellan, p. 113; H. Ryan and N. Cullinane, p. 119; H. Ryan and A. Connolly, p. 125; H. Ryan and J. O'Donovan, p. 131] than secondary amines or nitrosamines. It has been shown also that the phenylureas [H. Ryan and P. O'Toole, p. 139] resemble the urethanes in respect to difficulty of nitration at low temperatures and concentrations of the interacting substances.

In the present communication we describe the results of experiments we have tried with phenyl-methylurea, which form the last of the series of comparative experiments made in this laboratory on the nitration of nitrogenous aromatic substances.

Nitro derivatives of phenyl-methylurea have not hitherto been obtained, nor do they appear to be formed under the conditions which we employed in the following experiments.

Nitrous acid readily converts the urea into methylaniline, and such derivatives as we have isolated in our experiments can all be obtained from this base.

Nitrogen peroxide in the vapour phase converts phenyl-methylurea into tetryl, while in solution it forms on short action 4-nitro-phenyl-methyl-nitrosamine, and on more prolonged action 2·4-dinitro- and 2·4·6-trinitro-methylaniline are the chief products.

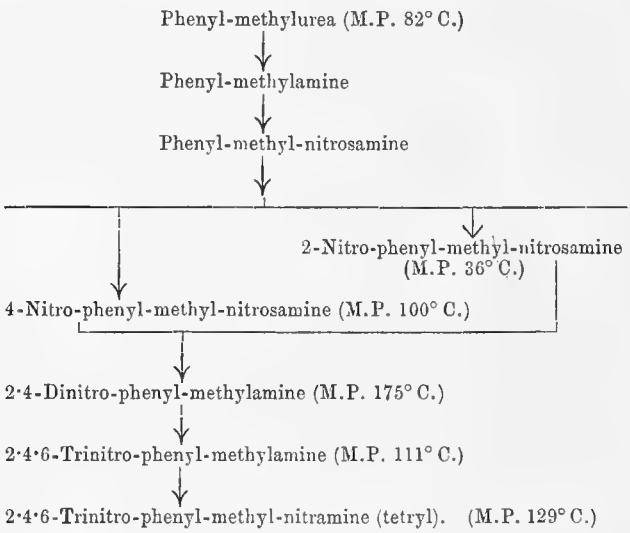
Nitrous anhydride acts on a solution of the urea in the same way as it does on the amine [cf. R. Stoermer and P. Hoffmann, Ber. d. Dtsch. Chem. Ges., xxxi (1898), pp. 2523–2541], forming on short action 4-nitro-phenyl-methyl-nitrosamine.

Pure nitric acid (in the presence of urea nitrate) at low temperatures and concentrations has little, if any, nitrating action on the urea, but in the presence of nitrous acid or at more or less high temperatures, especially in carbon tetrachloride solution, it behaves towards the urea in the same way as it does towards methylaniline, converting it into phenyl-methyl-nitrosamine, 4-nitro-phenyl-methyl-nitrosamine, 2·4-dinitro-methylaniline, 2·4·6-trinitro-methylaniline, and finally into tetryl.

The only nitrosamines we isolated were phenyl-methyl-nitrosamine and 4-nitro-phenyl-methyl-nitrosamine. The other nitrosamines known, which include 2 4-dinitro-phenyl-methyl-nitrosamine melting at 86° C. [R. Stoermer and P. Hoffmann, Ber. xxxi (1898), pp. 2523–2541] and 2·4·6-trinitro-phenyl-methyl-nitrosamine melting at 106·5° C. [E. Bamberger and J. Müller, Ber. xxxiii (1900), pp. 100–113], were not isolated. The same remark applies to the lower nitramine derivatives, which include the 2-nitro-phenyl-methyl-nitramine melting at 67° C. and the 4-nitro-phenyl-methyl-nitramine melting at 140° C. of E. Bamberger [Ber. d. Dtsch. Chem. Ges., xxx (1897), pp. 1248–1263].

Attempts to prepare nitro substitution derivatives of phenyl-methylurea from nitro derivatives of methylaniline and cyanic acid have not hitherto proved successful.

In conclusion, the course of the reactions between phenyl-methylurea and the oxyacids of nitrogen may be summarized as follows :—



Experimental.

A.—*Action of Nitrogen Peroxide on Phenyl-methylurea.*

I.—*In the Vapour Phase.*

Two shallow glass dishes containing 2 g. of phenyl-methylurea and liquid nitrogen peroxide were placed side by side on a glass plate under a bell-jar and allowed to remain for a month at the room temperature. When the dark reddish-brown liquid into which the phenyl-methylurea had been converted was allowed to remain exposed to the air overnight, it became crystalline. The solid, which melted at first about 118° C., on recrystallisation from alcohol and acetone gave faint yellow crystals, which melted at 126°–127° C., and proved to be identical with tetryl. A very small amount of a dark-coloured oil was obtained by concentrating the alcohol-acetone filtrates.

II.—*In Solution.*

(a) *Ether.*—Dry nitrogen peroxide fumes were passed at intervals through a solution of 2 g. of phenyl-methylurea in 75 c.c. of anhydrous ether, which was allowed to remain for about two months at the temperature of the room. About 1 g. of crystals separated, which melted at 111° C., and proved to be 2·4·6-trinitro-methylaniline.

By evaporating the ether filtrate and recrystallising the residue 2·4-dinitro-methylaniline was obtained in the form of yellow crystals, melting at 175°.

(b) *Alcohol.*—In another experiment 2 g. of phenyl-methylurea were dissolved in 10 c.c. of alcohol, and nitrogen peroxide vapours were passed through the solution, which was kept cold by immersing the tube containing it in water. The solution effervesced briskly and turned brown in colour. It was concentrated slightly. The yellow solid which separated, after recrystallisation from alcohol, melted at 99°–100° C., and, as its melting point was not affected by mixing it with 4-nitro-phenyl-nitrosamine, it was identical with the latter substance.

B.—*ACTION OF NITROUS ANHYDRIDE ON PHENYL-METHYLUREA.*

Fumes of nitrous anhydride, prepared by the action of nitric acid on arsenious oxide, were passed for about an hour through a solution of 2 g. of phenyl-methyl-urea in 10 c.c. of absolute alcohol, kept at the temperature of the room by immersing the flask containing the solution in water. The mixture effervesced, the colour changing through yellow to red. On allowing the solution to evaporate, 4 nitro-phenyl-methyl-nitrosamine separated in the form of yellowish crystals, melting at 99°–100° C.

C.—*ACTION OF NITRIC AND NITROUS ACIDS ON PHENYL-METHYLUREA.*

I.—*In the Absence of Solvents.*

About 8 c.c. of fuming nitric acid (sp. g. 1·51) were added slowly to 2 g. of phenyl-methylurea. The dark red mixture was allowed to remain overnight and was then poured into water. The yellowish solid which separated was filtered, washed with water and alcohol, and then recrystallised from acetone, from which it separated in the form of nearly colourless crystals, melting at 128°–129° C. The yield was very good. As its melting point was not affected by mixing the substance with pure tetryl, prepared from dimethylaniline by the method described by M. C. F. van Duin [Rec. de trav. Chim. Pays Bas, xxxvii (1917), p. 111], the two bodies are therefore identical.

II.—*In the Presence of Solvents.*

(a) *Water*: (α) *At ordinary concentrations.*—1. Phenyl-methylurea (13·6 g.) was dissolved in a mixture of 12 c.c. of concentrated hydrochloric acid and 24 c.c. of water, and to the cold solution 7·5 g. of sodium nitrite, dissolved in a small amount of water, were added slowly and with frequent stirring. The mixture rapidly became turbid, with separation of a yellowish oil, which was extracted with ether; the residue left on evaporation of the ether was reduced by means of tin and hydrochloric acid to methylaniline, which boiled at 192°–194° C. The aqueous solution, from which the nitrosamine had been separated by ether, contained a small amount of the hydrochloride of methylaniline.

2. An aqueous solution, containing 8·4 g. of nitric acid, was added slowly to a cold solution of 5 g. of phenyl-methylurea and 5 g. of sodium nitrite in 25 c.c. of water. A colourless oil first separated; but when the mixture was warmed for some hours on the water-bath the oily nitrosamine was gradually converted into a solid, which, when recrystallised from alcohol, melted at 99°–100° C., and proved to be 4-nitro-phenyl-methyl-nitrosamine.

In a similar experiment, in which, however, sodium nitrite was not added to the reaction mixture, the only change observed consisted of the formation of a small amount of methylaniline.

(β) *At low concentrations.*—Four solutions, each containing 1 g. of phenyl-methylurea in 50 c.c. of water, to which one, two, three, and four molecular amounts of nitric acid had been added respectively, were allowed to remain in stoppered bottles at the temperature of the room for about six weeks. The solutions, which had remained nearly colourless, were neutralized with sodium carbonate, saturated with sodium sulphate, and extracted with benzene. Almost all the phenyl-methylurea was recovered unchanged in each case.

(b) *Alcohol.*—As phenyl-methylurea was recovered unchanged from an alcoholic solution of it to which nitric acid containing urea nitrate had been added, and which had been allowed to remain at the temperature of the room for a couple of weeks, the experiment was varied by including nitrous acid in the interacting substances.

For this purpose aniline was converted into methylaniline by the method of G. T. Morgan (E.P. 102,834), and the methylaniline, without isolating it, was converted directly into its nitrosamine, which was separated from the simultaneously formed diazonium salt by means of ether; the phenyl-methyl-nitrosamine left on evaporation of the ether was further purified by distillation in a current of steam, and the product thus got was employed in the reactions described below :—

A solution of 5 g. of phenyl-methyl-nitrosamine in 100 c.c. of alcohol to which 0·75 c.c. of nitric acid (sp. g. 1·51) had been added was heated on the water-bath for five hours. The solid which separated on cooling melted at 99°–100° C., and consisted of 4-nitro-phenyl-methyl-nitrosamine.

(c) *Acetic acid.* (α) *At ordinary concentrations.*—1. To a mixture of 10 g. of phenyl-methylurea and 20 c.c. of glacial acetic acid 5·5 c.c. (two molecular amounts) of nitric acid (sp. g. 1·51) were added slowly. A solution of 5 g. of sodium nitrite in about 10 c.c. of water was then allowed to drop into the mixture, the temperature of which was kept below 30° C. during the addition of the nitrite. A crystalline solid separated from the solution, the latter becoming almost black in colour. After remaining overnight the solution was filtered and the solid was dissolved in hot alcohol, from which it separated in the form of almost colourless acicular prisms melting at 100° C., and consisting of pure 4-nitro-phenyl-methyl-nitrosamine.

On addition of water to the acetic acid filtrate a small amount of 2·4-dinitro-methylaniline was obtained.

The nitro-nitrosamine obtained in this experiment was heated with concentrated hydrochloric acid and alcohol under a reflux condenser until the evolution of nitrous fumes had ceased. The reddish yellow solution was cooled, diluted with water, and neutralized with sodium carbonate. The yellow solid which separated was filtered, washed with water, and recrystallised from alcohol. The yellow crystals thus got melted at 151° C., and consisted of 4-nitro-methylaniline.

2. In another experiment 20 c.c. of fuming nitric acid (sp. g. 1·51) were added to a solution of 5 g. of phenyl-methylurea in 50 c.c. of glacial acetic acid,

and the mixture was heated for an hour on the water-bath. Nitrous fumes were evolved, and a dark, somewhat oily solid separated when the solution was poured into water. When this solid was crystallised from alcohol it melted, but not quite sharply, about 111° C., and consisted of 2·4·6-trinitro-methylaniline.

3. Phenyl-methyl-nitrosamine (4·5 g.) was dissolved in warm acetic acid (10 c.c.), and to the solution 2·3 c.c. of fuming nitric acid (sp. g. 1·51) were added slowly. The mixture was heated to boiling for a few minutes, then diluted with water and cooled. The solid which separated was filtered, washed with water, and recrystallised from alcohol. About 3 g. of pure 2·4-dinitro-methylaniline melting at 175° C. were thus got; the parent liquid contained a small amount of an oily solid, probably consisting of the same substance.

(β) *At low concentrations.*—To four solutions of 5 g. of phenyl-methylurea in 100 g. of glacial acetic acid quantities of nitric acid corresponding to one, two, three, and four molecular amounts were added, and the solutions allowed to remain at the temperature of the room for about three months.

In no case was there any indication of the occurrence of any appreciable amount of reaction between the constituents. No solid separated from the mixtures, and when they were poured into water the solutions remained in all cases clear.

(d) *Carbon tetrachloride.—At low concentrations.*—As phenyl-methylurea is only sparingly soluble in cold carbon tetrachloride, it was found inconvenient in this case to carry out the reactions at the temperature of the room.

Four solutions were prepared, containing in each case 5 g. of phenyl-methylurea in 100 g. of carbon tetrachloride at 60° C. Quantities of fuming nitric acid (sp. g. 1·51) corresponding to one, two, three, and four molecular amounts were added to these respectively. In each case a vigorous reaction set in, with evolution of nitrous fumes and separation of a dark brown oily layer. The mixtures were allowed to remain at the temperature of the room for three months.

They were then shaken with water, the carbon tetrachloride was in each case separated, and the aqueous washings returned to the solid or tarry matter in the flask.

1. By distilling the deep yellow carbon tetrachloride solution from the flask to which one molecular amount of nitric acid had been added about 0·5 g. of tetryl was obtained.

The aqueous portion was made alkaline and extracted with ether. Methyl-aniline was extracted from the ether solution by means of hydrochloric acid, and after reconversion into the free base and extraction with ether boiled at 192°–194° C.

2. From the carbon tetrachloride solution to which two molecular amounts of nitric acid had been added we isolated, on the other hand, a small amount of 4-nitro-phenyl-methyl-nitrosamine; while from the mixture of the aqueous and undissolved portions in the flask we obtained a further quantity of 4-nitro-phenyl-methyl-nitrosamine and 2·4-dinitro-methylaniline.

3. In the case of the solution to which three molecular proportions of nitric acid had been added distillation of the neutralized carbon tetrachloride solution gave a small amount of a crystalline residue, which, when washed with ether, melted at 175° C., and consisted of 2·4-dinitro-methylaniline.

The mixture of solid and water in the flask was shaken with ether. The lower aqueous layer was filtered from a yellow solid (0·5 g.), which consisted of 2·4-dinitro-methylaniline (M. P. 175° C.), and the ether solution, after washing with dilute sodium carbonate, gave a further quantity (0·75 g.) of 2·4·6-trinitro-methylaniline.

4. By distilling the carbon tetrachloride solution from the mixture to which four molecular amounts of nitric acid had been added, a small amount of nearly colourless crystals of tetryl was obtained, and by fractional crystallisation from alcohol of the crystalline solid suspended in the aqueous portion we separated some pure 2·4·6-trinitro-methylaniline from the impurity (probably tetryl) with which it was mixed.

SUMMARY.

1. Under the conditions of our experiments phenyl-methylurea formed no nitro derivatives. When nitrous acid was present the phenyl-methylurea was converted into methylaniline, and hence the nitro substances we isolated were in all cases derived from the latter base.

2. Nitrogen peroxide in the vapour phase converted phenyl-methylurea into tetryl, but in solution it formed successively 4-nitro-phenyl-methyl-nitrosamine, 2·4-dinitro- and 2·4·6-trinitro-methylaniline.

3. In the presence of urea nitrate, nitric acid had little, if any, action on phenyl-methylurea, but in the presence of nitrous acid the phenyl-methylurea was converted into phenyl-methyl-nitrosamine, 4-nitro-phenyl-methyl-nitrosamine, 2·4-dinitro-methylaniline, 2·4·6-trinitro-methylaniline, and tetryl.

Incidentally it was found that tetryl could be obtained in a good yield and in a pure condition by the nitration of phenyl-methylurea or phenyl-methyl-nitrosamine.

4. So far as ease of nitration is concerned, phenyl-methylurea bears to *sym*-diphenylurea a relation somewhat similar to that of ethyl-o-tolylurethane to diphenylurethane.

In conclusion we wish to state that the above research was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for a grant in aid of the investigation.

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No. 20.

ON THE CAUSE OF ROLLING IN POTATO FOLIAGE; AND ON SOME FURTHER INSECT CARRIERS OF THE LEAF-ROLL DISEASE.

By PAUL A. MURPHY, Sc.D., A.R.C.Sc.I.,
Seeds and Plant Disease Division, Department of Agriculture and Technical Instruction for Ireland.

(Plate VI.)

[Read February 27. Printed May 15, 1923.]

Within comparatively recent years the designation "leaf-roll" has, by general agreement among phytopathologists, become restricted to a single specific disease of the potato plant. It is not necessary to discuss here the tangled history of the varying connotations of the term as used by different investigators since 1905. At the present time this name is the one applied by almost all plant pathologists exclusively to that very widely distributed disease of the potato plant which has as its most constant external symptom a distinctive thickening and upward marginal rolling of the leaflets of the lower leaves in the first instance, and which is accompanied by a more or less pronounced diminution in yield of new tubers—features which are persistent and not merely of seasonal or shorter duration.[1] As thus defined, the disease corresponds to the secondary leaf-roll of Quanjer.[2]

No general account of the disease need be given here except in so far as the results obtained by previous investigators have a direct bearing on the work now to be described. This deals mainly with some features of the physiology and histology of affected plants and with the transmission of the disease by certain classes of insects. The work was carried out principally at the Albert Agricultural College, Glasnevin, Dublin, in a laboratory and special plots provided for the purpose. Acknowledgments are due to Mr. R. McKay, A.R.C.Sc.I., who acted as Outdoor Assistant during the season of 1922, for the care of the plots and for carrying out some of the experimental details.

I.—*Previous work on the Translocation of Food Materials in Diseased Plants.*

It had already been surmised by a number of workers, including Sorauer, Spieckermann, Köck and Kornouth, Doby and Quanjer, that some disturbance in the mechanism of food transference in the plant underlay the disease described by them as "leaf-roll." Unfortunately it is difficult in many cases to establish with certainty the identity of the malady under discussion. The majority of

[1] It is desirable that the more indeterminate name "leaf-curl," which is still unfortunately retained in the official publications of the English Ministry of Agriculture, should not be used for this disease.

[2] Comparatively little attention was paid during the course of this work to primary leaf-roll, but it may be mentioned in anticipation that starch was found to accumulate to an abnormal extent in the upper leaves, which are the ones that first become rolled in this stage of the disease.

these investigators had in view the difficulty in upward transfer of food from the planted tubers to the developing shoot; and some evidence in support of the existence of this difficulty, based on chemical analysis, was brought forward by Spieckermann (14) and Doby (2). The same workers also found that at a later stage of growth there was an excess of food in the upper portion of the plant; whilst Quanjer (10) clearly expressed the opinion that the abnormal appearance shown by the foliage must be looked upon as being due in some way to a similar disturbance which interfered with the downward transfer of food.

According to Quanjer (11), Jordi (in 1913) was the first to record the presence of abnormal quantities of starch in the stems and petioles of plants affected with leaf-roll. It was not, however, until the publication of Neger's (6) first paper in 1918 that it was realised that the rolled leaves of diseased plants retained their starch to a very large extent instead of getting rid of it. In the following year Esmarch (3), Hiltner (4), and Quanjer (9) published the results of investigations in which each arrived independently at the same general conclusion. According to Esmarch (l.c., p. 17), the leaves of healthy potato plants when darkened become free from starch within a period of from 19 to 68 hours, depending on the temperature, weather conditions, age of the leaves, and individuality of the plants. The leaves of diseased plants, on the other hand, become depleted of their starch under the same conditions either not at all or only incompletely. In Esmarch's experiments the older leaves of diseased plants, after being darkened for from six to eight days, or in many experiments even for twelve days, still retained practically all their starch, in many cases the veins alone being more or less starch-free. In the younger leaves of diseased plants, which were still free from rolling, starch sometimes began to disappear at about the same time as in leaves of the same age from healthy plants; but generally it did so only after a period of from three and a half to eight days. Complete disappearance of the starch from young rolled leaves was seen only rarely. The older the leaf and the more pronounced the rolling the greater was the interference with starch translocation.

The experiments carried out by Neger gave very similar results. This worker employed cut shoots which, after removal from the plants, were placed in the dark with their ends standing in water. While this method is a very convenient one for demonstrating the difference between the amount of starch in the leaves of diseased and healthy plants (and was largely used for that purpose in the investigations now to be described), it is entirely unsuitable for experiments on the translocation or movement of carbohydrates in the plants. Consequently Neger's conclusions on carbohydrate translocation, based, as they were, on this method, are open to serious question.

II.—*Starch Accumulation in the Leaves Invariably Associated with Leaf-Roll.*

In taking up the further study of this subject it seemed necessary in the first place to determine whether excess of starch in the lower leaves of diseased plants was a universal concomitant of leaf-roll under Irish conditions. That this was so was established as a strong probability as a result of experiments carried out here with twenty-one varieties of potato in 1921. Two plants of each variety were selected, one diseased and the other healthy, and from a lower leaf of each, situated on the westerly side of the plant, corresponding leaflets were removed in the morning (10 a.m. to 11 a.m.), and were subjected to the well-known iodine test. In the case of all varieties but two there was a very marked difference in the amount of starch present, the affected leaflets containing an abundance of starch, while the healthy ones showed little or none.

The trials were continued in 1922 with a slight variation in method. The leaflets from each pair of plants of the same variety (diseased and healthy) were cut from corresponding lower leaves in the evening (5 p.m.), and were kept in the dark with their stalks in water for a period of from sixteen to forty hours. The latter period was generally used, and was found to give a better differentiation. In these experiments, as in the previous year, leaflets of twenty-three varieties were submitted to the iodine test during the months of June and July, and in all cases without exception a pronounced difference between the diseased and healthy leaflets was observed. In some cases whole leaves were used instead of leaflets, with the same results. The healthy leaflets were generally free from starch, but sometimes they showed a slight brownish mottling, indicating its presence in small quantities; the diseased leaflets, on the other hand, presented an intense blue-black colour, which, as a rule, was practically uniform all over. The bases of the mid-ribs and adjacent lateral veins sometimes contained no starch, and more rarely practically clear patches alternated with deep blue-black ones on other portions of the leaflets.

Most of the better-known varieties of potato were used in these experiments, including those of long standing and others of more recent introduction. Some of these were early, some mid-season, and some late sorts.

Since the varieties from which positive results were secured in 1922 included the only two exceptional ones of 1921 (when their condition in respect of leaf-roll infection was felt to be open to some doubt on account of the exceptional drought then prevailing), it seems safe to conclude that an abnormal accumulation of starch in the lower leaves always accompanies leaf-roll; and, incidentally, this circumstance provides a convenient symptom for the accurate diagnosis of the disease. Further, the iodine test has been tried and found to give satisfactory results on diseased and healthy lower leaves of the same variety purposely sent in by post in the ordinary way from the West of Ireland for examination. In such cases the difference in the colour of the two leaves, after boiling and treating with iodine, can be observed clearly by transmitted light without preliminary extraction of the chlorophyll. It was found previously that this could be done on material gathered direct and not sent through the post; and the omission of the bleaching part of the test saves both time and alcohol.

III.—*Effects of the Accumulation of Starch on the Conformation and Structure of the Leaves.*

A number of experiments were carried out to determine, if possible, the relationship in respect of cause and effect between starch accumulation and rolling of the leaves. A beginning was made by the examination during May and early June, 1922 (before any visible symptoms of leaf-roll appeared), of the starch content of the lower leaves of plants derived from diseased tubers.[1] All the plants examined, as was to be expected, afterwards developed normal symptoms of leaf-roll. With them were compared corresponding leaves from healthy plants, and, in most cases, similar leaves which had already begun to

[1] In the apparent absence of recognisable symptoms of the leaf-roll disease in the tubers, or at least of such as are of constant occurrence, the presence of infection in any given tuber can be assumed only when the plant of which it is the progeny was definitely known to be diseased in the previous season. Throughout this paper the expression "diseased tubers" is used in this sense. Similarly "healthy tubers" are such as were known to be produced in the preceding season by plants which were apparently free from leaf-roll, and which were as little as possible exposed to infection.

roll from other diseased plants. It should be explained that the time of onset of rolling of the leaves varies considerably, even in adjacent similar plants. The comparative examinations were repeated six times from May 25 to June 8, the variety used throughout being British Queen. As a general rule, corresponding leaflets from three plants of each category were used in each examination. They were removed at 5 p.m., and kept in the dark with their cut ends in water for from sixteen to forty hours, and were then submitted to the iodine test in the usual way.

The results of all the examinations may now shortly be summarised. In the first trials, on May 25, 26, and 27, there was no noticeable difference in the amount of starch present in such leaflets as came from healthy or diseased (but normal looking) plants after being kept in the dark for sixteen hours. Leaflets which had already begun to roll at this time, however, showed excess of starch; and the more pronounced the rolling the greater was the amount of starch present. On May 30 there was clearly more starch in leaflets which had not begun to roll, but which were taken from diseased plants, than in similar healthy leaflets, both being kept sixteen hours in the dark. When, however, the leaflets were kept in the dark for forty hours, there was no difference between the two lots. This shows that although starch accumulation in the diseased leaves had begun, it had not yet reached very large proportions. Confirmatory results were secured in the case of leaves from diseased plants which were tested on June 2. The last examinations, carried out on June 7, when the majority of the plants in the plot already exhibited symptoms of leaf-roll, showed a still more striking accumulation of starch in diseased lower leaves which had not yet begun to roll as contrasted with similar healthy leaves. In this case the difference was still easily perceptible after the leaves had been kept forty hours in the dark. This indicates that the amount of starch present in the diseased leaves had increased.

Corroborative results were secured by systematic microscopical examination during the same period of bleached material taken from the plant in the morning (10 a.m.), the diseased leaves being taken from the same plant throughout. Up to June 2 there was no perceptible difference between the diseased but non-rolled plants and healthy plants. The leaves of both contained about the same amount of starch, the lower ones being generally empty except for the guard-cells and "starch-sheath," and the upper ones containing a good deal of starch throughout the mesophyll. The first sign of increased starch-content in the diseased but non-rolled lower leaves was noticed on June 3. This feature had become very pronounced by June 6, there being by this time very much more starch present in the morning in the lower leaves (which had not yet rolled perceptibly) than in the upper leaves of the same plant.

A general examination of diseased plants showing the earliest symptom of rolling was made at this period in comparison with healthy plants, the material being collected at 10 a.m. and examined at once. The diseased lower leaf-blades contained an abnormal amount of starch, while their petioles were not distinguishable from those of healthy plants, both containing starch only in the "starch-sheath." This was also the case in the stems, but here there appeared to be appreciably less starch present in the "starch-sheath" of the diseased plant than in that of the healthy one. No necrosis of the phloem was seen in any part of the plants, although a careful search was made.

Although the methods used in the experiments described are not altogether free from objection, for the reason that the rolling of diseased leaves sets in gradually, thus rendering it impossible to say exactly when it begins, and because rolling follows rapidly after the beginning of starch accumulation,

nevertheless it is sufficiently clear that the lower leaves of diseased plants, which empty themselves of their starch normally at first, gradually accumulate it in the mesophyll at a later stage; and very soon afterwards begin to roll upwards. This conclusion is confirmed by the results of two further experiments, which will now be described.

Artificial retardation of starch accumulation and leaf-rolling.—At 10 a.m. on June 1, 1922, before any signs of rolling in their leaves were visible, two plants of the variety British Queen growing in the field from "sets" derived from diseased tubers, were darkened by covering them with comparatively light-tight boxes. At that time the lower leaves were found to be practically free from starch; and they were entirely free from it after a further period of twenty-four hours, with the exception of the merest trace in the "starch-sheath" and guard-cells. Carbohydrate translocation may, therefore, be assumed to have been normal at that time. The plants remained covered except for occasional short periods, when the boxes were removed for the purpose of allowing a small amount of photosynthesis to take place. These periods generally extended from 5 p.m. to 9 a.m., but sometimes the plants were uncovered for shorter intervals in the daytime. Under those abnormal conditions the plants assumed, of course, a somewhat sickly and chlorotic appearance; but the leaves remained free from roll, and starch accumulation did not occur until a period of from thirty to thirty-six days had elapsed after all the similar, neighbouring, non-covered, diseased plants had shown both rolling of their leaves and starch accumulation. One of the covered plants was first observed to show decided rolling of its leaves on July 11, this occurring after an unduly prolonged exposure to light. Some of the leaves on the other covered plant began to roll very slightly on July 12, also following a period of exposure. The two plants were again covered on the dates mentioned, and they were not again exposed to light until seventeen days had elapsed in one case and twenty-one days in the other. In both cases periodical tests were made, and they showed that a large proportion of starch was retained by those more or less rolled leaves which remained green. The leaves which became yellow eventually showed no starch. While the badly rolled leaves of the first plant, after it had remained covered for seventeen days, turned entirely black with iodine, the bases of the veins of the slightly rolled leaves of the other covered plant began to clear at that time, and after twenty-one days the basal halves of the leaflets of this plant were quite clear, while their upper halves were full of starch (fig. 9, Pl. VI). When it was finally uncovered, the second plant developed still more pronounced symptoms of leaf-roll within twenty-four hours.

Rolling of leaves and starch accumulation artificially produced.—In the second experiment each of four healthy tubers of the variety Up-to-Date was cut in two, and the resulting pairs of four "sets" were planted in two parallel rows as far removed as possible from all likely sources of leaf-roll infection. The four "sets" in one row were planted in mounds of soil about one foot above the general ground-level. Farmyard manure was previously placed at the bottom of each mound, and stakes were provided to which the resulting plants were afterwards tied. The corresponding four "sets" were planted in the other row in the usual manner, i.e. in a drill in which the manure had been placed. All the plants grew well, and appeared normal. On July 7 the soil was carefully removed from the bases of the four plants growing on the mounds, and the stolons (which were just developing), together with most of the axillary buds and shoots, were removed from them.[1] The immediate effect of this treatment was

[1] This operation was repeated once during the course of the experiment.

the occurrence of a peculiar upward rolling of the blades of the leaflets of the upper leaves, and within three days this feature was very marked on the four treated plants. The four untreated controls remained quite normal (figs. 1 and 2, Pl. VI). The rolled leaves of the treated plants still retained their dark green colour; and tests of their starch-content made on the third day after treatment showed an accumulation of starch after they were kept in the dark for forty hours with their stems in water. This excess became more pronounced as time went on. On the seventh day, for example, the "artificially" rolled top leaves, when removed from the plants in the evening, contained obviously more starch than corresponding normal leaves taken from the untreated control plants. The starch disappeared from the normal leaflets when kept in water in the dark within sixty-four hours (perhaps in a shorter time), but within this period the similarly treated rolled leaflets had only cleared slightly at their tips (fig. 6, Pl. VI). In seven days not more than one-quarter of the area of the "artificially" rolled leaflets was free from starch, the clearing proceeding from the tip downwards (fig. 7, Pl. VI). An actually diseased and rolled lower leaf from an affected plant, kept at the same time under the same conditions, began to clear, however, at its *base*. Similar results were obtained on the seventeenth day of the experiment, when whole leaves were used in place of leaflets.

The presence of an abnormal quantity of starch in the "artificially" rolled leaves was confirmed by microscopical examination. The excess of starch was not confined to the lamina of the leaf, but was also very noticeable in the parenchyma, and particularly in the "starch-sheath," of the petiole, in which the starch grains were unusually large.

Up to the seventeenth day after excision the treated plants remained in the condition described, but the rolling had gradually become more pronounced (see photograph made on the seventeenth day, reproduced in fig. 3, Pl. VI), and had extended downwards on the plant so as to involve practically all the leaves but the lowest. At that time these plants presented a marked contrast with their controls. The symptoms of rolling obviously differed from those which accompany primary leaf-roll, the most pronounced distinction being the fact that the "artificially" rolled leaves retained a very dark green colour, and showed a tendency on the part of the leaf as a whole to curve downwards. On the seventeenth day, however, one of the treated plants showed suspicious symptoms of true primary leaf-roll,[1] and it was immediately cut off at ground-level in the hope of preventing the infection of the remaining three healthy plants, from which it was desired to secure healthy tubers or cuttings. The soil, therefore, which had previously been removed, was heaped up about the bases of the stems of these plants, and they were allowed to grow in the normal way.

The hope of keeping them in a healthy condition was, however, only partially realized. Primary leaf-roll continued to appear in plant after plant, both treated and control, and each plant was removed as it became infected. On the twenty-fourth day of the experiment only three plants were left—one control, which was normal, and two of the treated plants, which were now forming tubers with extreme rapidity, and from which the "artificial" rolling of the leaves had almost disappeared. Two days later the leaves of the then only remaining treated plant had entirely lost their rolled appearance (see figs. 4

[1] It should be stated that during the period of the experiment primary leaf-roll had become common in neighbouring healthy plants in the same plot. All these plants were much infested with capsid bugs (*Calocoris bipunctatus*), which came from an adjacent weedy hedge, and infection was attributed to them.

and 5, Pl. VI),[1] and when removed from the plant on this day and subsequently tested at intervals they were found to lose their starch fully as quickly as corresponding leaves from an untreated healthy control plant of the same variety (see fig. 8, Pl. VI), as the following details will show :—

	Amount of starch present after being in dark room for—				
	22 hours.	25 hours.	29 hours.	45 hours.	50 hours.
Top leaflets of previously "artificially" rolled Up-to-Date plant.	Some present.	Little present.	Practically gone.	None.	None.
Top leaflets of healthy untreated Up-to-Date plant.	A little more than above.	A little more than above.	A little more than above.	Trace.	None.

It appears that Vöchting (15) long ago carried out a somewhat similar experiment, but not, of course, with reference to leaf-roll, and no mention seems to have been made of any resulting change in the form of the leaves, although it is apparent from some of the figures that a certain amount of rolling set in. That it was not more pronounced is probably due to the formation of aerial tubers, which provided an outlet for the large amount of starch that accumulated. Quanjer *et al.* (11) also refer to the presence of excess of starch in the stems and leaves of plants from which the tubers have been removed, and in those the stolons of which are attacked by *Corticium vagum*; but no reference is made to any rolling of the leaves following these occurrences.

From the results of the experiments described in this and in the preceding sections of this paper it is concluded that one of the earliest discernible effects of the leaf-roll disease is an abnormal accumulation of starch, principally in the lower leaves of affected plants. It has been shown that the starch disappears at the normal rate from these leaves at first. At a somewhat later period there is a gradual retardation of this process, and, following rapidly on this, the leaflets begin to roll upwards at their margins. Seeing that in the case of affected plants rolling appears to follow starch accumulation, and that the rolling of the leaves of diseased plants can be prevented for comparatively long periods by the exclusion of light, and by thus greatly reducing the amount of carbon assimilation; and further, in view of the fact that rolling and starch accumulation can be induced in healthy plants when they are deprived of their storage organs and most of their growing points—there is little room for doubt but that the rolling of the leaves of diseased plants is a direct consequence of the accumulation in them of an excess of starch.[2]

Distention of the spongy parenchyma.—This accumulation of carbohydrate in the tissues of affected leaves leads to an abnormal distention of the spongy as compared with the palisade parenchyma. Owing to the fact that the spongy parenchyma is comparatively free to expand in three directions, but is restricted

[1] An unusual feature of some of the treated plants may be noted in fig. 4—namely, the prolongation of the ultimate branches of the floral shoot into shoots which bore small foliage leaves and developed new flower buds at their tips. The floral axes on these plants were unusually stout and well developed, and in one case fruit was set, which is a very rare occurrence in the case of Up-to-Date. These phenomena are no doubt related to the presence of an abnormal amount of food materials in the upper portion of the treated plants.

[2] It is quite probable, as appears from Neger's work, that an abnormal amount of sugar is also present in rolled diseased leaves. This point was not investigated.

on its upper side by its attachment to the palisade cells, a downward and lateral extension follows, which has, as one of its consequences, the upward rolling of the margins of the leaflets.

As is well known, rolled leaves give the impression of being thicker than normal ones; and on measurement this is generally found to be the case. It is not invariably so, because it occasionally happens that the growth in length of the palisade cells of affected leaves is interfered with, and the consequent reduction may be of such a magnitude as more than to counterbalance the extension of the spongy parenchyma. It does seem to be a general rule, however, that the spongy parenchyma of diseased leaves is thicker than normal, as is proved by comparing the thickness of this tissue (including the lower epidermis, which does not vary) in similar leaves, diseased and healthy, of the same variety; or by determining what percentage of the whole thickness of the leaf is made up of spongy parenchyma in the two cases. This percentage was found to vary in mature healthy leaves between 47 and 54 per cent., while in corresponding diseased leaves it varied between 57 and 74 per cent. It is believed that this demonstrated expansion of the cells of the spongy parenchyma in a downward direction must be accompanied also by a certain amount of lateral extension, and this seems to be confirmed by the apparently more rotund shape of the cells and by a reduction in the sizes of the intercellular air-spaces between them.

In view of the obviously more compact and less elastic nature of the palisade layer, to which the lower and less rigid tissue is firmly attached, the result must be an upward rolling of the leaves, just as a pair of metal strips of which the lower has the greater coefficient of expansion must, if firmly fastened together, roll upwards with a rise in temperature. The fact that a similar distention of the spongy parenchyma was observed, though not to such a marked degree, in the "artificially" rolled leaves of healthy plants is further evidence in support of this view. In the course of three determinations, each including five measurements, it was found that in the "artificially" rolled leaves of these healthy plants the spongy parenchyma and lower epidermis very regularly made up 57 per cent. of the total thickness of the leaf, while in corresponding normal leaves this figure varied from 46 to 53 per cent., the average being just under 50 per cent. Similar measurements made after the "artificially" rolled leaves had become normal showed that the spongy tissue had practically returned to its original size, the percentage it then formed of the total thickness of the leaf being 51, as against 50 per cent. in the case of similar leaves which had never rolled.

Excess of starch in the leaves not confined to leaf-roll.—There is still further evidence to connect the rolling of the leaves with the presence in them of an excessive amount of starch. Rolling of potato leaves, as is well known, is not confined to the leaf-roll disease. It may accompany other diseases, and may also occur as a result of mechanical or other injury to the plant; and in some at least of these cases the rolled leaves contain an excess of starch. This was proved to be the case in the upper rolled leaves of certain stalks of healthy plants of the variety Barley Bounty, which were partially broken across at the base, where aerial tubers were being produced in the leaf axils just above the injury. The remaining stalks and their leaves were normal in appearance. When such rolled and normal leaves were kept in the dark with their petioles standing in water for forty-two hours the normal leaves were found to be practically free from starch, except for a little, principally along the basal margins of the lower leaflets. The leaf-blades and petioles of the rolled leaves, however, contained obviously more starch at this time. It was estimated that.

on the average, about one-quarter of each rolled leaflet had become clear in irregular patches, the clearing being most pronounced in the terminal leaflet as a whole, and generally in the tops of the others (fig. 10, Pl. VI).

The same experiment was repeated more than once with plants of the variety Up-to-Date, which were free from leaf-roll, but which proved on examination to be affected with black-stalk rot (*Bacillus atrosepticus*). The rolled leaves from the top of such plants, while they retained their green colour, contained more starch than similar leaves from healthy plants; and when cut and placed standing in water in the dark became clear at about the same rate and in the same way, from tip to base, as in the case of rolled leaves from broken stalks of healthy plants.

Again, excess of starch was demonstrated in the upper rolled leaves of plants of the varieties Up-to-Date and Ally, the rolling being due to obscure and apparently temporary causes, for the plants afterwards recovered.

It will be observed that in all these cases an upward rolling of the leaves is an accompanying feature of starch accumulation; and, in the case of the rolling due to the breaking of the stalk at least (the only one in which the relation was determined), an increase in the depth of the spongy parenchyma was found to be another accompaniment. In the rolled leaves of this plant the spongy parenchyma and lower epidermis made up 52 per cent. of the thickness of the leaf, while the corresponding figure for healthy leaves was 47 per cent.

IV.—*The Cause of Starch Accumulation in Rolled Leaves of Diseased Plants.*

Since disorganization or necrosis of the phloem has been shown by Quanjer *et al.* (10) to be an accompanying feature of leaf-roll, and since it is believed by most plant physiologists that the carbohydrates of the plant are translocated through this tissue, the assumption was soon made that such necrosis was the cause of the accumulation of starch in the leaves; and that, in fact, the association of these two phenomena furnished a settlement of the old question as to the channel through which carbohydrates are principally distributed in the plant.

Investigation was made as to the extent to which the phloem is disorganized in plants affected with leaf-roll, and it was found to be very variable. It depends apparently on the susceptibility of the variety to the disease, the severity of the attack in the particular plant under examination, the period of the season at which the examination is made, and probably on many other factors. Thus varieties which commonly show an aggravated form of leaf-roll, such as President (which appears to be synonymous with the Dutch variety Paul Kruger) and Black Skerry, are also generally characterized by severe phloem necrosis, at least in the later stages of the disease. The same may be said for British Queen, and probably for other varieties, when the attack is unusually severe. On the other hand, when the disease occurs in its normal intensity on British Queen, Up-to-Date, and most of the varieties which are in common cultivation in Ireland, the amount of disorganization which can be seen in the phloem during the height of summer does not appear to be sufficient to account either for the external symptoms of disease or for the failure to translocate carbohydrates. This seems to be beyond question for the earliest recognizable stage of the disease. Examination made at the end of May and early in June, 1922, of plants in which starch accumulation had just begun in the lower leaves, which were showing the first signs of rolling, failed to reveal any trace of alteration in the phloem, either in the petioles of the affected leaves or in the stem below their insertion. This examination was made repeatedly

in comparison with similar tissues of healthy plants, and always with the same result, thus corroborating the work of Oortwijn Botjes (8), and ruling out phloem necrosis as the original or principal cause of the abnormal accumulation of starch in the leaves of diseased plants.

Further, it is to be observed that there appears to be no spatial relationship between the situation in which starch accumulation first occurs (in the mesophyll of the lower leaves) and the place at which disorganization of the phloem is supposed to be first noticeable, namely, the lower part of the stem. If the necrosis were actually the cause which precluded carbohydrate translocation, it would be reasonable to expect to find excess of starch just above the point of obstruction. This was not found to be the case. For, not only was the accumulation of starch at that stage confined to the blades of the lowest leaves, but, as has been stated (p. 166), less starch was found at 10 a.m. in the stems of diseased plants showing the first signs of rolling than in similar healthy stems.

It is also to be noticed that the disappearance of starch from the very vigorously assimilating upper leaves of affected plants is not interfered with at this time, although the destination it reaches is presumably the same as would have been reached by the starch in the lower leaves were this capable of being moved.

Again, when rolled leaves are kept darkened for a considerable time, whether remaining on the plant or standing with their cut ends in water, the starch vanishes first from the neighbourhood of the lowest part of the midrib and the base of the leaflet (fig. 9, Pl. VI). This circumstance shows that the hindrance to translocation, whether mechanical, physico-chemical, or otherwise, is less likely to be found a considerable distance away in the stem than in the leaf itself.

A similar conclusion may be drawn from the fact that in the last stages of a very severe attack of leaf-roll, such as is common in the varieties President and Black Skerry, practically the only starch to be found in any part of the plant (with the exception of the tubers) was in the leaves. A systematic examination of plants of the varieties mentioned was made on July 25, 28, 31, and August 2, 1922, the material being taken at 10 a.m. to 1 p.m., and examined at once; and practically the same results were secured throughout from the two varieties. The plants selected were very badly diseased, being dwarfed and chlorotic, with upstanding leaves, which were practically all rolled, and showed considerable pinkish coloration and tip-injury. The greenest of the more or less well-developed leaves were used in the examinations.

There was a great accumulation of starch in the leaves, and this was largely or entirely confined to the palisade layer. No starch was found, as a rule, in the veins; but in those portions of the spongy parenchyma furthest away from them starch was sometimes present. The midribs and petioles contained traces of starch in the form of small grains in the "starch-sheath," and sometimes as isolated grains in other parenchymatous cells. The total amount of starch present in the stem was small, and was confined to the "starch-sheath," with the exception of an area some little distance below the growing point, where grains occurred in the parenchyma of the principal vascular bundles and in the pith. In proceeding from the top of the plant downwards the initially small amount of starch present in the "starch-sheath" became gradually less, until at about ground-level it finally disappeared. In the underground organs, with the exception of the tubers, but including the stem, stolons, and roots, starch was either entirely absent or a trace occurred in the cortex of the stolons. It should be noted that in these cases the phloem in the lower half of the stem was considerably disorganized.

In contrast with this state of affairs, corresponding healthy leaves examined

at the same time showed a normal distribution of starch, there being less in the palisade layer than was observed in diseased leaves, and more present elsewhere. Similarly in healthy stems, while there was sometimes little difference in the starch-content of diseased and healthy plants near the tip, as a general rule a great deal more starch was present throughout than in corresponding tissues of diseased plants. This was particularly so in the underground portions of the stem, the stolons, and the roots. Furthermore, the lower portions of the stems of diseased plants above ground, when cut off in the afternoon and tested at once with Benedict's solution, gave no reaction for sugar, while similar portions of healthy stems gave a strong reaction.

The view is put forward, based on these results, that in the case of plants seriously affected with leaf-roll the exhaustion of starch in the regions of the leaf nearer the larger veins and the almost complete absence of starch (and sugar) along the vascular bundles in the stem in the track of movement, are probably to be explained by the fact that all the carbohydrate which can be moved has been drawn on for the most part and translocated. The remaining starch, principally in the palisade cells, is apparently not readily mobile, the reason for which is at present unknown. As a result of this, no more carbohydrate (or very little) can be produced in the leaves, owing to previous starch accumulation and the disturbance it set up. The consequence is that at least in the extreme cases now being considered the plant as a whole gradually dies from starvation. This view of the case, which supposes the seat of the disturbance of starch translocation in the leaf (whatever and wherever its cause may be) to lie in the leaf-blades rather than in disorganized distant tissue in the stem, is believed to be correct, because the disturbance begins and ends in the leaves; but the problem is admittedly a complicated one.

In other stages of the disease and in other varieties, when the plants are not so severely attacked and are still growing fairly vigorously, there is a very large and abnormal accumulation of starch in the petioles of the lower rolled leaves and in the stem near their points of insertion. This in itself might perhaps be accounted for by assuming it to be due to an obstruction in the phloem a little further down. But such a supposition would not account for the fact that the accumulation is known in these cases also to start in the blades of the leaves. On the whole, it seems more reasonable to consider the matter as a gradual extension of the disordered condition from the leaf-blades, where it originated, although it is possible that phloem necrosis, once it has set in, may add to the difficulty of translocation.

Influence of low temperature on carbohydrate translocation.—A different theory to account for the interruption in the translocation of carbohydrate is put forward by Neger (7), and is based on experiments from which it appeared that the starch in diseased leaves—and in general also in leaves of healthy plants belonging to (assumed) susceptible varieties, or of healthy plants of (assumed) individual susceptibility—is translocated with difficulty at low temperatures (10° C.), and under conditions of poor aeration of the leaves. The further conclusion was reached by this investigator that high temperature and good aeration promoted the translocation of starch from rolled diseased leaves. From these findings the general inference was drawn that the leaf-roll disease was the result of cold nights or of cold wet weather, and was merely the expression on the part of certain plants or varieties of their inability to translocate starch normally under such conditions. The experiments of Neger, which were carried out entirely with shoots cut from the plants, will be further discussed below. Before doing so, an experiment to test the effect of low temperature on starch translocation in a growing plant will be described.

A healthy plant of the variety Up-to-Date (which is quite susceptible to leaf-roll) was grown in a flower-pot and placed in a position where it was as little as possible exposed to leaf-roll infection through insect agency. As a matter of fact, neither it nor its neighbours ever became infected. On August 8, when the potted plant was well developed, normal in appearance, and just showing its flower-buds, it (together with its pot) was placed during the night (from 5 p.m. to 9 a.m.) in a small ice-chamber, the temperature of which varied between 5·5° C. and 7·2° C. In the evening, when the plant was first brought in, its leaves contained a normal amount of starch, but after a night in the refrigerator they were found to contain none, and the plant was apparently in no way injured or altered by the treatment. It was then exposed to full daylight in its original position, and again placed at night in the ice-chamber, and this treatment was repeated for eleven consecutive days. The range of temperature at night was the same throughout. Periodical tests of the starch-content of the leaves were made during and at the end of the experiment, but an accumulation of starch was never found in the morning, and no rolling of the leaves or any other abnormal symptom appeared.

In seeking for an explanation of the different results secured by Neger under apparently similar conditions, it is to be observed that this worker used cut shoots and not the whole plant in his experiments. When a normal potato shoot or leaf is cut off and placed with its end standing in water in the dark, it loses its starch in a comparatively short time; but the process is one of hydrolysis of the starch and eventual consumption of the resulting sugar in respiration, and not of translocation in the generally accepted sense of the word; for there are no normally functioning organs of storage or growth by which the carbohydrate can be absorbed, and no soluble carbohydrate passes out of the stem into the water. Even the apparent transference of starch from the leaf-blades to the petiole is largely illusory, although some translocation may perhaps take place, because there is a gradual but less rapid diminution in the total amount of starch present in the petiole also. In the case of larger shoots there would, of course, be a correspondingly more extended field in which such limited translocation might take place, but the conditions are so unnatural as to render doubtful the applicability of results secured under them to the normal growing plant. An even more rapid disappearance of starch is to be observed (as Neger also noted) in the case of cut leaves and shoots which are not put standing in water, and which wilt in consequence. The obvious conclusion, however, that translocation could not account for the disappearance in this case was not drawn by this worker.

A similar process of hydrolysis and respiration goes on, but probably more slowly, when diseased shoots and leaves are allowed to stand in the dark with their cut ends in water; but it is not possible to measure by means of the iodine test the relative rates of carbohydrate translocation in healthy and diseased leaves or shoots kept in this way, because the starch-content of the leaves is very different to begin with, and because the diminution in starch-content is not solely or in the main due to translocation. While practically all the starch contained in a healthy leaf cut off in the evening may be dissolved during the night and retained as sugar, with the exception of that used up in respiration, it is obvious that a diseased leaf cannot under the same conditions be in a position to become free from starch in the same time, even if the starch in it were equally hydrolysable, because of the greater amount of it originally present. A further complication would also ensue, since the resulting sugar, for which there is no outlet but that of respiration, would be present in greater quantity, and would be likely to retard or prevent the final disappearance of the starch. It there-

fore appears that Neger's conclusions do not follow from his experiments. The latter refer in the main to the hydrolysis of starch in the leaves, and only in a minor degree, if at all, to the translocation of the resulting carbohydrate. The only point proved by them is that rolled leaves of diseased plants contain much more starch than similar healthy leaves.[1]

V.—*Histological and other Symptoms of Leaf-Roll.*

During the course of a systematic examination of the starch-content of various portions of diseased and healthy plants certain histological differences were discovered or re-investigated. As was first pointed out by Schander and v. Tiesenhausen (12), necrosis of the phloem, which Quanjer regards as being the underlying cause and principal symptom of leaf-roll, is not confined to plants affected with the leaf-roll disease. These two workers showed that similar injury to the phloem may be found in plants affected with a number of other diseases, including curly dwarf, black-stalk rot, and blight; and that it may even occur in maturing healthy plants. It has also been pointed out elsewhere by the present author (5) that a very thorough disorganization of the phloem (as well as of other tissues) occurs in streak disease. Recent work has again confirmed the fact that this type of injury is not confined to leaf-roll. It was found during the past year to occur in the lower portions of the stalks of potato plants attacked by a disease possibly due to eel-worms, and it has also been seen in the lateral veins, midribs, and petioles of potato leaves attacked by *Phytophthora infestans*. In neither case were the plants affected with leaf-roll. The disorganization in these diseases was not, of course, confined to the phloem, but the effect on that tissue was exactly of the same kind as in leaf-roll, only rather more pronounced. The attack began in the oldest cells, the walls of which became brown in colour, and afterwards collapsed. The walls of such cells no longer gave a cellulose reaction, but the nature of the change in composition was not further inquired into. In the blighted plants the death of the phloem was apparently due to a toxic substance, which operated some distance in advance of the parasite itself. The first cells to be killed in the petiole were those in the epidermal and sub-epidermal layers (giving rise to the brown stripes characteristic of the disease on stems and leaf-stalks), and from them the parenchymatous ground tissue and the phloem groups nearest the surface of the stalk were attacked in turn.

A difference manifests itself in the behaviour of diseased and healthy leaves in the matter of starch evacuation which does not seem to have been sufficiently emphasized. It seems to be a general rule that the disappearance of starch begins at the bases of diseased leaflets—that is, if any noticeable clearing whatever takes place—and proceeds for a less or greater distance towards the apex. This feature is most clearly seen when diseased leaflets in the earliest stage of rolling are darkened for prolonged periods (fig. 9, Pl. VI). In such cases a sharp line divides the tissue which is free from starch from that which retains it in apparently undiminished quantity. So far as our experience goes, it also seems to be invariably true that the starch begins to disappear from healthy leaflets in

[1] In a subsequent paper, a note of which has just been seen (Centralbl. f. Bakt. Abt. II, 54 Band, 1921, p. 512), Neger records an experiment in which living plants diseased with leaf-roll were kept at night under different conditions. From this it appears that those kept during the night at 20° C. became entirely healthy, and showed normal ("good") translocation, as measured by the application of the iodine test to the leaves. However this may be (and it should be noted that the respiration factor seems to have been left out of account), it does not prove the author's contention that leaf-roll, or the liability of a plant to it, is an expression of inability to translocate carbohydrate at a low temperature.

the first instance at or near the tip, and then gradually vanishes from the lower portions. Here there is not the same sharp line bounding the starch-free area, and the progress of hydrolysis is not so regular, but the direction in which it proceeds is unmistakable. Furthermore, in the case of healthy leaves the starch disappears first from the tip of the terminal leaflet, then from the tips of the next pair, and so on to the base of the leaf; each leaflet at any one time showing a somewhat larger area still full of starch than the leaflet immediately above. This method of evacuation is also followed by healthy leaves and leaflets which contain an excess of starch, due to causes already discussed, and which roll upwards in consequence (figs. 6 and 7, Pl. VI).

The brown areas which generally arise in the course of time on rolled leaves appear to originate in the death of a single cell either of the upper or the lower epidermis, this being most frequently a guard-cell, but not invariably so. The walls of the cell become brown as well as the contents, and the discoloration spreads to the wall of any neighbouring cell. The cells involved collapse and fall in, particularly when the affected area starts on the lower surface of the leaf. All the subjacent tissues of the mesophyll are liable to be attacked in turn, until the lesion extends from one side of the leaf to the other. No parasite was seen in connexion with this injury. It may be remarked that the cells of the epidermis of diseased leaves contain apparently more vigorous nuclei, more abundant cytoplasm, and a greater quantity of starch than corresponding cells of healthy leaves. The difference is more marked in the case of the upper epidermis.

VI.—*Insect Carriers of Leaf-Roll.*

A survey, which was unavoidably somewhat hurried, was made by my colleague, Mr. Rhynehart, during July, 1921, of the common insects which occurred in the experimental potato disease plots; and the writer wishes to acknowledge the help thus given. The following insects were found on the plants, and were presumed to be feeding on them :—

Calocoris bipunctatus (Capsid Bug). Abundant.
Typhlocyba Ulmi (Jassid). Abundant.
Philaneus spumarius (Frog-hopper). Abundant.
Psylliodes affinis (Potato flea-beetle). Abundant.
Aphides (Genus and sp. not determined). Fairly common.
Lygus pratensis var. *campestris* (Capsid). Fairly common.
Typhlocyba sp. (Jassid). Fairly common.
Anthocorus sylvestris (Anthocorid). Scarce.
Aetorhinus angulatus (Capsid). Scarce.
Idiocerus sp. (Jassid). Rare.
Bythoscopus sp. (Jassid). Rare.

Among these insects aphides certainly appeared to be of secondary importance. They were unevenly distributed in the plots, being almost absent in some parts. This may have been due to the great prevalence of ladybirds (*Coccinella* sp.) and their larvae in the month of June, these being practically the only insects then to be found on the foliage. The same prevalence of these beetles and their larvae and scarcity of aphides was noted in 1922. Estimating the aphides individual for individual against the other kinds of insects, they should probably be classed as "fairly common" on the average in 1921.

Infection experiments with insects other than aphides.—Experiments to test

the capacity of the four commonest of the insects listed above to carry leaf-roll were undertaken at once by transferring individuals taken from diseased plants to healthy plants, the latter being protected by muslin cages which had been placed in position soon after the plants appeared above ground. Each caged healthy plant was provided with a caged control plant, the two being derived from the two halves of one tuber. The caged plants were separated by a distance of four yards of unplanted ground from the nearest potatoes, which were for the most part healthy. It was found that under local conditions, and at least in the abnormal season of 1921, the caging of a healthy plant with muslin was not a guarantee of protection against leaf-roll infection unless the plant was also placed at a certain distance from diseased plants. This distance need not apparently be very great, probably because a small space of clear ground is a considerable help in preventing infestation with aphides, while the other insects (which, it will be seen, were proved to be carriers also) are comparatively easily excluded, although much more active. The experimental infestation of the caged plants with the various kinds of insects took place on July 27, 1921, the latter being collected from diseased plants, and one lot of each, from five to twelve in number, being introduced into a cage. No insects were put in the adjacent cages containing the control plants. Owing to circumstances which were beyond control, it was not possible to note the behaviour of the plants during the latter part of the season, but the tubers of each of them were dug and saved separately at the usual time.

The tubers were all planted in separate lots in the open field in 1922. All those from the control cages produced healthy plants, except those from cage No. 3 (two in number), both of which were diseased with leaf-roll. The plants from tubers from the corresponding cage, to which frog-hoppers had been introduced in 1921 (again two in number), were also both diseased with leaf-roll. Unfortunately there appeared in this exceptional control cage in 1921 a "volunteer" plant from a tuber which survived in the ground from a previous crop of the same variety as the experimental plant (Up-to-Date). This crop was very badly affected with leaf-roll in 1920; and although the plant was removed as soon as noticed, under the circumstances it is thought better to exclude this part of the experiment from consideration.

The remaining results were as follow:—

1921.	1922.
Healthy plant experimentally infested with 12 capsid bugs (*Calocoris bipunctatus*) taken from affected plants—	produced 5 plants, all diseased with leaf-roll.
Control plant, from half of same tuber, not infested—	produced 4 plants, all healthy.
Healthy plant experimentally infested with 12 jassids (*Typhlocyba Ulmi*) taken from affected plants—	produced 3 plants, all diseased with leaf-roll.
Control plant, from half of same tuber, not infested—	produced 4 plants, all healthy.
Healthy plant experimentally infested with 6 flea-beetles (*Psylliodes affinis*) taken from affected plants—	produced 4 plants, of which one was diseased with leaf-roll, and 3 were healthy.
Control plant, from half of same tuber, not infested—	produced 5 plants, all healthy.

Where the disease occurred in 1922 in this experiment it appeared early in the season in the pronounced secondary form. It was clearly visible at the time of the first detailed examination of the plots for leaf-roll on June 20; and the contrast between the produce of the infected and the control plants was then striking. This is illustrated in the photographs taken on July 10, reproduced in figs. 11 and 12, Pl. VI, which show one of the plants infected through the agency of capsid bugs and one of the still healthy controls beside it, the two being reduced to the same extent. The plants infected by means of jassids and their corresponding controls presented an entirely similar contrast at that time. The starch-content of the lower leaves of the diseased plants was compared with that of similar leaves from the healthy control plants, and the usual difference was evident.

While the above results show hardly more than a suspicion that the potato flea-beetle can act as a carrier of leaf-roll, there is little room for doubt that both capsid bugs and jassids act as efficient transmitters. This is important as showing that there is no exclusive specific relationship between aphides and the actual cause of leaf-roll, which is presumably an organism. This finding might indeed have been expected in the case of capsids from the work of Oortwijn Botjes (8), if it be assumed that the German common name "Wanze" refers to a species of capsid. This author seems to have been in doubt about his result, because in one experiment with these insects no infection followed, while in the other, in which it did, it was considered equally attributable to aphides, which accidentally found their way into his cages.

General observations in the plots made in 1921 and 1922 showed the comparative scarcity of aphides in both years; and yet the ease and rapidity with which infection was carried in quantity over comparatively long distances were clear. Hence it appeared that some other and more active carrier must be concerned in the matter, and that this in fact was the main problem. It may be noted that in many of the original and later experiments of Quanjer, van der Lek, and Oortwijn Botjes (10 and 8) the removal of healthy plants to a distance of from 2·5 to 4 metres from diseased individuals frequently protected them to a very large extent, if not entirely, from infection. Similar results were obtained by the writer (5) in Eastern Canada. Under the conditions under which the experiments now being described were carried out such a comparatively small degree of isolation was of little or no avail, and mass infection occurred over very much greater distances. In 1921 it is believed that jassids were the principal carriers. On account of the proximity of a number of elm trees, these insects were so abundant that if a potato plant in the vicinity of the trees were disturbed on a sunny day in July they flew into the air almost as thickly as bees in a swarm. Fortunately they appeared to be entirely absent from the plots in 1922, which were in a different situation. Capsid bugs, however, were present in that year in greater numbers than in the preceding one. This was particularly so near a hedge bordering on the plots. On a day following a period of heavy rain as many as nineteen of these insects, feeding voraciously, were counted on the exposed portions of leaves of one plant in such a situation. How many more there may have been concealed amongst the foliage was not determined.

Insect infection through sprouts.—The presence of aphides feeding in late winter and in spring on the sprouts of seed potato tubers before they are planted is apparently not uncommon in some places in Ireland as well as in Great Britain, and probably elsewhere. It was known previously that they occurred thus on the farm on which the experiments described here were caried out; and since the lots of potatoes selected for planting in the plots were stored alongside of each other in small chip baskets ("punnets"), and included healthy tubers as

well as others from plants which had suffered from various diseases, it became a matter of urgency to determine whether aphides could carry infection from the sprouts of diseased tubers to those of healthy ones. This was proved to be the case so far as leaf-roll, at least, is concerned.[1]

The details of the experiment are as follows. The sprouts on a number of tubers which had been kept on a dish in a lobby, and which originally came from England, the past history of the tubers being not known, were found in February, 1922, to be strongly infested with aphides. One of these tubers, which was assumed to be healthy, but which gave rise later to a plant affected with leaf-roll, was selected, and eighteen aphides (*Myzus Persicae*, Sulzer)[2] from its sprouts were transferred to the sprouts of one-half of a tuber from a plant known to have been affected with mosaic in 1921. The other half of this mosaic tuber was kept separately, and no aphides were placed on its sprouts. Similarly, eighteen aphides from the same original source were placed on the sprouts of a half tuber derived from a healthy plant which was caged in 1921, while the other control half received no aphides. On two subsequent occasions further lots of twenty-five and twelve aphides respectively were transferred from the same source to the sprouts of the same two half tubers, because those originally transferred did not multiply with sufficient rapidity.

From the sprouts of the mosaic half tuber aphides were transferred, as their numbers increased and permitted (about twelve to twenty-four were used in each case) to the sprouts of three half tubers derived from healthy plants caged in 1921. The corresponding three half tubers were kept free from aphides. Similarly, from the sprouts of the healthy half tuber first infested from the original source aphides were placed on the sprouts of three further healthy half tubers, the corresponding halves of these receiving no aphides.

Each of the half tubers was covered almost completely with sterilized soil which half filled a flower-pot, the terminal sprouts, on which the aphides fed, alone projecting. The half tubers and their sprouts were in each case enclosed in a wide glass cylinder, pressed down nearly two inches below the soil-level, and closed on top with a triple layer of muslin. The non-infested sprouted half tubers were similarly protected, and remained free from aphides.

The left-hand portion of accompanying figure diagrammatically illustrates the transference of the aphides from the sprouts of the original tuber (centre) to those of the mosaic and healthy half tubers, and from both of the latter to the three healthy half tubers in each case. The controls (not infested) of all these are shown with the cut surfaces facing in the opposite direction in the top and bottom rows. The original state of health of all the tubers is indicated by shading.

When the aphides had fed on the sprouts for from 13 to 52 days (and still longer in the case of those first infested), the sprouts, including those of the control half tubers, were thoroughly fumigated with a commercial greenhouse

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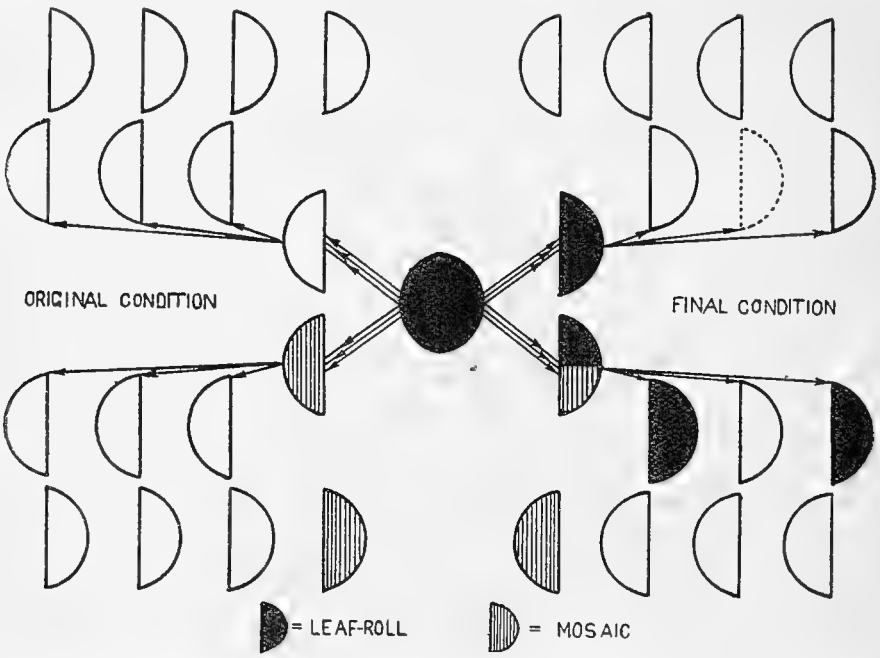
[1] Owing to unavoidable circumstances, the experiment about to be described and another similar one miscarried before any results were secured regarding the transmission of mosaic; but that this disease may also be carried in the same way is only to be expected. This conclusion can perhaps be drawn from an experiment of Schultz and Folsom (13), in which sprouts were infected with leaf-roll and mosaic respectively by means of aphides taken from the foliage of diseased plants. These authors state that "this experiment is probably not duplicated by natural conditions"; but storage conditions in milder climates may be such that the transmission by means of aphides of infection from the sprouts of unplanted diseased tubers to the sprouts of healthy tubers may occur and be of serious practical importance.

[2] Thanks are due to Messrs. Rhynehart, Theobald, and Laing for identifying the aphides used.

fumigator, the chimneys being removed. The pots were then filled up with sterilized soil, so that the sprouts were covered, and the plants were allowed to develop. They were divided into four lots, depending on the probability of successful infection, which were separated a considerable distance from each other out of doors on the flat roof of the College of Science building in the centre of Dublin. The plants were kept under close observation, and whenever, as happened, a few aphides appeared on the foliage of any of them, they were all fumigated again, still in separate lots. That these aphides can have had no part in the successful infections which followed is clear from their insignificant numbers and immediate suppression; from the early development of leaf-roll; from the fact that the disease appeared at once in the secondary form; and from the unexceptional behaviour of the control plants.



The results of the experiment are diagrammatically represented in the right-hand portion of the figure already alluded to. The original tuber was evidently affected with leaf-roll from the beginning, the resulting plant being badly diseased. The leaf-roll disease was transferred from this to both the mosaic half tuber and the healthy half tuber first infested, for the resulting plants showed leaf-roll (secondary) combined with mosaic in the one case and leaf-roll (secondary) alone in the other. The aphides taken from the former infected with leaf-roll (secondary), two out of the three plants arising from healthy half tubers on the sprouts of which they were placed. The third plant remained apparently healthy; but it should be stated that observations ceased abruptly on June 26, when the experiment had to be abandoned. Up to that date also leaf-roll had not appeared in either of the two plants derived from healthy half tubers on the sprouts of which aphides from the originally infested healthy half tuber had been placed (second horizontal row from the top on right of diagram). The third tuber in this row failed to produce a plant. In spite of

the experiment being brought to a premature close, the net result was that of the seven plants produced by half tubers the sprouts of which had been infested with aphides from diseased tubers, four developed symptoms of leaf-roll within about one month of their appearing above ground.[1] All the controls remained entirely healthy throughout, except, of course, the plant from the half tuber known to have been diseased with mosaic.

The diagnosis of leaf-roll was in all cases confirmed by making a test of the starch-content of the leaves; and it was found that all the plants which showed symptoms of leaf-roll also showed starch accumulation in the lower leaves, while the upper leaves showed none. Corresponding leaves, both upper and lower, of the plants which showed no signs of leaf-roll, when tested at the same time, reacted normally.

It would appear, therefore, that the potato may be in danger of infection, certainly with leaf-roll, during its resting period. This is particularly the case if tubers are stored in houses during the winter, or are sprouted in boxes before planting. In all probability the more delicate and etiolated are the sprouts the better the nidus they provide for the insects; for some cases have come under observation in which improperly sprouted tubers became so infested that at planting time they were almost covered with the cast cuticles of aphides, and were slimy with their exudate. On the other hand, sprout-infestation does not appear to occur in the case of pitted potatoes, although, of course, sprouts may develop on the tubers.

In the older English literature concerning "Curl"—a term which would appear of necessity to have included leaf-roll, at any rate so far as Great Britain and Ireland are concerned—several observers recorded the fact that potatoes stored in houses suffered more from the disease than similar potatoes stored in pits. Atanasoff (1) refers to one of these cases; and it is possible that under certain conditions the correctness of the observation might be less debatable than would appear on the surface.

The presence of aphides on the sprouts of seed potatoes may be of importance in another way, because it was observed that in the course of their development such sprouts can carry the aphides above ground, and thus, no doubt, give rise to the first seasonal infestation of the foliage with these insects. Where this occurs, the assumption made in many quarters that the infestation of potato foliage arises from the migration of aphides during the summer from an alternate host, such as plants of the order Rosaceae, is obviously an unnecessary one. Even when rose bushes infested with aphides are in the immediate vicinity of potato plants, the amount of migration that takes place is sometimes negligible, for the potatoes may remain practically free from aphides. This was the case in 1922 in the writer's garden, where two small patches of potatoes were under observation during June, July, and part of August. A large "rambler" rose

[1] As has been stated, the disease appeared at once in the secondary form, and therefore the dictum that secondary leaf-roll results only from the planting of tubers from infected plants requires modification. There was another exception of common occurrence in the dry, hot summer of 1921—namely, the development of the full symptoms of secondary leaf-roll on plants which had previously shown clear primary rolling, and which previous to that had all the appearance of health—all within the course of the same season. The disease took this course apparently when plants were infected through the foliage in June and July. The rolling and discoloration characteristic of the primary phase appeared first in the top of the plant, and then extended gradually downwards so as to involve all the leaves. At this stage such plants were indistinguishable from secondarily diseased plants produced from infected tubers, and carbohydrate translocation from their lower leaves appeared to be similarly affected. The course of the disease here described is evidently different from that following infection through the sprouts.

bush, eight feet high, which was heavily infested with aphides, grew within ten feet of the potatoes; and from this the insects dispersed to some extent to neighbouring comparatively non-infested rose trees, but only to a very slight extent to the potatoes.

An effective fumigant for aphid destruction.—It has been stated that the development of aphides was feared during winter storage on the sprouts of the various seed potatoes intended for the experimental disease plots. This unfortunately began to take place early in March, 1922; and after recourse had been had to a twice-repeated fumigation with commercial fumigants without completely satisfactory results, a successful method of treatment was discovered. The sprouting boxes containing the seed potatoes were placed in a small room having a capacity of 1,700 cubic feet. One pound of tetrachlorethane was then distributed in small lots in earthenware saucers in various parts of the room and allowed to evaporate. The room was then closed up, and was not opened for three days. The effect was immediate and lasting, for no more living aphides were seen up to the time planting was concluded, about thirty-three days later. The vapour of this chemical does not injure the tubers or sprouts, and it is perfectly safe and not unpleasant to use.

VII.—*Summary.*

It was established that the presence of an excess of starch in the rolled leaves of diseased plants is a constant symptom of leaf-roll.

The rolling of the leaves of diseased plants was found to be preceded by the accumulation of starch in the mesophyll.

The artificial darkening of diseased plants before their leaves rolled, and the consequent reduction of photosynthesis to a minimum, was found to prevent the rolling of the leaves for long periods.

Temporary rolling of the leaves of healthy plants was brought about by depriving the latter of most of their growing points and storage organs. Accompanying the rolling a great excess of starch was found in the rolled leaves. The rolling and excess of starch afterwards disappeared when normal growth was allowed to proceed.

It is concluded that rolling of the leaves is a direct consequence of the presence in them of an abnormal amount of starch, and probably of other carbohydrate, and that it is caused by the distention of the spongy parenchyma, which was demonstrated.

Starch accumulation in the leaves accompanies rolling due to some other causes, such as injury to the base of the stalk, attacks of black-stalk rot, and other obscure disturbances.

Evidence is presented to show that the seat of the disturbance in the translocation of carbohydrate from the leaves of diseased plants resides in the blades of the leaves, where the accumulation of starch begins and ends, and not in the disorganization of the phloem in distant tissues.

Low temperatures were found incapable of causing healthy leaves of a living plant to accumulate starch or to roll.

The presence of disorganized phloem was established in plants attacked by *Phytophthora infestans* and in others apparently suffering from an attack of eel-worms.

The disappearance of the starch in diseased leaflets proceeds from base to tip, but in healthy leaflets from tip to base.

The brown spots which develop on affected leaves originate in the death of a single cell of the epidermis.

It was proved that capsid bugs (*Calocoris bipunctatus*) and jassids (*Typhlocyba Ulmi*) act as carriers of leaf-roll in the field.

Aphides (*Myzus Persicae*), when they occur on the sprouts of unplanted tubers, were also shown to be carriers of leaf-roll, and to be capable of giving rise to the earliest infestation of the foliage with these insects.

The vapour of tetrachlorethane was found a safe and efficient medium for ridding sprouted tubers of aphides.

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EXPLANATION OF FIGURES.

Plate VI.

Fig.

1. Leaf-rolling "artificially" induced in healthy Up-to-Date plant by removal of tubers and axillary shoots. Photographed on fifth day after treatment.
2. Untreated control plant corresponding to plant illustrated in fig. 1. The two were derived from the halves of a single tuber, and were photographed at same time and to same scale.
3. "Artificially" rolled leaf from healthy plant, as illustrated in fig. 1. Photographed on seventeenth day after original treatment of plant.
4. One of the previously "artificially" rolled plants, showing normal foliage after tubers and axillary shoots had been allowed to develop. Note development of foliage leaves on floral shoot.
5. Leaf from previously "artificially" rolled healthy plant after disappearance of rolling.
6. Leaflet from "artificially" rolled healthy plant (on right), and from normal control plant (on left), both having been kept for sixty-four hours in dark room. The leaflet from the treated plant contains a great excess of starch, and has cleared only very slightly at the tip. The normal leaflet is free from starch.
7. Another pair of leaflets, one from "artificially" rolled plant (on right) and the other from normal control plant (on left), showing further progress of starch disappearance after a period of seven days in dark room.
8. The resumption of normal starch translocation in previously "artificially" rolled foliage. Leaflet on right, from previously rolled plant, contains slightly less starch than leaflet on left from normal control plant. Both kept for twenty-nine hours in dark room.
9. Leaflet on left taken from diseased plant which showed early stage of rolling, and was then darkened in field for twenty-one days. Note large amount of starch still present, and progression of clearing from base upwards. Leaflet on right, from healthy plant of same variety which was darkened in field for twenty-four hours, is almost free from starch. Variety, British Queen.
10. Accumulation of starch in leaves accompanying injury to base of stalk. Leaflet on left was taken from top of injured stalk, and shows excess of starch. Leaflet on right from top of normal stalk of same plant. Both forty-two hours in dark room. Variety, Barley Bounty.
11. Plant (of season 1922) diseased with leaf-roll, being one of five diseased plants which were the progeny of a caged healthy plant on which capsid bugs (*Calocoris bipunctatus*) taken from diseased plants had been placed in 1921.
12. Healthy control plant (of season 1922), corresponding to plant shown in fig. 11. This was one of four healthy plants which were the progeny of a caged healthy plant from which insects were excluded in 1921. The two original plants of 1921 were derived from the halves of one tuber. Both plants photographed to same scale. Variety, British Queen.

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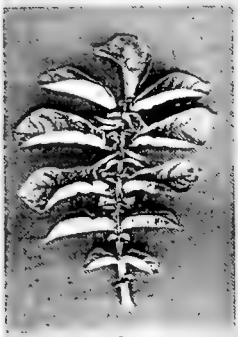

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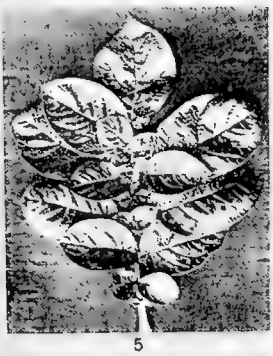

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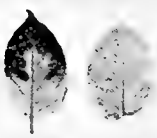

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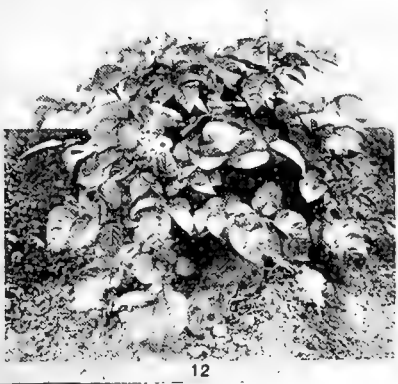

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No. 21.

ON THE CHANNELS OF TRANSPORT FROM THE STORAGE ORGANS OF THE SEEDLINGS OF *LODOICEA, PHŒNIX,* AND *VICIA.*

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(Plates VII–XI.)

(Read January 23. Printed June 18, 1923.)

Reasons have been given elsewhere (3 and 4) why it is difficult to accept the generally received view that the bast or phloem is the main channel by which organic substances are transmitted from place to place in the higher plants. The suggestion was there put forward that probably the wood may be more properly regarded as this channel, and evidence which favoured this view was adduced.

The general presence of tracheae in the connecting channels of the organs of mature plants can possibly be explained by the exigencies of water-supply; hence we cannot draw conclusive inference on this point from the development of tracheae in the bundles connecting the assimilating and storage organs of these plants.

In the case of some seedlings, however, the conditions are different. Here the storage organs are connected with the embryo, which either has an independent water-supply from the radicle, or is adequately provided for in this respect of imbibition. Hence, on the assumption that the phloem is responsible for the transport, we might expect that the xylem, being superfluous, would either not be developed at all in these connecting organs, or would be represented by merely vestigial traces.

It was then with the object of collecting evidence on this point that we have investigated the structure of the organs connecting the stores with the embryo in some seedlings.

Lodoicea sechellarum.

The general course of the germination of this palm has been described by several observers. As is well known, the seed is of remarkable size, weighing, according to Sir William Hooker, 20-25 lbs. (6). It is deeply bilobed, and is covered by a hard black shell—the fruit. In germination the embryo emerges from the depression between the lobes. It develops as a cylindrical mass, 1·5-3·0 cm. in diameter, and, turning upwards for a little, then pushes its cylindrical

body in a more or less horizontal direction. After a horizontal growth of 50-300 cm. the end turns downwards and buries itself in the ground. The buried lower tip now develops as a root, while the part just above the level of the soil greatly increases in diameter. Soon a longitudinal split in this enlarged region allows the developing plumule to emerge. These relations are very well seen in fig. 1, Pl. VII, which is a photograph of a fruit of Lodoicea germinating in Kew. We are indebted to Dr. A. W. Hill, F.R.S., for this interesting picture.

The whole growth of the embryo until it forms connexion with the soil is evidently made at the expense of the material stored in the seed and transmitted through the cylindrical body to the growing parts. Even for a long time after the root has entered the soil much nutriment is conveyed to the embryo from the seed. Farmer (5) records a case in which connexion was maintained between the seed and the young plant for five years.

Morphologists seem agreed that the organ connecting the young plant and the seed is the cotyledon (14). The basal part of this leaf forms a complete circular attachment at the first node of the embryo, and is excavated into a conical cavity overarching the plumule. Above this cavity the walls converge and form a solid cylindrical petiole, which is continued into the seed. Within the seed its distal end expands into a bilobed, rounded mass—the haustorium. The surface of the haustorium is corrugated and thrown into many folds and ridges; secondary folds imposed upon these make the surfaces of the larger corrugations densely papillose. This papillose surface is in close contact with the store material filling the seed, and exposes a very large area to the latter, both for the excretion of dissolving enzymes and for absorption.

By the kindness of Dr. A. W. Hill, we were able to make a histological examination of a germinating seed. The seed was one of four which germinated during 1922 at the Royal Gardens, Kew; and, having shown signs of retarded growth, was most kindly placed at our disposal.

The embryo was then protruding from the shell about 30 cm. It had a twisted and seared appearance. When the seed was opened, the haustorium was found to be about 10 cm. in diameter. It was of an irregularly rounded form, deeply grooved and papillose. It was creamy white, and contrasted with the bluish white of the endosperm. A narrow neck connected the haustorium with the petiole, apparently deeply constricted by the shell of the fruit. The distal part of the petiole with which this neck was connected was flattened by the pressure of the two lobes of the fruit. Once clear of these lobes, the petiole became approximately circular in section.

The outer surface of the petiole was marked with irregular longitudinal grooves and ridges. It was brown in colour, with irregular white blotches. Its transverse section was limited by 4-6 layers of parenchymatous cells, the contents of which had disappeared, and the walls alone of which persisted. These cells in transverse section are about $0{\cdot}02 \times 0{\cdot}02$ mm. They are about 0·07 mm. in length. Within them there follow 4-6 layers of sclerenchymatous cells, about 0·01--0·02 mm. in diameter and 0·06--0·08 mm. in length. Their thickened walls almost completely obliterate their lumen. From the sclerenchymatous layer inwards the mass of the petiole is composed of thin-walled parenchyma built of cells, which in the outer regions are comparatively small, viz., $0{\cdot}01\text{-}0{\cdot}03 \times 0{\cdot}06\text{-}0{\cdot}08$ mm., while in the inner regions the dimensions are $0{\cdot}05 \times 0{\cdot}10$ mm. or more. Scattered among these cells were many tannin-sacs, and throughout the tissue were inter-cellular spaces. There was no starch in any of these cells.

Embedded in this fundamental tissue were the vascular bundles to the number of 800-1,200 in one cross-section. They exhibited the usual structure of the vascular bundles of xerophytic monocotyledons. One of them is repre-

sented in cross-section in fig. 7, Pl. VIII. The sclerenchymatous sheath of each bundle was largely developed, occupying about 75 per cent. of the area of the cross-section. Sunken in the outer surface of this sheath, rows of small cells containing siliceous spherules, as is common in palms and orchids, were observed (2 and 13).

The phloem and xylem within the sheath were normally developed, the ratio of the areas of their cross-sections being on the average about 6 : 5. The vessels of the xylem having a diameter of 0·05-0·07 mm., were strengthened by annular and spiral supports. The diameter of the sieve-tubes averaged about 0·01 mm., their length about 0·15 mm. In the specimen examined many of the vessels contained a homogeneous slime-like substance.

Here and there among the normally developed vascular bundles were found greatly reduced bundles, consisting of one or two tracheae, and a very few sieve-tubes surrounded by a thick sheath of sclerenchymatous fibres. Often all the woody elements, and even all the cellulose ones, were suppressed, and the bundle consisted of fibres alone (fig. 2, Pl. VII). These reduced bundles are probably branches of the larger bundles, and end blindly in the fundamental tissue, as has been described in the stem of *Vanda teres* (2). Their function appears to be mechanical only.

Above the constriction already noticed, the cotyledon expanded within the seed to form the haustorium. Once within the seed, the cells of the superficial layer presented a different appearance. They were isodiametrical, about 0·01-0·03 mm. in diameter (figs. 12, 13, 14, Pl. X). Their walls were thin, their cytoplasm largely vacuolated, and their nuclei conspicuous. They formed a uniform layer of cells, following the lobed and papillose surface of the haustorium, generally without discontinuities. On the distal surface of the haustorium the superficial cells were somewhat smaller, and their cytoplasm was less vacuolate. They stained more deeply. In this region they presented somewhat the appearance of a columnar epithelium.

Enclosed by this layer was the general mass of the haustorium, in the main composed of thin-walled fundamental tissue, built of cells with rounded contours, which were smaller, and closely packed in the outer region. In the inner parts of this tissue were enclosed large intercellular spaces, and the component cells were formed like those of the spongy parenchyma of a leaf. The spaces in the central regions were filled with air, but the smaller ones close to the surface were often infiltrated by a slimy material resembling the *débris* of the endosperm on the outer surface of the epithelium. This material appears, during the growth of the haustorium, to force its way between the epidermal cells into the intercellular spaces (fig. 12). In the specimen examined the fundamental tissue was in many places traversed by the hyphae of the non-septate mycelium of a fungus, which had developed subsequently to the opening of the fruit, or possibly had established itself at some earlier stage. These hyphae, being very rich in protoplasm and multinucleate, are easily distinguished from the tubular cells which will shortly be described. The cells of the fundamental tissue contained many starch grains, and smaller starch grains were occasionally found in the superficial layer.

The cells of the inner fundamental tissue are comparatively large, the dimensions of their cylindrical branches being about 0·05 mm. × 0·10 mm. They are often constricted where they come into contact with one another, and the partition walls have wide pits. Here and there in this large-celled parenchyma are to be seen giant cells, having about the same diameter, but a much greater length, so that in one section they may show a length of 0·80 mm. or more (figs. 3 and 4, Pl. VII). They are tolerably straight, and seldom branch. As they pass between the other cells, they make contact with them. The areas of

contact are provided with large pits. These giant tubular cells very often form straight or slightly curved linear series connecting one bundle with another, starting in contact with or very close to the thin-walled sheath. So far as our observations go, they never contain starch, but possess a readily seen protoplasmic lining, in which is embedded a single nucleus. Often several of these tubular cells run along side by side through the loose cells of the parenchyma. The walls of these adjacent tubes cohere together, and where they cohere are marked with pits.

Apparently quite distinct from these giant cells of the fundamental parenchyma, but sometimes associated with them, are narrow tubular elements, which traverse the intercellular spaces of the fundamental tissue. They have a diameter of about 0·004-0·007 mm. and a length of about 0·10-0·15 mm., and form very long linear series originating from the bundles. They pass out from the bundles into the cortical tissues, or penetrate through the inner tissues of the haustorium. Each member of the series has a nucleus. Figs. 10, 11, Pl. IX, show a sheaf of these elements which has branched in the cortical region, and has sent one branch outwards through the cortex to the epidermis and another into the deeper tissues of the haustorium. The whole length of this tract was about 2 mm. Fig. 11 shows the inner end of this tract more highly magnified. When these tubular elements reach the outer tissues of the cortex, where the intercellular spaces are injected with the *débris* of the endosperm, their ends enlarge (figs. 12, 13, Pl. X), and sometimes they push their way between the epidermal cells and come directly in touch with the food supply (fig. 14, Pl. X). Sometimes one finds their ends in intercellular spaces still within the tissue. In this case they have a rounded termination, reminding one of the tip of a root-hair or a rhizoid.

The connexion of these tubular elements with the bundles may be seen in a section cutting a bundle at right angles, just at the point where these tubules emerge from it (fig. 5, Pl. VII). Here the tubules may be seen as continuations of the groups composed of two, three, or more small angular elements, which are scattered like small islands in the phloem and in the xylem-parenchyma.

Fig. 9 is a tangential section of a bundle in the haustorium, and shows the transverse section of a group of these tubules emerging through the bundle-sheath. Fig. 8 is a transverse section of such a sheaf of tubules as it passes across the fundamental tissue. It also shows the tubules running in an intercellular space.

The giant cells and the tubules seem often to accompany one another, but they also occur quite separated from one another.

The tubular form of both kinds of elements and the distance of the transverse partitions in them from one another suggest that in them diffusion, or possibly some other means of translocation, will take place less hampered than in the isodiametrical parenchyma.

From a causal point of view one is tempted to surmise that the exceptional abundance of organic supplies has led to a hypertrophy of two different cell-categories to form rhizoid-like internal organs.

On entering the haustorium, most of the bundles bend appropriately to distribute themselves in the layers of fundamental tissue immediately next the surface. In this region they bifurcate and anastomose to form a network, the meshes of which are about 1 mm. or less across. Very often these bundles lie immediately beneath the grooves separating the papillae of the haustorium from one another, and from these bundles many of the tubular cells just described extend into the tissues of the adjacent papillae, and running along immediately beneath the epidermis, emerge at the surface between the epidermal cells (fig. 14, Pl. X). Some bundles do not turn outwards towards the surface, but after

entering from the petiole strike across the central tissue of the haustorium, and make connexion with the network formed by the others at the distal surface of the haustorium.

On leaving the petiole, the bundles soon lose their sclerenchymatous sheath, and are continued as a double strand of xylem and phloem, surrounded by an ill-defined sheath of elongated, prismatic, thin-walled cells (fig. 6, Pl. VIII). On the outside these adjoin the spongy parenchyma. Within the sheath the phloem is composed of sieve-tubes, companion-cells, and bast-parenchyma. The latter occupies the greater part of the phloem. Its cells are comparatively large, having a diameter of 0·02-0·03 mm., and being 0·10-0·20 mm. in length. The sieve-tubes and companion-cells are in small groups, and associated with them are the groups of tubular elements already noted, among the phloem-parenchyma. The whole cross-section of the phloem contains about 120 elements. In the cross-section of the xylem, on the other hand, 12-18 elements are seen. They are spiral and annular tracheae and xylem-parenchyma and one or two groups of the tubules. The ratio of the cross-section of the phloem to that of the xylem in the bundles of the haustorium is about 8 : 1, which is a marked contrast to the same ratio in the petiole, which is 3·0 : 2·5 (cf. figs. 6 and 7, Pl. VIII).

The haustorium of the specimen we examined was embedded in the softened tissue of the endosperm. This was in turn surrounded by the outer layers of the endosperm which were still white, and of stony consistency. The inner layers next the haustorium were yellowish and pasty, and could be scooped out with a spoon. Both the soft and hard layers gave a blue reaction with iodine and sulphuric acid, and the soft part reduced Benedict's solution after standing some days with toluene (cf. 9, 10).

Microscopic examination showed that in the pasty substance cell-structure was more or less obliterated. The wall-substance was jellified and the protoplasm indistinguishable. The hard tissue was still composed of very thick-walled prismatic cells, arranged perpendicularly to the surface of the seed. Their ends were bevelled. In the comparatively small lumen there was visible a distinct lining of cytoplasm, in which was a nucleus. The vacuole contained varying quantities of mucilage. Deep and wide pits extended into the secondary layers of wall-substance to the limiting membrane of the end and side walls (compare Gardiner's fig. 20, in 7). The outer diameter of the endosperm cells ranged from 0·09-0·13 mm. The diameter of the lumen was 0·08-0·03 mm. The lengths of the cells varied from 0·40 mm. to 1·20 mm.

Phœnix dactylifera.

The general course of the germination of *Phœnix dactylifera* is well known (8, 11, 12).

There are about eight or nine bundles in the cross-section of the petiole of the cotyledon. Most of these bifurcate before entering the haustorium, so that there are about sixteen at the level of the constriction below the haustorium (fig. 17). On entering the haustorium they diverge from one another, and run along just under its convex surface towards its margin. The haustorium itself is in the early stage button-shaped—oval in outline and slightly concave on its distal aspect. Figs. 16 and 17, Pl. XI, show the distal and proximal surfaces of the haustorium of an embryo just emerging from the seed. Just below the margin the bundles branch again, so as to give rise to 35-40 bundles which turn over the margin, converge, anastomose irregularly, and closely follow the concave surface towards the middle of the haustorium. Thus, close beneath the surface of the haustorium there is a network of vascular bundles, connected by about 16-20 main bundles with the vascular system of the petiole, and ultimately with

that of the embryo. No bundles traverse the central regions of the haustorium, as they do in Lodoicea.

The tissues of the margin and distal aspect of the haustorium retain their meristematic phase later than those towards the proximal aspect, so that in the earlier stages of germination cell and nuclear divisions are frequently found in the fundamental tissue there, and among these dividing cells the bundles are represented by procambial tracts. From these latter the tracheae are developed earlier than the sieve-tubes.

When the haustorium expands in size it becomes bilobed, and the lobes turn over and overarch the depressed central concavity. In this condition the outer surface is near the surface of endosperm, while the central mass of that tissue is embraced by the haustorium, and is in contact with its inner surface. In the middle of the haustorium, between its inner and outer surfaces, a large intercellular space develops. During this enlargement the network of conducting tracts, which at first had irregular meshes formed of sinuous bundles, becomes more regular and the bundles straighten. Meanwhile the surface becomes corrugated and papillose.

In the younger stages of germination the epidermis of the haustorium is composed of approximately cubical cells, with dense and finely granular contents, which almost entirely fill their cavities. A large nucleus is visible. They present the appearance of the secreting cells of a columnar epithelium (fig. 19, Pl. XI, ep.). As the haustorium enlarges, their vacuoles grow and their protoplasm becomes more scanty, and loses its affinity for stains. At the same time the nucleus diminishes in size, and its granules become less conspicuous (cf. 10).

The bundles in the haustorium lie extremely close to its surface, so that their outer elements are often separated from the epidermis by one or two layers only of cells. As in the case of Lodoicea, the ratio of the area of the cross-section of the phloem in the haustorium to that of the xylem is much greater than that in the petiole. In this case the ratio is about 5 : 1 in the haustorium and 1 : 1 in the petiole (figs. 18 and 19, Pl. XI). There are about twenty elements in the phloem both of the haustorium and of the petiole; about ten of these are sieve-tubes. In the xylem there are about four in the haustorium and twenty in the petiole.

In the haustorium the sieve-tubes are comparatively wide (0·01-0·003 mm. in diam.), and their walls stain deeply with haematoxylin. Their length is 0·06-0·09 mm. They occupy the outer part of the phloem strand, and are separated from the xylem by several layers of cambiform cells. The tracheae of the bundles are few, and have a diameter of 0·03-0·01 mm.

Phœnix canariensis and P. silvestris.

Later stages of germination were observed in these two palms. In the example of the first-named species which was examined there were 8--10 bundles in the transverse section of the petiole of the cotyledon. In that of *P. silvestris* there were only 6. Between some of these bundles there were reduced bundles (2 or 3 in all), consisting of fibres only, like those found in *Lodoicea sechellarum*. These died out before entering the haustorium. As in Lodoicea and in *P. dactylifera*, the strong sheath which accompanies the bundles in the petiole ceases immediately as the bundle enters the haustorium. In none of the three species of Phœnix did bundles cross the central tissue of the haustorium; as they enter it they diverge and bifurcate, keeping close to its surface. Then turning over its margin and arriving on its concave surface, they converge and re-unite to form a network on its distal aspect (fig. 15, Pl. X).

The bundles after entering the haustorium consist of 8--10 tracheae and a few wood-parenchyma cells, forming their xylem; their phloem is composed of large phloem-parenchyma cells, which separate the two or three groups of sieve-tubes. Between this phloem and the xylem there are two or three layers of cambiform cells.

Epidermal cells of haustorium, diameter ...	0·01--0·06 mm.
Sieve-tubes, diameter	0·01--0·02 mm.
Sieve-tubes, length	0·10--0·15 mm.
Tracheae, diameter	0·01--0·03 mm.

Vicia faba.

With a view to throwing some further light on this subject, the anatomy of the cotyledons of the broad bean, *Vicia faba,* was also studied. In the plant, as is well known, the cotyledons do not emerge from the seed-coat, but function solely as storage organs; and, being normally below the ground, they do not transpire.

Sections of a seed which had been soaked in water for twenty-four hours were first examined. The bulk of the cotyledons is composed of large parenchymatous cells, which are closely packed with starch and protein granules. Between these cells there is a considerable development of intercellular spaces. Where the neighbouring cells come in contact with one another there are numerous large pits in the intervening walls, which possibly facilitate diffusion from cell to cell. The cotyledons are traversed by vascular bundles, which even at this stage show fully developed vessels with spiral thickenings; but there is no trace of sieve-tubes, the remainder of the bundle being composed of elongated parenchymatous cells filled with protoplasm and containing large nuclei. This early development of wood in the cotyledons may be contrasted with the conditions obtaining in the radicle and plumule, in which the presence of xylem cannot be recognized until some time after germination.

At a later stage, when the main root has reached a length of about 12 cm., the wood in the cotyledons shows a considerable increase in amount, and well-developed pitted vessels are present. The exact time at which the sieve-tubes are differentiated in the cotyledons is difficult to determine. At this stage they are certainly present in the petioles, and at a later stage can also be seen in the laminae of the cotyledons. The mature sieve-tubes can be easily recognized by the presence of the peculiar ellipsoidal slime-masses in the vacuole of the cell. These bodies were described by Strasburger (13) and Beccarini (1) in members of the Leguminosae, and are particularly well-developed in Vicia. They appear to be quite free in the sieve-tube, and in most cases they lie close to one of the sieve-plates.

The anatomy of these cotyledons, in so far as it has a bearing on the problem of translocation, may be briefly considered. The storage tissue through which the soluble organic materials must pass by diffusion is constructed so as to facilitate this function as far as possible. The vascular bundles, on the other hand, probably provide the conduit for transport to the growing points. The early formation and later development of the xylem in the cotyledons, where transpiration is negligible, is significant. It may, of course, be considered that the xylem is a purely vestigial structure, but its extremely marked development hardly seems in accordance with this view. If, however, it is functional, it would seem that it must be of use in translocation. We know that organic substances must at times be able to pass with comparative freedom from cell to

cell, and therefore must pass through the protoplasmic lining of the cell. Consequently, unless we regard the cells lying next to the vessels as being relatively more impermeable, there seems to be no difficulty in understanding how organic substances can be injected into the vessels.

On the other hand, the living cells of the vascular bundles seem ill-adapted to the task of translocation. The sieve-tubes are late in development, and even when fully formed would offer a greater obstruction to diffusion than would the storage tissue, which, in addition to a large cross-sectional area, is better provided with pitted cell-walls.

With a view to obtaining some evidence on the normal course of the current in the xylem of the cotyledons, the following experiments were conducted:—

A seedling of *Vicia faba,* with a root 5·5 cm. long, was used. The cotyledons were cut across in a direction parallel to the direction of the root, and the cut surface was placed under a solution of eosin. The root was surrounded with moist Sphagnum moss. After twenty-four hours all the bundles of the cotyledons were injected with eosin, and traces of eosin were found in the root down to about 1·5 cm. below the origin of the plumule. During this period the root had elongated 0·5 cm.

In another experiment a seedling with a shoot 12 cm. and a main root about 20 cm. long was used. The root was cut across and immersed in an eosin solution for one and a half hours, and at the end of that time the whole of the wood in the cotyledons was deeply injected with eosin.

Such experiments, unfortunately, give no indication of the normal course of the current in the wood. They do show, however, that if water or a solution of eosin can be drawn into the plant at any point it will be transported with an equal facility in an upward or downward direction, according to requirements.

That the cotyledons have the power of absorbing water is shown by the rapidity with which the dry seeds swell up when immersed in water. It seems probable, therefore, that water continually passes in through the cotyledons during germination; and, unless the cells lying next to the vessels are specially impermeable, a solution of organic substances would travel by means of the wood from the cotyledons in the direction of the growing points.

Summary.

1. In the seedlings of the palms examined there is a network of vascular bundles close below the absorbent surface of the haustorium, which is embedded in the endosperm. This network is connected with the growing embryo by bundles which traverse the basal parts of the haustorium and the petiole longitudinally.

2. Well-developed xylem, consisting largely of lignified tracheae, is found in the vascular bundles of organs connecting embryos with their stores.

3. Sclerenchymatous sheaths and cords of sclerenchymatous fibres are also often found in these organs. These sclerenchymatous elements are not continued into the haustoria.

4. The total area of the cross-section of the phloem of all the bundles in the haustoria is much greater than that of the bundles in the connecting organs or petioles. There is not the same disproportionality in the cross-section of the xylem in the two organs. Sometimes the total area of the cross-section of the xylem of the bundles of the petiole approximately equals that of the bundles of the haustorium, e.g., in *Phœnix dactylifera.*

5. In *Vicia faba* tracheal elements are differentiated in the petiole much earlier than are sieve-tubes. Before the latter appear, considerable transport of organic substances to the embryo must take place.

6. Material produced by the disintegrated endosperm finds its way into the outer intercellular spaces of the haustorium of *Lodoicea sechellarum*, passing between the epidermal cells.

7. Tubular cells taking their origin at or near the sheath of the vascular bundles of the haustorium of *Lodoicea sechellarum* pass between the isodiametrical cells of its spongy tissue. They form connexions with these cells, and their walls are pitted at the points of contact. They often form connexions between the sheaths of the vascular bundles.

8. Groups of narrow tubular elements are found in the phloem and xylem of the bundles of the haustorium of Lodoicea. Here and there sheaves of these pass out of the bundles into the intercellular spaces of the surrounding tissues. Sometimes they turn inwards and push their way among the cells of the central fundamental tissue; more frequently they turn outwards and traverse the cortical region. When they come immediately beneath the epidermis, their ends expand, and may even push out between the epidermal cells to the surface.

CONCLUSIONS.

1. The presence of the vascular network in haustoria and of conducting tracts in the transmitting organs suggests that the vascular bundles are the channels by which the embryo receives the organic supplies from its storage organs.

2. The development of tracheal tubes in transmitting organs of seedlings, where the transport of water is unnecessary, is in conformity with the view that these tubes convey organic store material.

3. Experiment shows that these tubes will convey fluid either in a basal or in a distal direction, according to the position of the source and sink.

4. The lateness in the differentiation of the sieve-tubes in the vascular strands of the petioles of the cotyledons of *Vicia faba* shows that much transport of organic substance down the petiole is effected without their assistance. During this period tracheae are available.

5. Transport of organic substance through the parenchymatous cells of the haustoria and through those of storage cotyledons has to be attributed to the permeability of the protoplasm of these cells. It seems gratuitous to assume that the cells adjacent to the tracheae are the only cells in these organs which are impermeable. If they are permeable, the tension set up in the tracheae will secure movement of organic fluids into and along the tracheae.

6. The great development of the phloem in the haustoria compared with its mass in the transmitting organ—the petiole—suggests that this tissue is chiefly concerned with the preparation of the organic substance absorbed and its transmission into the tracheae.

7. It may be pointed out that sieve-tubes in their mature state are by no means ideally constructed for the transmission of organic substances. The pores in their sieve-plates are exceedingly fine, and they are mostly or entirely blocked by protoplasm or callus. Bearing in mind the semi-permeable nature of protoplasm, it would appear that the sieve-plate would present a greater obstacle to the flow of most solutions and every sol than would a simple cell-wall. It seems quite possible that we should regard the sieve-tubes rather as minute reservoirs than as conduits.

8. The contrast of appearance of the phloem-parenchyma (cambiform cells) in this connexion is suggestive. The bundle endings in leaves and in growing points, as is well known, are without well-differentiated sieve-tubes, and their phloem is composed of cambiform cells only. These may be supposed to be effective in transmitting the products of photosynthesis into the tracheae and at the growing points to extract these supplies for the growth there.

9. The companion-cells of the phloem are completely filled with finely granular and easily staining protoplasm. Their nuclei are comparatively large. Their appearance is that of secreting cells. Hence we may provisionally assume that to them is assigned the function of secreting substances (probably enzymes) to prepare the organic materials and render them suitable for introduction into the tracheae and for transmission in these tubes.

10. These secretions may be stored in the sieve-tubes, whose sieve-plates prevent their passage along the bundle. The sieve-plates may be regarded as partitions which allow the protoplasm to be withdrawn through their pores, but at the same time have been rendered practically impermeable by the remnant of protoplasm and the callus left blocking the pores.

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DESCRIPTION OF FIGURES.

PLATE VII.

Fig.

1. Germination of *Lodoicea sechellarum*, photographed at Kew, January 19th, 1923. The curved petiole of the cotyledon is bent down in front of the bilobed fruit. The split in the base of the petiole shows the plumule about to emerge.

2. *Lodoicea sechellarum.* Transverse section of sclerenchymatous strand in petiole of cotyledon. × 500. e.s., sac containing siliceous nodule; t.w., thin-walled elements.

3. *Lodoicea sechellarum.* Transverse section of haustorium. × 50. v.b., vascular bundle; f.p., fundamental parenchyma; g.c., giant-cell.

4. *Lodoicea sechellarum.* Transverse section of haustorium. × 150. Showing giant-cell and its attachments; *a*, with parenchyma-cells.

5. *Lodoicea sechellarum.* Transverse section of vascular bundle in haustorium at the level where some tubules are emerging; i.s., intercellular space; p., phloem; t., tubules; x., xylem.

PLATE VIII.

6. *Lodoicea sechellarum.* Transverse section of vascular bundle close to surface of the haustorium. × 420. x., xylem; t., tubules; s.e., sieve-tubes and companion-cells; p.p., phloem-parenchyma cell; i.s., intercellular space filled with slime.

7. *Lodoicea sechellarum.* Transverse section of vascular bundle in petiole of cotyledon. × 300. e.s., sac containing siliceous nodule; t.s., trachea containing slime; p., phloem; x., xylem; s.s., sclerenchymatous sheath.

PLATE IX.

8. *Lodoicea sechellarum.* Transverse section of haustorium, showing tubules (t.) passing down intercellular space (i.s.). × 250.

9. *Lodoicea sechellarum.* Longitudinal tangential section of bundle sheath in haustorium, showing sheaf of tubules (t.) cut transversely. × 250.

10. *Lodoicea sechellarum.* Transverse section of haustorium. × 48. e.n., remains of endosperm; ep., epidermis; v.b., vascular bundle; t., sheaf of tubules.

11. *Lodoicea sechellarum.* Inner part of the same sheaf of tubules (t.) represented in fig. 10, showing its bifurcation. i.s., intercellular space. × 250.

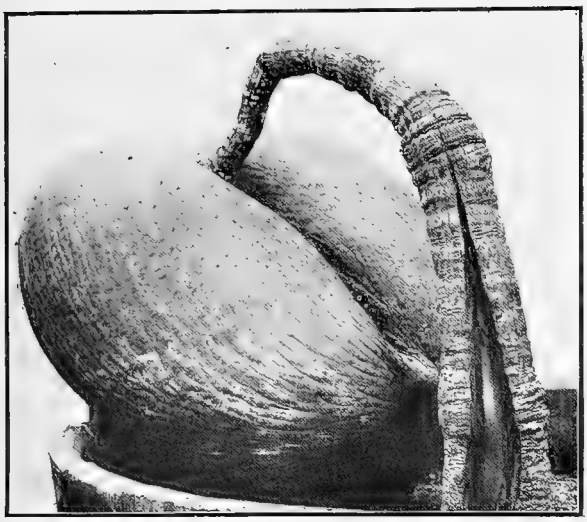
Plate X.

Fig.

12. *Lodoicea sechellarum.* Transverse section of haustorium. en., remains of endosperm; ep., epidermis; i.s., intercellular space filled with slime from endosperm; t., tubule in intercellular space. × 335.
13. *Lodoicea sechellarum.* Transverse section of haustorium. en., remains of endosperm; t., enlarged end of tubule filling intercellular space under epidermis (ep.).
14. *Lodoicea sechellarum.* Transverse section of haustorium. en., remains of endosperm; t., end of tubule filling sub-epidermal intercellular space and emerging between two epidermal cells (ep.). × 550.
15. *Phœnix silvestris.* Longitudinal section of more mature haustorium and petiole (p.). v.b., vascular bundles; i.s., intercellular space. × 7.

Plate XI.

16. *Phœnix dactylifera.* Distal surface of young haustorium, showing developing vascular bundles (v.b.) through the outer tissues. × 24.
17. *Phœnix dactylifera.* Proximal surface of young haustorium and transverse section of petiole. v′.b′., vascular bundles in section, and v.b., through the outer tissues. × 24.
18. *Phœnix dactylifera.* Transverse section of vascular bundle in petiole of young cotyledon. s.s., sclerenchymatous sheath; p., phloem; x., xylem. × 380.
19. *Phœnix dactylifera.* Transverse section in young haustorium. ep., epidermis; p., phloem; x., xylem. × 380.



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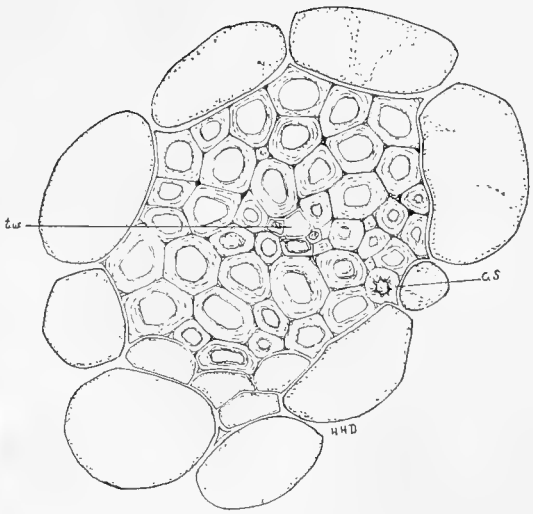


FIG. 2.

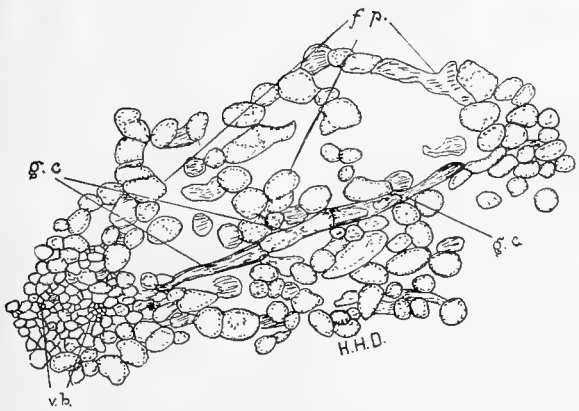
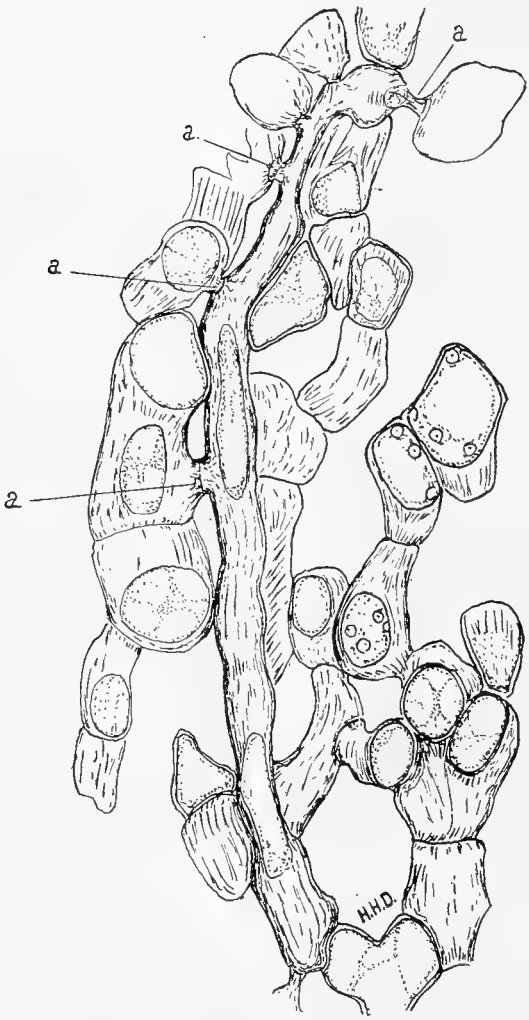


FIG. 3.



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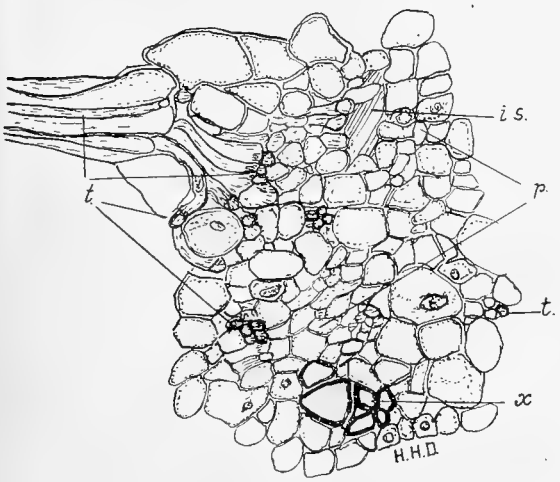


FIG. 5.

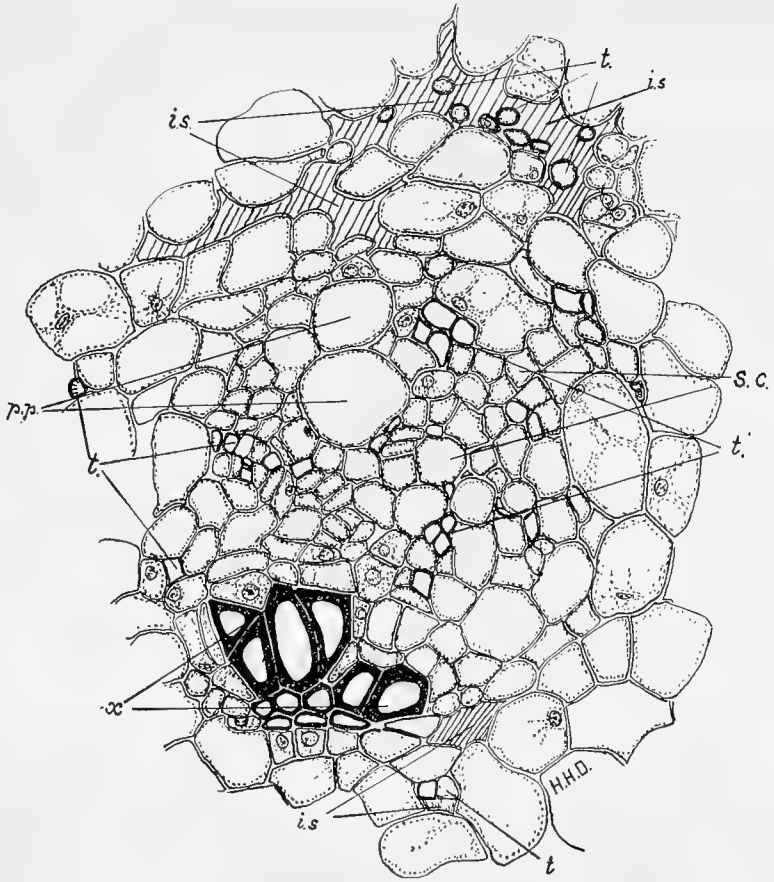


Fig. 6.

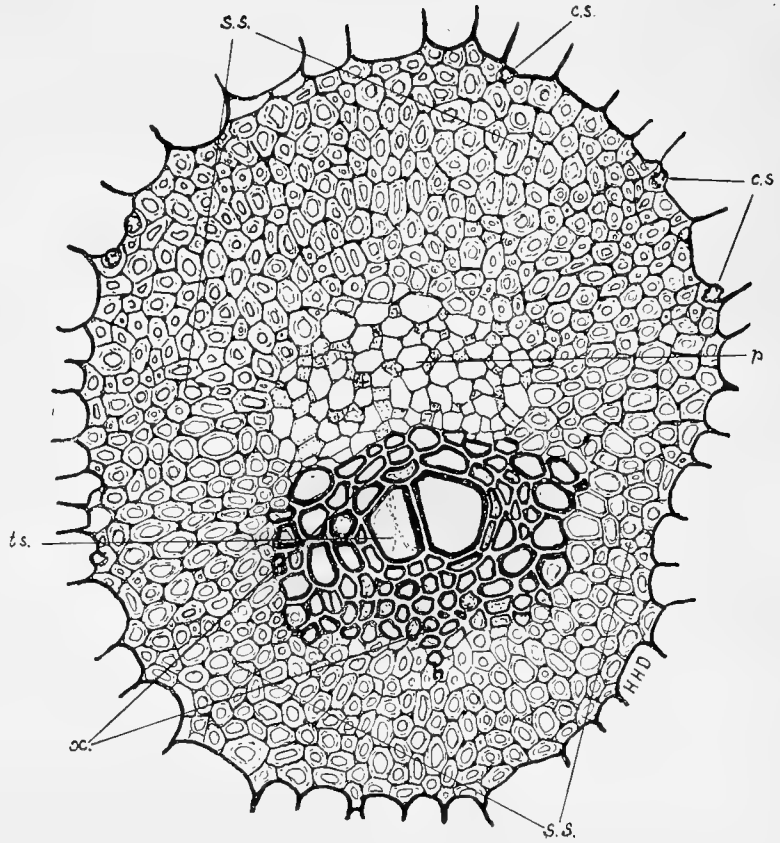


Fig. 7.

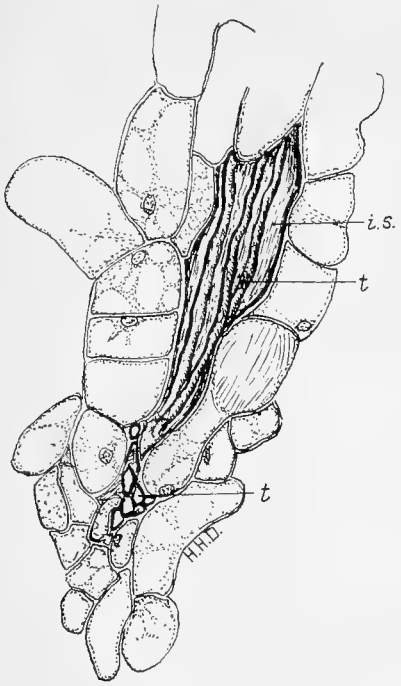
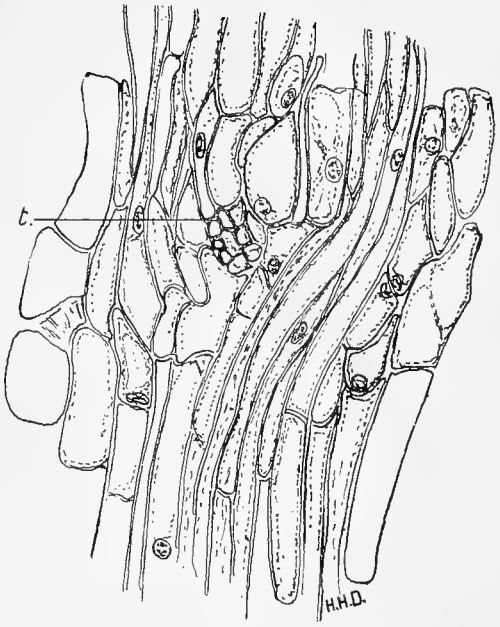


FIG. 8.



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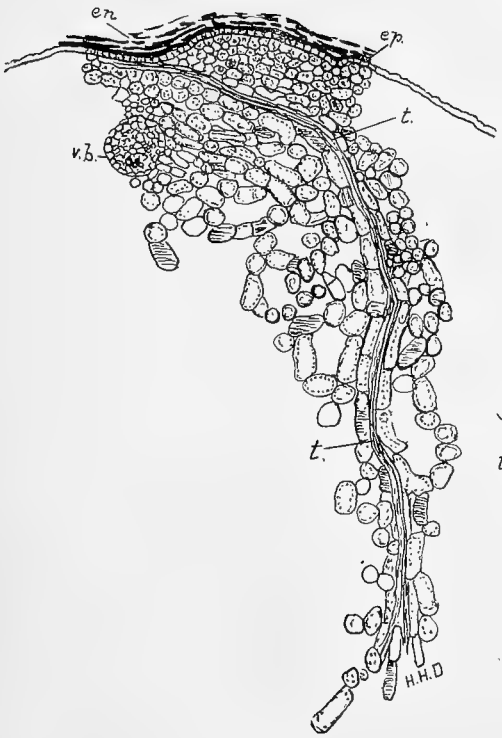
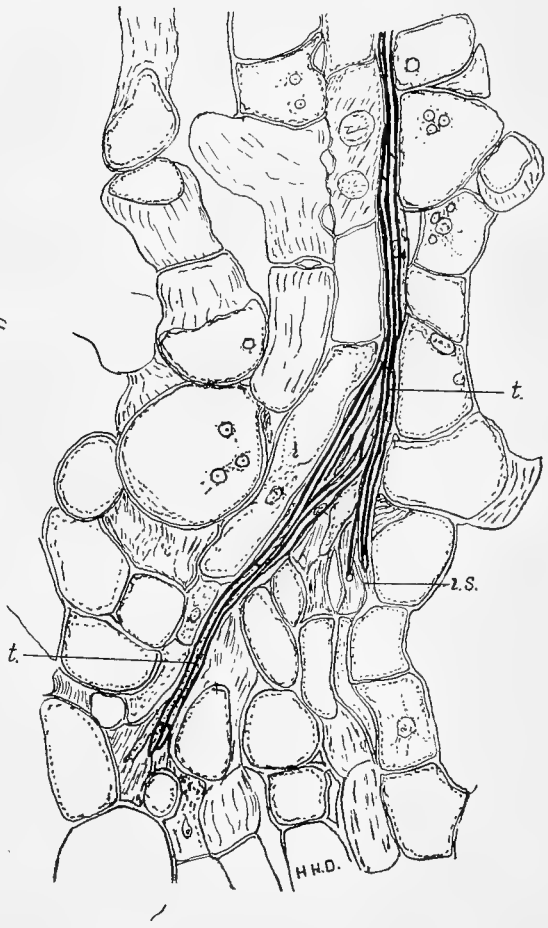


FIG. 10.



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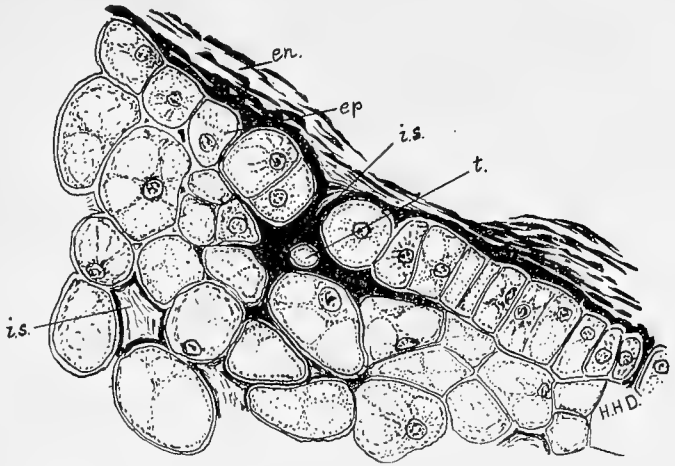

FIG. 12.

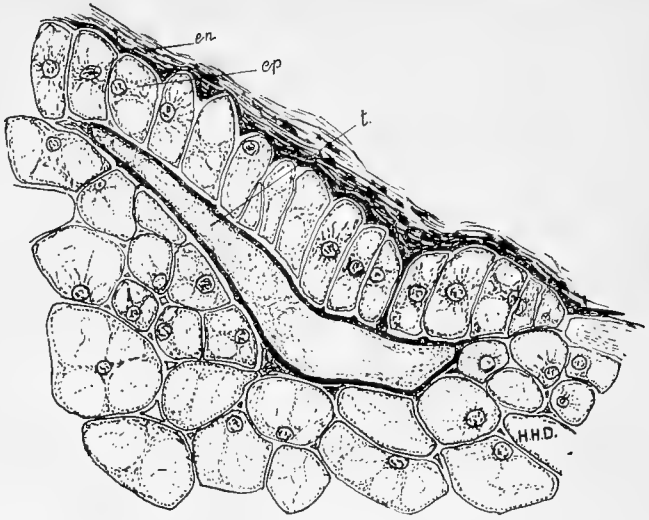

FIG. 13.

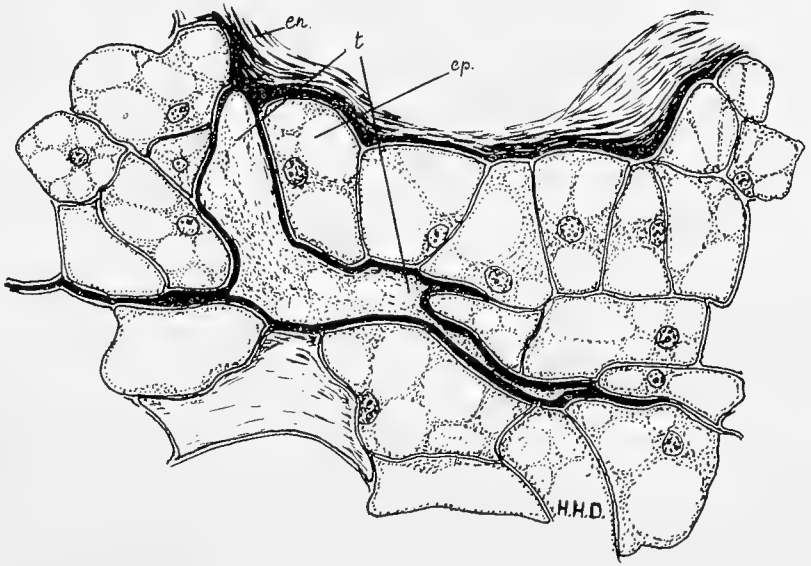

FIG. 14

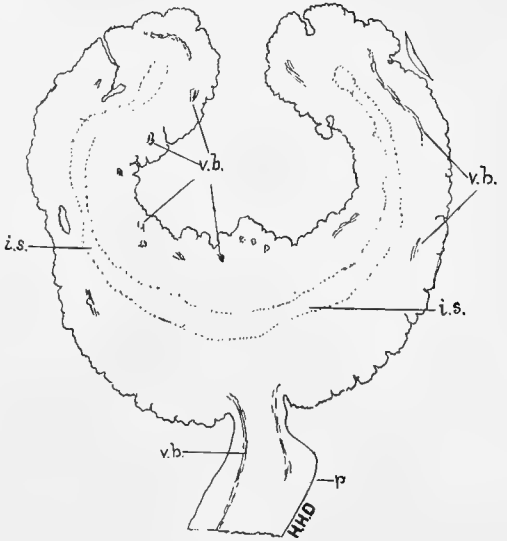

FIG. 15

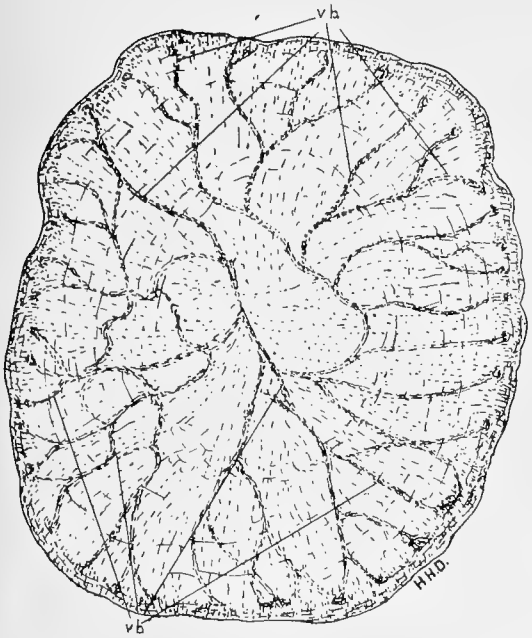

Fig. 16.

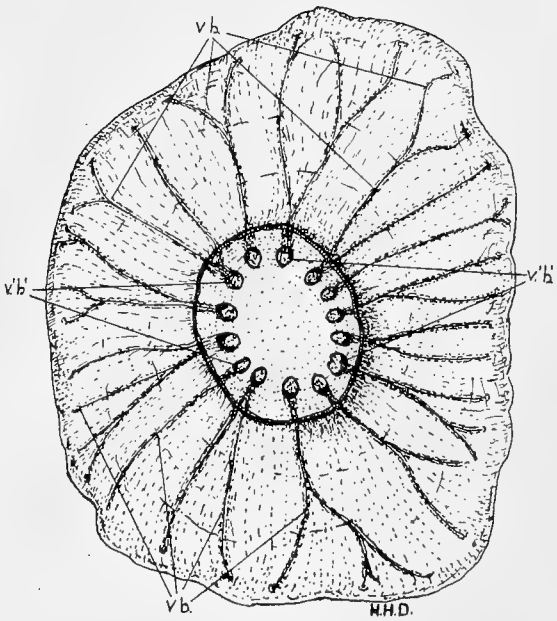

Fig. 17.

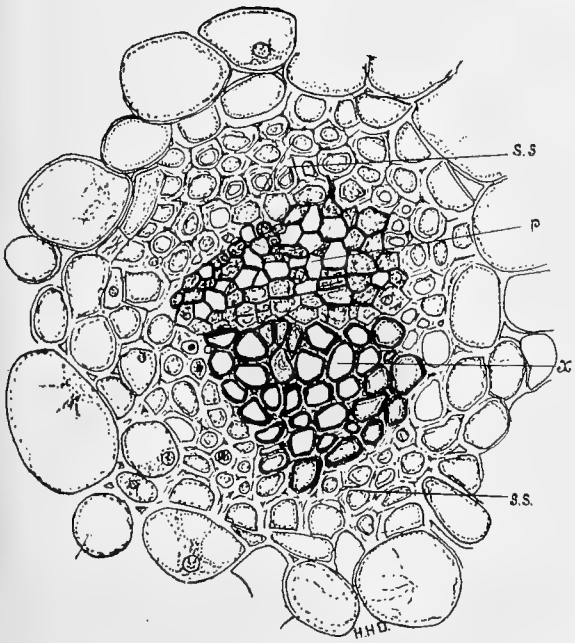

Fig. 18.

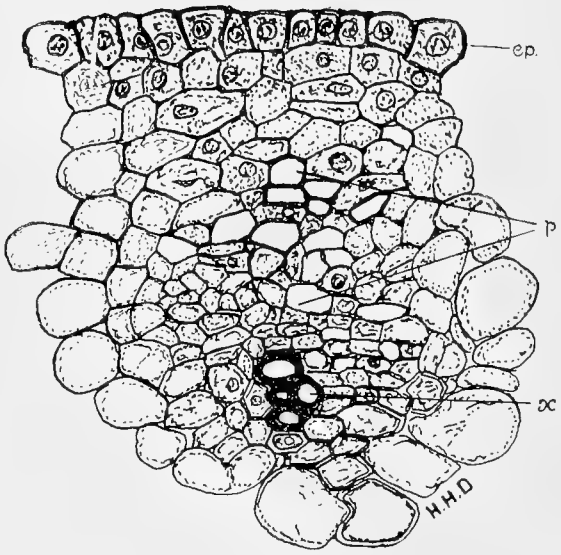

Fig. 19.

No. 22.

IRREGULARITIES IN THE RATE OF SOLUTION OF OXYGEN BY WATER.

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AND

E. F. PEARSON, A.R.C.Sc.I.,
Research Student.

(Read March 27. Printed June 19, 1923.)

In the course of some previous work one of the authors noticed that when experiments on the absorption of gases by water were made by exposing columns of air-free water to the atmosphere and then analysing the gas-content of these columns after different periods of time, various small irregularities appeared in the results, which were greater than the experimental errors involved. The effect indicated seemed to show that instead of the process of absorption of the air by the water being steady and uniform, as it is when the water is gently mixed, it varied suddenly at different times, especially towards the saturation point.

This effect, though too small to be of importance on the large scale, was considered worth investigating further, with a view to elucidating the process by which a soluble gas is absorbed and distributed in a liquid. To do this it was necessary to use a method which allowed of observing the amount of gas absorbed by the liquid at any time during the whole course of the aeration. At the same time it was thought desirable to eliminate temperature variation, and also the variation in the humidity of the gas in contact with the liquid, so that the absorption of the gas would take place under conditions which were as constant as possible, and any effect due to these causes would be excluded.

The apparatus shown in fig. 1 was designed for the purpose. It consists of two glass bulbs, V_1 and V_2, about 30 mm. diameter, and of a capacity of 60 and 200 c.c. respectively. The bulb V_1 is provided with a capillary tap (A), and is fused on to one end of the water-manometer M. The other end of the manometer is fused to a four-way piece on the top of the bulb V_2. The remaining two branches of the four-way piece are fused to a capillary and tap (H) and a gas-reservoir R. The amount of gas absorbed at any time was shown by the reading of the manometer, and this could be re-set to zero at any stage of the process by allowing mercury to flow into the reservoir to replace the gas which had been absorbed. The whole apparatus was enclosed in a water-jacket 2″ in diameter, through which a stream of water from a thermostat, kept constant to a tenth of a degree, was circulated by means of a pump.

Previous to an experiment the three taps (H), (A), and (K) were connected by means of a three-way piece, and the whole apparatus thus exhausted by a good filter-pump. The manometer was kept at zero during this process by manipulating the taps (H) and (A). When the apparatus was completely exhausted, oxygen from a gas-holder was allowed to enter through the same connexions until the pressure was again atmospheric. These operations were repeated three times in succession in order to ensure that no appreciable amount of air would remain in the apparatus. The oxygen used was taken from a cylinder of the gas, and was filtered through a tube containing glass-wool to retain traces of dust, and then passed through a small flask immersed in the thermostat, and containing a little water, in order to saturate the gas with water-vapour at the working temperature (25·8° C.).

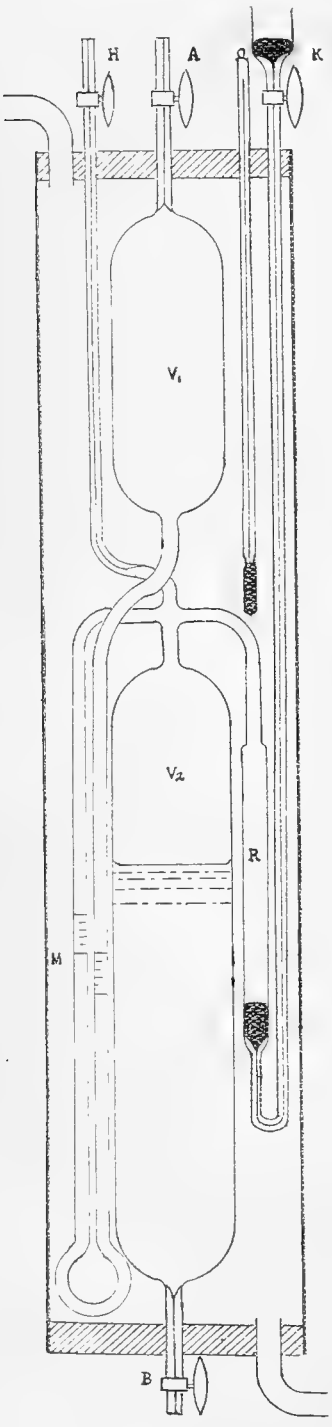

Fig. 1

When the apparatus was full of oxygen, the air-free water (which had been prepared by boiling distilled water in an apparatus, previously described, and stored in a bulb of about 250 c.c. capacity over mercury) was allowed to enter. The bulb containing the water was attached to the tap (B) and the apparatus exhausted; on opening the tap, the water was displaced over and allowed to rise to the 60 c.c. mark. The oxygen was then allowed to enter until the pressure was atmospheric, when the taps (H) and (A) were opened simultaneously and closed after about two seconds, thus rendering the pressure in both bulbs exactly the same, and the observations of the manometer were then started. The barometer was read at the time of starting an experiment, and the volume of gas absorbed calculated by means of the formula given in a previous paper.

It was originally intended to make a very complete study of the phenomenon by means of a large number of experiments over a long period of time, but the occupation of the College of Science for purposes other than scientific interfered with this scheme, and finally interrupted the work completely. As it will not be possible to resume the work for some time, the results so far obtained are presented in this paper, since they are of interest in that they clear up some points which were previously in doubt.

The results of six experiments are given in the table, and the variation of gas-content with time is shown in fig. 2. The points indicated by dots were obtained with an apparatus which was supported rigidly on a slate wall-bracket (such as is used for balances), and braced in every direction with wire stays to eliminate vibration as completely as possible. The points indicated by crosses were obtained with an apparatus supported in the ordinary way by a retort-stand.

TABLE OF RESULTS.

Time.	% Sat.	Time.	% Sat.	Time.	% Sat.
EXPERIMENT 2*a*.		EXPERIMENT 10*a*.		EXPERIMENT 13*a*.	
5·5	10·0	1·5	2·2	1·0	2·5
22·5	34·0	3·0	5·9	18·75	30·0
24·5	36·0	19·75	31·0	20·5	32·7
26·0	39·2	23·0	34·8	21·5	34·3
EXPERIMENT 3*a*.		25·0	37·6	23·25	36·0
1·0	2·2	28·0	42·0	43·0	64·0
2·0	3·0	43·75	57·0	44·0	64·2
3·0	5·5	45·75	58·8	EXPERIMENT 11*b*.	
4·25	7·3	47·75	60·0	16·0	40·8
5·0	8·4	51·25	62·0	18·0	41·7
22·0	35·5	67·75	80·0	19·5	44·7
EXPERIMENT 12*b*.		68·75	82·0	22·0	48·0
17·5	38·6	74·0	84·0	23·5	50·7
22·5	47·0	91·75	94·5	40·0	72·4
24·4	49·0	96·5	96·0	44·75	73·3
42·5	68·8	99·5	96·5	47·5	75·0
45·5	72·4	115·75	98·0	66·75	80·8
66·5	79·8	118·0	98·6	112·5	86·0

The curves drawn on the graph are reference curves, calculated by means of the formula $w = 100\ (1 - e^{-bt})$, which has been shown to apply when the water is mixed, however gently. The value of the coefficient "*b*" for the upper curve is 0·028, and that for the lower curve is 0·018, and these values represent the upper and lower limits of the rate of solution under these conditions.

It will be seen that up to a value of about 60 to 70 per cent. of saturation the experimental values agree well with the logarithmic curves, but beyond that the divergences are wide. In the case of the upper curve this is shown by a marked falling-off in the rate of solution, while in the case of the lower curve a marked increase followed by a falling-off is recorded.

The fact that the process follows the logarithmic curve during the early stages of the absorption would seem to indicate that the water is kept slowly but steadily mixed during this stage, while the uncertain behaviour after this

shows that the force causing the mixing has become so extremely small as to be capricious in its action.

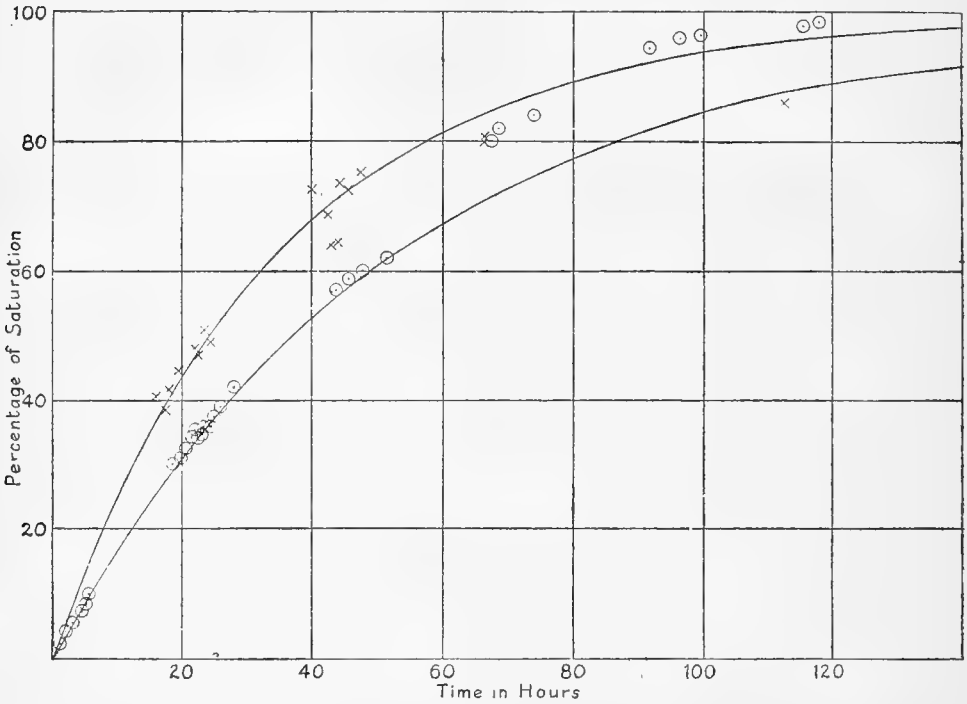

Fig. 2.

In considering the possible agencies which could affect the process under the experimental conditions, it must be remembered that these were kept as constant as possible.

The mass of gas used was completely enclosed, and both gas and water were maintained continually at the same temperature. The gas was given the correct humidity for the working temperature before admission to the apparatus, and therefore evaporation from the surface layers of the water was prevented. The possibility of slight convection currents, due to cooling of the surface layers, which might occur when the water is exposed to the atmosphere, is eliminated.

All the more obvious causes which might produce mixing have thus been excluded in these experiments, and the fact that it is present shows that the cause must be more fundamental in its nature, and further careful experiments will be necessary to discover its precise nature.

Whatever the cause, these experiments clearly show that pure distilled water exposed to oxygen at a uniform steady temperature absorbs the gas in such a way as to indicate that very slow mixing of the water occurs even under these conditions during the early stages of the absorption, but that towards the saturation point this mixing tends to become uncertain in its action.

Chemical Laboratory, College of Science, Dublin.

No. 23.

THE HYDROGEN ION CONCENTRATION OF THE SOIL IN RELATION TO THE FLOWER COLOUR OF *HYDRANGEA HORTENSIS* W., AND THE AVAILABILITY OF IRON.

By W. R. G. ATKINS, O.B.E., Sc.D., F.I.C.

(Read March 27. Printed June 18, 1923.)

Flower Colour and Soil Reaction.

When studying the relation between the distribution of plants and the hydrogen ion concentration of the soil (Atkins, 1922, 1), it was noticed that pink hydrangeas were found on alkaline soil and blue on acid. A number of situations were examined to test the validity of this observation; the results are given in the following table:—

Locality.	Colour of hydrangea.	*pH* of soil.	Notes.
Cornwood, Devon. .	Blue. . . .	6·0	Several plants.
,, ,, .	Pink. . . .	7·6	One plant, blue when in previous site.
Plymouth Hoe. .	Pink. . . .	8	Several plants, various sites.
Falmouth, near sea.	Pink. . . .	7·5	One plant.
Dublin. . .	Pink. . . .	8·0	One plant.
Mt. Edgcumbe, Cornwall.	Blue. . . .	5·75	Numerous plants, blue.
,, ,, .	Pink and blue. .	5·9	One plant.
Antony, Cornwall. .	Pink; some blue, and intermediate.	7·3	Two plants.
Templemichael, Co. Waterford.	Blue, pink, and intermediate.	6·2	Plant, when bought and planted, had bright pink flowers only.

There is in these examples undoubted proof of the relation between soil reaction and flower colour. They include the case of a plant originally bearing blue flowers changing to pink when in an alkaline habitat, as at Cornwood, and the converse change, pink to blue and some pinkish, at Templemichael.

Some plants bear blue and pink flowers; these are found in situations with a reaction of *pH* 7·5 and under. It must be added that the soil tested was from

the first four inches, which is insufficient to get near the absorbent portions of the roots.

Sap-soluble Pigments as Possible Indicators.

Since the sap-soluble colouring matters of many plants act as indicators, as shown by Haas (1916), it seemed possible that the diverse colours in hydrangeas were due to varying degrees of acidity, the more so as it is known that certain colour changes from red to blue, according as the flower fades, are explained correctly by this hypothesis. As against this must be set the remarkable constancy in *pH* value given by the leaves, stems, and roots respectively of members of the same species.

To test the matter directly, pink and blue flowers were treated with dilute acetic acid, but the pink did not change to blue. In fact, no very marked change was noticed in either case. Furthermore, flowers were obtained from a single plant bearing both pink and light mauve or blue. Petals were crushed with an agate pestle, and two drops of water, and one of indicator, added to each. Using brom phenol blue as indicator, both appeared to be close to *pH* 4·0, and were indistinguishable. This indicator is, however, dichroic, so an exact comparison in a turbid drop is not easy to make. Methyl orange covers a somewhat similar range, and with it both the pink and blue petals were ascertained to be at *pH* 4·2, and were, as before, indistinguishable. It is accordingly clear that the colours of hydrangea flowers are not due to the natural pigment acting as an indicator.

Flower Colour and Availability of Iron Salts.

Among gardeners the practice of potting with iron nails is well known as a means of producing blue hydrangeas. It therefore seemed probable that the solubility of iron salts might be the direct cause of the production of the blue. Attempts were made to induce cut flowers to change from pink to blue by placing the stalks in dilute solutions of ferrous and ferric salts. A deep dark green appeared in the stems, and spread slowly into the petioles and veins of the petals. The flowers then withered. Possibly with iron salts in much smaller amount a blue might have resulted.

Culture experiments by Duggar (1920) have shown that certain salts ordinarily considered as insoluble are quite effective as plant nutrients, since the solids yield a continual supply of a minute amount in solution. There is, however, a limit to the availability of "insoluble" compounds, as shown by McCall and Haag (1921) to be the case with ferric salts. These workers found that culture solutions containing ferric salts were adequate for nutrition of wheat when the reaction was *pH* 4·0 or more acid, but solutions from *pH* 4·0 to *pH* 7·0 gave rise to chlorosis in the plants grown in them. Patten and Mains (1920) have shown that ferric hydroxide is precipitated in quantity between *pH* 3·5 and *pH* 6·0, at which the process is complete. In nature, however, few, if any, soils are as acid as *pH* 3·5; yet plants grow in natural solutions, according to species, up to *pH* 8 or 9. As previously suggested by the writer (1922, 1), this is probably due to the presence of iron in the ferrous condition. Further work (1922, 2) has shown that ferrous hydroxide does not begin to be precipitated until *pH* 5·1; and though it comes down in quantity at *pH* 5·5 to *pH* 6·5, yet even beyond *pH* 7·1 a small amount exists in the solution, and is slowly precipitated as it becomes oxidised to the ferric state. It thus appears that under the reducing conditions met with in soils, especially perhaps in badly aerated acid soils, in which the colloids are not aggregated as in the presence of calcium bicarbonate, iron may be readily available at *pH* 6, and even, though in

greatly diminished amount, at *pH* 7 to 8. It accordingly seemed probable that the blue colour of the flower stalks and petals, shown in habitats of the more acid type, was due to the presence of traces of ferrous salts not required in the general metabolism of the leaves. The ferrous salt in excess may then react with the natural anthocyan pigment, which is pink, giving the blue colour which is observed.

This conclusion, as it has since been found by the writer, was previously arrived at by Molisch (1897), whose work is discussed in a following section. On this view the hydrangea flower may be taken as an indicator of the availability of iron, and indirectly of the soil reaction. Such variations of flower colour with habitat do not appear to be common.

An interesting example has been examined by Boresch (1920), who found that the Cyanophycean *Phormidium Retzii* var. *nigro-violacea* has normally an olive-green colour; this, however, becomes violet or yellowish-brown in cultures deficient in iron salts, addition of which restores the usual colour.

Qualitative and Quantitative Tests for Iron in the Hydrangea Flower.

The facts and considerations of the preceding pages rendered it of interest to see whether it was possible by direct tests to demonstrate the presence of a greater amount of iron in blue hydrangea flowers than in pink. For this purpose the delicate hæmatoxylin test of Macallum (1897) was tried. Dried flowers (no fresh were available) were boiled in twice distilled water containing a dilute solution of well-washed hæmatoxylin crystals. Such a solution is a pale yellow, and iron, where present, acts as a mordant for the stain. Pink flowers and others, blue to mauve, all from the same plant, were used. Control tests without hæmatoxylin showed that the blue flowers always appeared slightly darker than the pink long after their natural colour had been extracted. With hæmatoxylin there was, however, a very definite darkening, amounting in parts to the development of the typical blue purple of iron hæmatoxylin, but in the blue or bluish flowers only. No trace of the blue purple was ever seen in the pink flowers, though some possibly showed an almost imperceptible darkening. Since this test is only given by iron in inorganic, or in at least ionisable condition, the absence of such iron in the pink hydrangea and its presence in the blue may be taken as demonstrated. It seemed that quantitative results were desirable, so flowers, including the blue or pink portions of the stalks, were dried and incinerated. The almost white ash was then dissolved in hydrochloric acid, and after oxidation with nitric acid was treated as usual for the precipitation of ferric hydroxide. None could be seen, however, so the white precipitates were redissolved, made up to the same concentration with respect to ash, and the traces of iron estimated colorimetrically with ammonium thiocyanate. Standard N/10,000 ferric iron was diluted, and it was seen then that the solution from the blue hydrangea ash lay near a 0·5 dilution, viz., 2·8 milligrams of iron per litre, whereas that from the pink corresponded to 0·3 dilution, or 1·7 milligrams. Since the volume of the blue hydrangea solution was approximately one-eighth of a litre, the iron in the ash was only about 0·35 mgrm., an amount too small for gravimetric analysis. The ash of the blue hydrangea flowers was found to be 5·9 per cent. of the material taken after drying at 95° and over sulphuric acid. It contained 0·24 per cent. of iron. The pink hydrangea gave 4·3 per cent. of ash, of which iron amounted only to three-fifths as much as in the blue, namely, 0·14 per cent. It seems probable that both types of flower have a certain amount of iron in the form of complex organic substances, but in the blue only is there an excess available to combine with the natural sap-soluble pigment.

Calculated on the dry weights of the flowers used, the foregoing analyses give approximately 140 parts of iron per million for the blue flowers and 60 p.p.m. for the pink, when the difference in ash-content is taken into consideration. The analyses of Maquenne and Cerighelli (1921) show that the iron in plant tissues varies from about 20 to 150, rising exceptionally to much higher values, such as 362 p.p.m.; thus the quantities found for hydrangea flowers are quite normal. These workers draw attention to the fact that iron accumulates in tissues such as bark, leaves, etc., as they grow old. Such a difference, however, can hardly be held to explain the varying iron-content of two flowers in full bloom. Moreover, were this the cause, flowers which open pink should later on become blue, but such a change does not occur—the pink remain pink and the blue remain blue, though the intensity of colour may change somewhat. Furthermore, the evidence of the hæmatoxylin test is against it, as young blue flowers often give the reaction intensely.

Correlation of Results with those of Previous Workers.

It had long been recognised that certain soils possessed the property of producing a blue colour in the hydrangea; and Charles Darwin recorded that alum influenced the flower colour.

Molisch grew no less than four hundred hydrangeas in various soil mixtures with or without added salts and metallic iron and iron oxides. Since the flowers were normally all red, it is evident that his standard earth must have been alkaline. The addition of iron and iron oxides failed to induce any alteration in colour. This is in keeping with the results for alkaline soils. In peaty soils the plants grew excellently, and produced blue flowers. Various metallic salts were tried, and either had no effect or were poisonous to the plant. Ferrous sulphate, however, led to the production of blue flowers, as did also alum and aluminium sulphate. The action of these salts is to increase the acidity of the soil, for quite dilute ferrous sulphate solution may be as acid as *pH* 4·8. Pure aluminium sulphate in dilute solution gives an acidity of about *pH* 4, the value for alum being almost identical, as previously mentioned.

Since iron in some form is always present in the soil, an increase in soil-acidity renders more iron available for the plant, so the addition of alum or aluminium sulphate increases both the soluble iron and aluminium salts. To which of these, then, is due the production of the blue flowers? As already mentioned, ferric salts are precipitated as hydroxide between *pH* 3·5 and *pH* 6·0, ferrous from about *pH* 5·1 onwards to about *pH* 8; aluminium, as hydroxide, begins to be precipitated at *pH* 3·9 to 4·2, a precipitate is still obtained at *pH* 5·4, but on filtering the solution the filtrate at *pH* 6·4 fails to give any further precipitate or trace of turbidity when rendered less acid. The quantity of aluminium salts in solution at *pH* 6 to 6·4 must therefore be very minute, though hydrangeas are normally blue at *pH* 6, tending towards mixed colours at *pH* 6·4. These considerations, taken in conjunction with the qualitative and quantitative tests for iron in the flower, render it probable that iron rather than aluminium is the metal that reacts with the pink anthocyanin to give the blue in the growing plant. An experiment performed by Molisch certainly does not leave this conclusion entirely free from doubt. Having failed, as did also the writer, to obtain an extract of the pigment, which quickly becomes decolorized, Molisch found that longitudinal sections of the flower stalks gave a blue colour when treated with alum, aluminium, sulphate, or ferrous sulphate.

Since indicator changes in the pigment have been ruled out, the following possibilities present themselves as interpretations. Firstly, that under the

conditions of the experiment iron and aluminium form blue salts with the red anthocyanin. Secondly, that the action of the aluminium is in part responsible for the blue colour in nature, though iron also plays a large, or possibly the main, part. Thirdly, that the action of the acidity of the alum and aluminium sulphate was to liberate iron already present either as an insoluble deposit (which in some plants has been shown by Gile and Carrero (1916, 1) to exist) or from organic combination.

Variation in colour due to the addition of salts to a number of anthocyanin pigments has been studied by Shibata, Shibata and Kasiwagi (1919). The extracts examined by them do not include that of the hydrangea, yet they state that "the colour change of hydrangea and other flowers caused by iron salts and alum . . . is nothing but the complex formation, as we see with the extracts containing anthocyanins."

Culture experiments carried out by Kraemer (1906, 1909) with *Hydrangea otaksa,* which normally has red flowers, showed that plants grown with alum gave blue flowers, as did also those with aluminium sulphate and calcium hydroxide. In the latter case the excess of the aluminium was probably the active portion of the mixture.

Kraemer further discovered that the addition of potassium carbonate to plants grown in sand resulted in the production of blue flowers. It appears, therefore, that the availability of iron may somehow be increased by a markedly alkaline reaction after passing through a minimum value. It is desirable that such experiments should be repeated, and the *pH* value of the soil examined at intervals during the growth of the plants. The possibility of an increase of the absorption of iron in the region of marked alkalinity is in keeping with the results obtained by Arrhenius (1922) for the intake of salts in general. Working with wheat and radish in well-aerated water cultures, he found that, at maximal growth, the intake of the salts is at a minimum.

Chlorosis and Availability of Iron Salts.

The relation between chlorosis and a deficiency of iron was established as long ago as 1843 by the work of Eusèbe Gris. The disease became of considerable economic importance in France through its manifestation in American vines, and numerous researches showed that treatment with ferrous sulphate sprayed on the leaves was beneficial. The subject has been reviewed at length by Roux (1900). Roux's own experimental cultures are of great interest, and a series of photographs illustrates the growth of nine species in soils containing from 0 to 25 per cent. of calcium carbonate.

More recently Tansley (1917) has demonstrated how in calcareous soils *Galium saxatile* becomes chlorotic, and either dies or cannot withstand the competition of *G. sylvestre,* when the two are grown in absence of other plants.

Chlorosis has received attention also from Gile and Carrero (1916), who conclude that iron is not easily translocated from old leaves where it has accumulated. They (1916, 1920), moreover, made an elaborate study of the chlorosis of the rice plant, and showed that the ash of chlorotic rice plants was low in iron; the condition was met with in calcareous soils with a normal amount of water, though not when the same soils were submerged. They suggested that special roots better fitted to absorb iron were developed. An explanation in keeping with present knowledge of the *pH* values at which ferrous and ferric salts are precipitated is as follows. The water standing over the rice tends to lessen soil aeration, to intensify the reducing action of the soil, and possibly slightly to increase its hydrogen ion concentration in

the immediate neighbourhood of the roots, through retention of carbon dioxide in solution; as a consequence, ferrous salts become more available, and chlorosis disappears. It appears, therefore, that chlorosis in certain plants and the development of hydrangeas with pink flowers are closely related phenomena, since both depend on the same factor, the low availability of ferrous salts in the soil. Limestone soils may have *pH* values from 8·2 downwards to *pH* 7·6, or even less. In these the solubility of ferrous salts are much reduced compared with soils at *pH* 6 to 7, and so chlorosis may develop. The work of van Alstine (1920) and of Jones and Shive (1921) is of interest in this connexion, and experiments showed that ferric phosphate gave rise to chlorosis at *pH* 4·1, though satisfactory growth could be made with ferrous sulphate up to much higher *pH* values. Further work in this line has been carried out by Arndt (1922).

Heretofore the *pH* values for precipitation of hydroxides have been considered; but in culture solutions, and even in the soil, phosphates may have to be considered in this connexion. It was mentioned previously that M'Call and Haag (1921) found their nutrient solutions free from all traces of ferric iron below *pH* 3·14, yet Patten and Mains (1920) give *pH* 3·5 to 6·0 as the limits for precipitation of ferric iron. The explanation lies in the presence of phosphate in quantity sufficient to precipitate the iron completely. Aluminium, too, has a highly insoluble phosphate, for, according to V. D. Elst (1922), it is precipitated at *pH* 2·6 to 3·7, the range for the hydroxide being given as *pH* 3 to 5. In the writer's own experience this hydroxide is not completely precipitated till beyond *pH* 5·4. Conner (1921), too, states that at *pH* 3·9 aluminium is more completely precipitated as phosphate than it is at *pH* 6·0 as hydroxide. Beyond *pH* 6·4 the writer could not obtain any further precipitate of aluminium hydroxide.

The work of Lipman (1921) on the relation of the soil to chlorosis in citrus trees appears to indicate that the lack of available iron may not always be the cause of chlorosis.

Effects of Excess of Soluble Iron Salts in the Soil.

There are a number of records of an excess of iron salts being the cause of injury to growing plants, and the subject has been considered experimentally by workers mentioned previously. An effect on the soil itself remains to be considered. Swedish chemists have studied the processes taking place under acid humus soils. The rain-water percolates, and, according to Arrhenius (1922), becomes acidified and dissolves iron compounds, so that in time a bleached earth is found below the humus. The water, as it penetrates, reaches a less acid region, where its iron and aluminium salts are deposited, giving rise to an "ortsten" layer. Since peat gives to aqueous extracts acidity equivalent to *pH* 4·6, and the subsoil is far less acid and acts as a buffer, the reason for precipitation is clear. Further, under the reducing conditions of the humus, most of the iron is probably in the ferrous condition. Frosterus (1914), in his study of the soils of Finland, gives illustrations in colour of these changes, as well as analyses of the different layers. The contrasts are very striking. In a schematic presentation of his results Frosterus distinguishes between the red iron "podsol" and the brownish humus "podsol." The red earth is low in humus, rich in iron and bases, but poor in aluminium. The brown is richer in humus, containing 3 to 11 per cent., considerably more acid, richer also in aluminium. Now, since ferric salts are precipitated as hydroxides from about *pH* 3·5 to 6·0, and aluminium salts from *pH* 3·9 to somewhere above

pH 5·4 and below *pH* 6·4, one would expect that they would be washed out in much the same manner. Arrhenius, indeed, in describing the process, classes iron and aluminium together. It is interesting, therefore, to find that, in the two types of podsol analysed for Frosterus, the iron and aluminium have to a certain extent been separated. The explanation appears to be that the iron present as a ferrous salt is not precipitated at all till over *pH* 5, and appreciable amounts are still in solution at *pH* 7. It is, therefore, carried further into the soil before precipitation is complete.

The explanation of iron podsol formation advanced by Arrhenius may be employed to explain the formation of iron pan in this country. The conditions under which it is formed are described by Hall (1910). At about the level to which the soil is ordinarily aerated, a layer of hydrated ferric oxide accumulates in acid clay and sandy soils, which are apt to be water-logged. The conditions prevailing in certain land in the Ballyhoura Mountains, Co. Cork, may be cited as an example. For these details the writer is indebted to Mr. A. C. Forbes: "The soil consists chiefly of glacial drift on Old Red Sandstone. A thin covering of peat has been more or less removed from this soil for fuel purposes, leaving the surface almost entirely bare of vegetation. A few inches below the surface the soil becomes very compact, water resting on it throughout the greater part of the winter, but becoming very dry in summer. Iron pan is more or less universal. Trees planted on this soil make very little growth for several years, spruce especially showing little power of recovering, while pines, especially Corsican and Maritime, thrive in many places when once established. There is no lack of depth, from six to ten feet of drift lying over the solid rock."

As already mentioned, peat gives a solution at *pH* 4·6 with great constancy. Soil from various places where the peat had been removed and trees planted was found to be at *pH* 5·05, 5·25, 5·4, and 5·6. Here undoubtedly are the conditions for solution of iron salts, which begin to be deposited as they percolate slowly into the glacial drift. The ferric salts are the first to come down, and, moreover, once *pH* 5 is surpassed, the ferrous begin to be deposited. Where sufficiently near the air, they may in time become oxidised to the ferric condition. Indeed, according to McBain (1901), it is the minute amount of hydrolysed ferrous salt which is, in all ferrous solutions, the most readily oxidised constituent. Again, the strong buffer action of the clay ensures that the acid water quickly loses its acidity as it percolates; accordingly the precipitation takes place over a relatively narrow vertical range. There is, however, a secondary result of precipitation through oxidation of ferrous salt to ferric, namely, the regeneration of the acid previously combined with the precipitated iron. This regeneration has been shown (Atkins, 1922, 2) to occur, as ferrous sulphate solutions originally at *pH* 4·8 become on standing as acid as *pH* 2·6, a heavy deposit of ferric hydroxide meanwhile appearing. More ferrous iron may thus be brought into solution in the soil, and in time a small amount of acid may result in the solution and—through oxidation—of the redeposition of much iron. There is thus an additional reason why the region of deposition should be in the zone accessible to oxidation, where in fact processes of oxidation and reduction are both in progress.

SUMMARY.

1. The common garden hydrangea, *H. hortensis*, produces blue flowers when grown in soil at *pH* 5·7 to 6 or slightly over. In less acid habitats some flowers may be pink and others blue on the same plant, but above about *pH* 7·5 pink flowers only are the rule.

2. Ferrous salts remain in solution after ferric salts have been precipitated or rendered completely insoluble as the hydroxide. The precipitation of ferrous hydroxide does not begin until about *pH* 5·1, and even at *pH* 7·1 an appreciable amount remains unprecipitated. Since plants grow at *pH* values beyond the limits for the complete precipitation of ferric salts, they must, under these conditions, utilize ferrous salts only.

3. The difference between the pink and blue flowers of hydrangea is not due to acidity, since both kinds from the same plant were found to be at precisely the same hydrogen ion concentration, *pH* 4·2.

4. The use of Macallum's hæmatoxylin test shows that blue hydrangea flowers give the typical purple and brown reactions for "inorganic" iron, whereas pink flowers have only minute traces of "inorganic" iron.

5. Colorimetric estimations with ammonium thiocyanate show that the ash of the pink flowers contains only 0·6 as much iron as that of the blue. Calculating on the dry weight of the flowers themselves, the blue contain about 140 parts per million of iron and the pink about 60 p.p.m.

6. Dilute solutions of alum and of aluminium sulphate give a reaction of about *pH* 4, ranging up to about *pH* 3·6 for more concentrated ones. These substances therefore are convenient reagents for increasing soil acidity without risk of attaining injuriously high hydrogen ion concentrations by accident. To the increase in acidity, and consequent liberation of iron, is due the blue colour of hydrangeas found by Molisch to result from this treatment. It is possible, however, that the aluminium, as well as the iron, may form a blue complex with the anthocyanin, which is pink in absence of excess of these salts.

7. The precipitation limits for ferrous and ferric salts given in section 2 of this summary throw light upon the availability of iron in the soil and on the occurrence of chlorosis in certain plants when grown on alkaline soils.

8. The formation of iron pan may also be considered from this view-point, and the lessened solubility of iron salts when converted into the ferric condition is of importance in explaining the formation of pan where an acid soil solution percolates into a less acid region which is still sufficiently near the surface for oxidation of the precipitated hydroxide to proceed; ferric salts, if present, would be precipitated slightly before the ferrous, as would also those of aluminium.

The author wishes to record his indebtedness to Mrs. E. W. Sexton for drawing his attention to the problem of the flower colour of the hydrangea; also to those friends who so kindly provided him with soil samples. The reagents necessary for the hydrogen ion determinations were supplied out of a grant from the Department of Scientific and Industrial Research; and thanks are due to the Director of the Marine Biological Laboratory, Plymouth, for general laboratory facilities.

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No. 24.

THE COMPARATIVE VALUES OF PROTEIN, FAT, AND CARBOHYDRATE FOR THE PRODUCTION OF MILK FAT.

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While the composition of milk yielded by an animal depends primarily on the nature of the inherited milk producing factors, it must be conceded that certain constituents of milk are subject to alteration by feeding. Jordan, Hart, and Patten (1906) found that on a low phosphorus ration the percentage of fat in cows' milk was noticeably reduced, and *vice versa*. The author (1922) showed that the percentage of fat in the milk of goats fed on an insufficient ration was increased by adding protein, fat, or carbohydrate to the ration, and that the reverse change took place in the milk fat when the ration was considerably reduced. Eckles and Palmer (1916) were unable to affect the composition of the milk of cows by feeding superabundant rations, but, by feeding rations insufficient to supply the cow's requirements for maintenance and milk yield, they did effect a change in the percentage of fat and protein. Evidently the percentage of fat in milk is influenced to an extent by feeding, and there is some evidence that the percentage of nitrogen is subject to variation by feeding also. Our knowledge of the physiology of milk fat production is not yet complete, but there is fairly conclusive evidence from the experiments of Meigs, Blatherwick, and Cary (1919), and the author (1921), that the precursor of milk fat in the organism is some soluble phospholipoid, in which form fat is absorbed from the blood by the mammary gland cells, to be synthesised into the glyceride esters of fatty acids found in milk. Body fat may be produced from fat in the food—Lebedev (1882), and from carbohydrates—Lawes and Gilbert (1852); and there is strong evidence in favour of its formation from protein—Lusk (1909): the direct formation of fat from protein with the omission of the carbohydrate stage is suggested by the recent work of Atkinson, Rapport, and Lusk (1922). Milk fat, likewise, is derived from more than one source. That ingested fat is converted, at least partly, into milk fat in a lactating animal is shown by the fact that the nature of the milk fat produced, after the consumption of a particular fat, is modified to resemble the latter—Rosenfeld (1902-03). The experiments of Jordan (1897 and 1901) and his collaborators showed that the carbohydrate of the food may be converted into milk fat. In a recent paper (1922) and in the present one the author produces evidence to show that the protein of the food may be utilized for the same purpose.

In this paper data are presented which enable the comparative values of protein, fat, and carbohydrate, as sources of milk fat, to be determined. It is obvious that sufficient protein must be supplied in the diet of a lactating animal to provide for the nitrogen requirements of the body, and also to supply material for the milk protein, if nitrogenous equilibrium is to be maintained. Surplus proteins are de-aminised; the nitrogen is excreted in the form of urea, and the remainder of the molecule converted into carbohydrate. The comparative feeding value of protein, fat, and carbohydrate, for milk fat production, was

therefore tested on animals already receiving at least the minimum protein requirements referred to. Three lactating goats were used, and the tests continued from the beginning of May, 1922, to the middle of December, 1922. The yield of milk, the percentage of milk fat, and the total milk fat from each goat were determined daily, and the animal's weight was recorded weekly. Curves were drawn of the total milk yield, percentage of fat in the milk, total fat in the milk, and weight of animal; the figures 1, 2, and 3 represent the result from the individual goats, and show the daily ration supplied to them. By examining the milk and fat curves in conjunction with the curve of body weight for successive periods during which comparative foods were fed, it is possible to observe the effect produced on the milk fat by the comparative diets. It is known that the proportion of sugar in milk is nearly constant, and that the changes in the percentage of protein are small and irregular—Paton and Cathcart (1911), and Eckles and Palmer (1916): consequently the consideration of the sugar and nitrogen content of the milk can be reasonably neglected. Each animal was separately fed and housed. A basal daily ration of hay 1 lb., mangels 10 lbs., crushed oats ½ lb., and white fish meal ½ lb. was given. From previous experience with the same goats, this ration was deemed barely sufficient to maintain weight and give a moderate flow of milk; and it was calculated that the ration contained sufficient protein to supply the animal's protein requirements and also to provide for the protein secreted in the milk. In a previous paper—Sheehy (1922)—the author showed the effect on the milk fat of increasing this ration, so that particular test was excluded in this case, and the basal ration was supplemented from the beginning by fat, carbohydrate, or protein. The fat used was hydrogenated soya bean oil, the carbohydrate was maize starch, and the protein was casein. As the milking period advanced, the fat, starch, and casein were interchanged and the effects produced on the fat yields noted.

The total yield of milk in all three cases gradually increased after parturition; it maintained a high level for some time, and declined towards the end of the lactation period. Fortunately the individual milk yield remained constant for considerable periods, and by effecting changes in the food during these periods of constant milk yield it was possible to show very definite effects on the milk fat from the change in food. Unfortunately on other occasions changes in the diet synchronised with variations in the milk yield; but, since the effect on the total fat of the variation in the milk yield could be easily calculated, the part played by the food was, even in these cases, determined. It will be noticed that in fig. 2 there is a considerable fall in yield at the beginning of period C; this is due to illness from which the animal suffered during periods C and D. In comparing period A with B in figs. 2 and 3, reference must be made to fig. 1, which serves as a control in this connexion. Fig. 1 shows the natural inclination for the yields of milk and butter fat, and it will be noticed that, while the graphs in fig. 3 (periods A and B) follow a similar course to those of fig. 1, the percentage and total milk fat curves in fig. 2 are raised in period B. In the case of each animal the daily ration was reduced to the basal level during the last period of the experiment. This change caused a considerable drop in both the percentage of, and the total milk fat, which shows that the previous increase was not the consequence of advance in lactation period, but rather of change of diet. All three goats increased rapidly in weight for the first three or four weeks, after which the increase in weight was very slight but fairly regular right up to the end of the lactation period. The small irregularities in the weight curve are principally due to slight variations in health, caused by irregularity of the action of the bowels to which goats continuously confined to the house are subject.

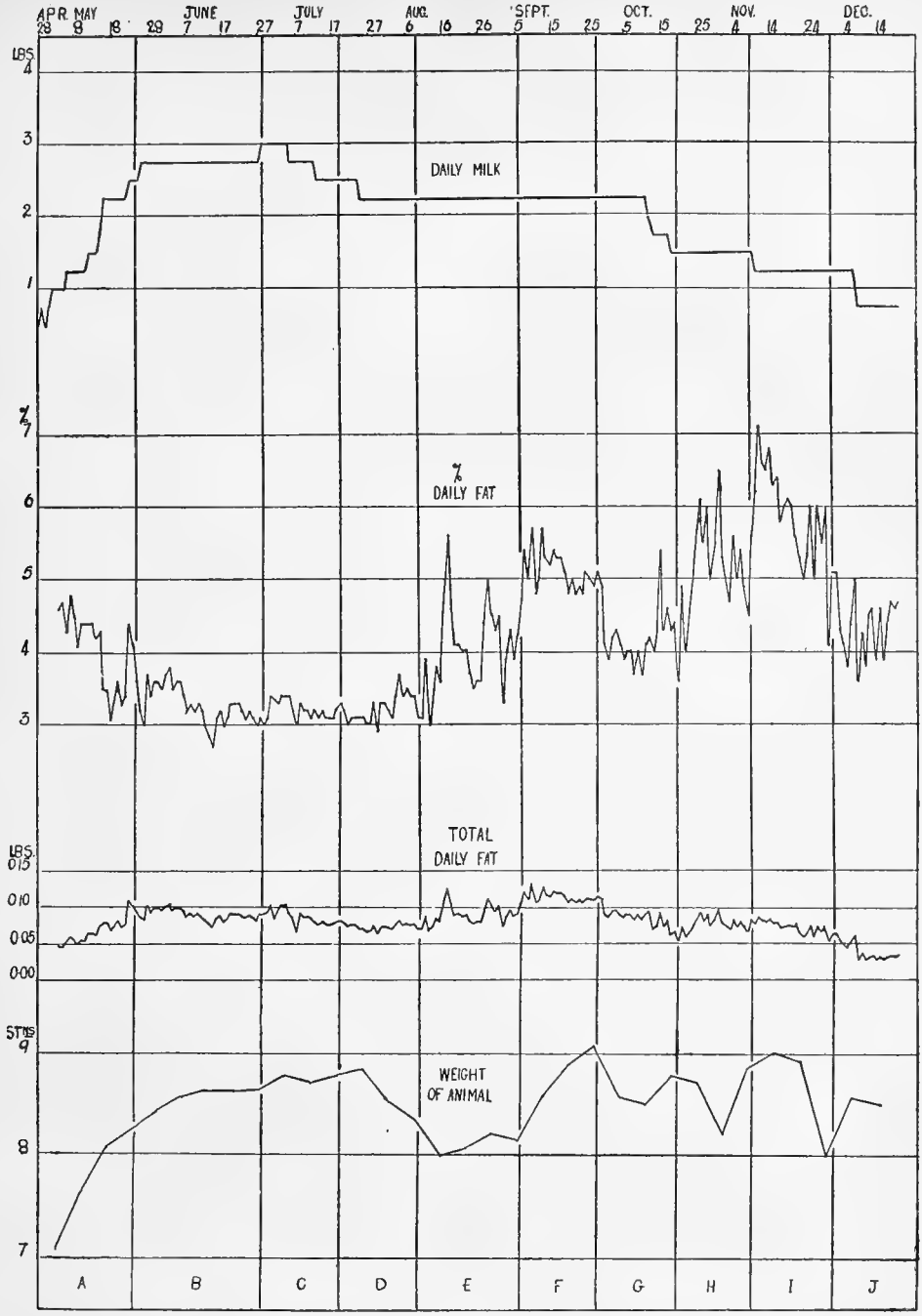

FIG. 1.—GOAT 1.

DAILY RATION.

A. Starch, $1\frac{1}{4}$ lbs.
B. Starch, $1\frac{1}{4}$ lbs.
C. Casein, $1\frac{1}{4}$ lbs.
D. Starch, $1\frac{1}{4}$ lbs.
E. Starch, $1\frac{3}{4}$ lbs.
F. Fat, $\frac{1}{2}$ lb.
G. Starch, $2\frac{1}{4}$ lbs.
H. Starch, $1\frac{1}{4}$ lbs.; fat, $\frac{2}{5}$ lb.
I. Fat, 1 lb.
J. ————

In addition to the above, a constant basal ration (see p. 212) was fed throughout the entire experimental period.

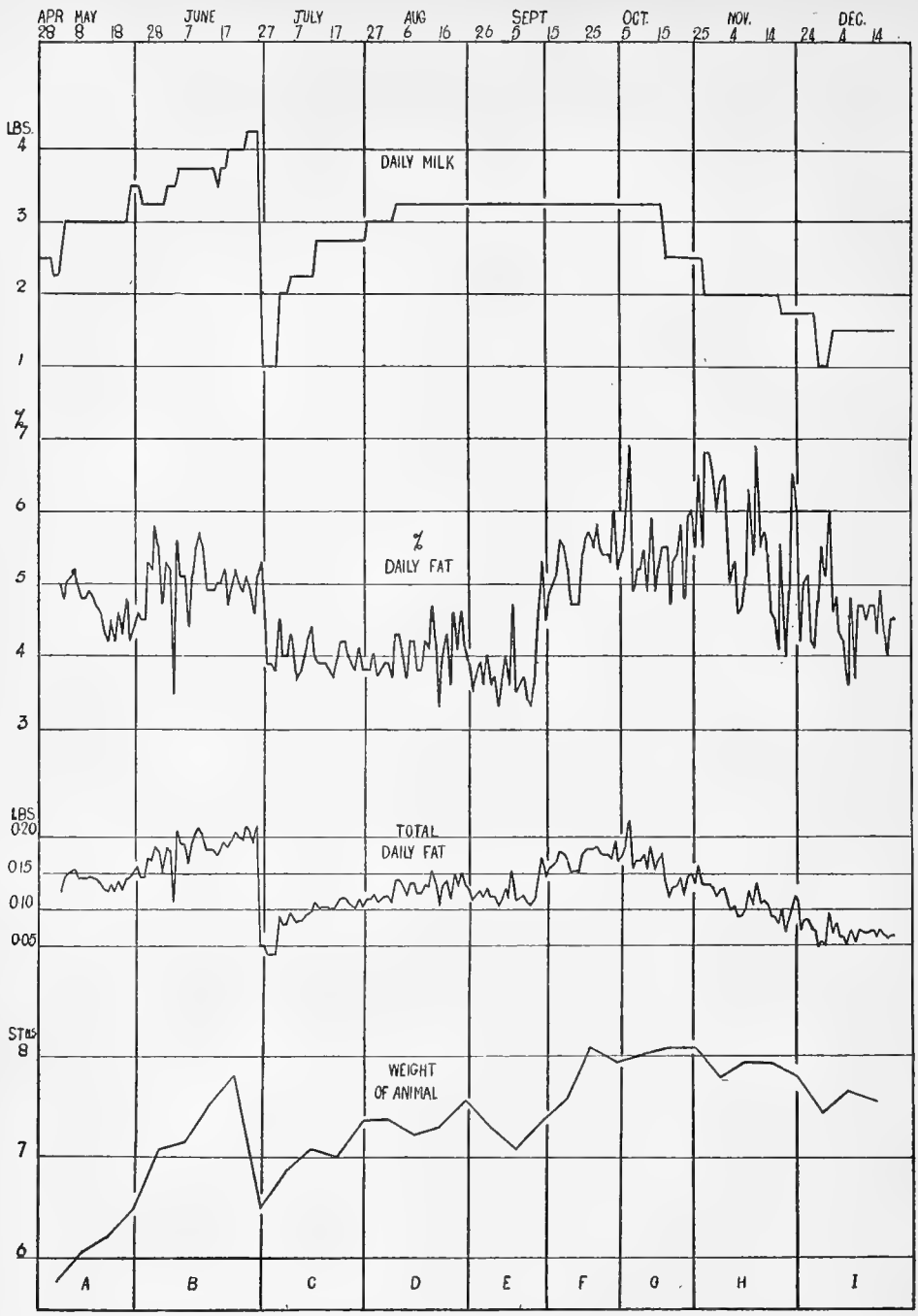

FIG 2.—GOAT 2.

DAILY RATION.

A. Starch, $1\frac{1}{4}$ lbs.
B. Fat, $\frac{1}{2}$ lb.
C. Starch, $1\frac{1}{4}$ lbs.
D. Fat, $\frac{1}{2}$ lb.
E. Starch, $1\frac{1}{2}$ lbs.
F. Fat, $\frac{1}{2}$ lb.
G. Fat, $\frac{1}{4}$ lb.
H. Starch, $1\frac{1}{2}$ lbs.; Fat, $\frac{1}{16}$ lb.
I. ————

In addition to the above, a constant basal ration (see p. 212) was fed throughout the entire experimental period.

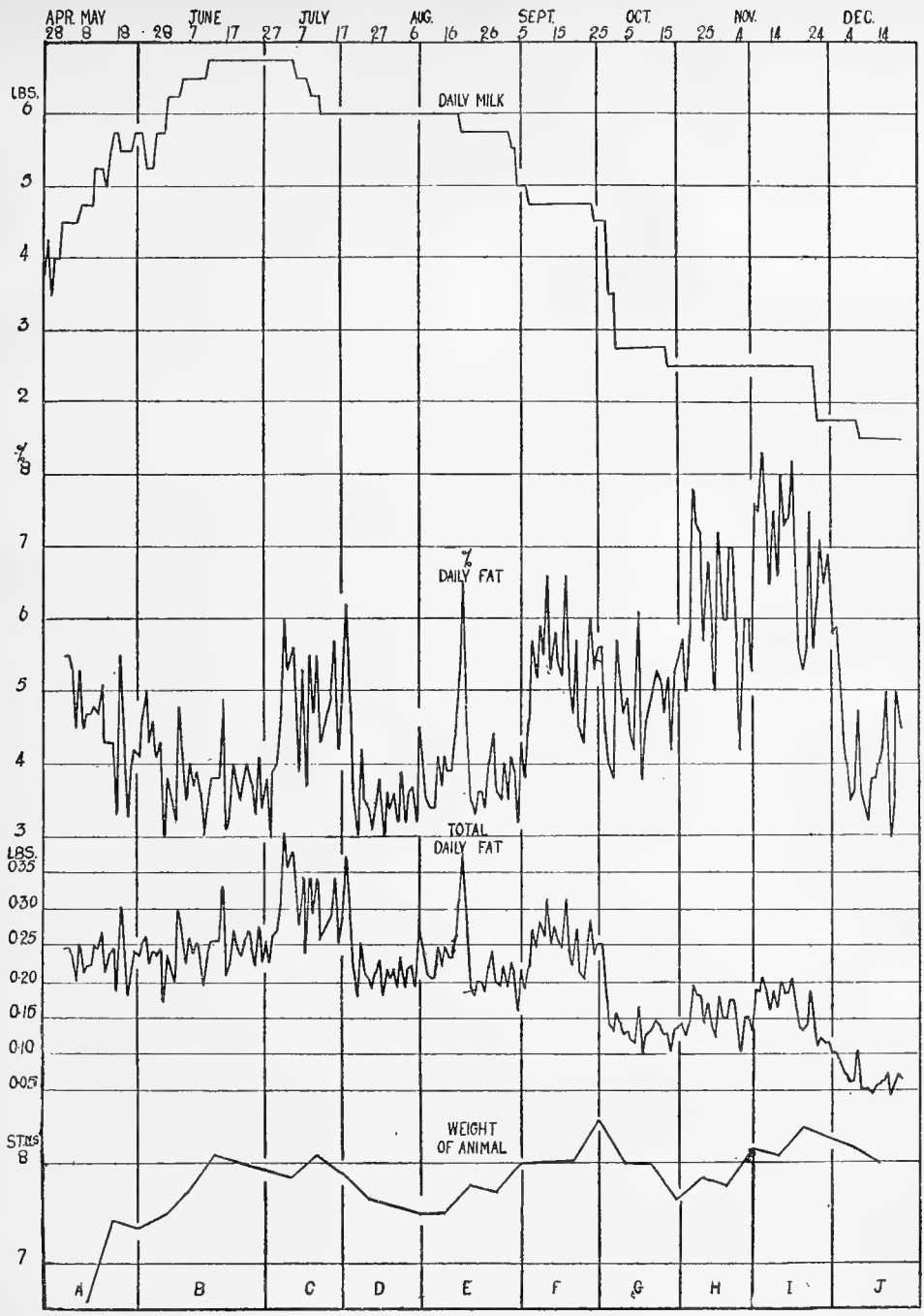

FIG. 3.—GOAT 3.

DAILY RATION.

A. Starch, $1\frac{1}{4}$ lbs.
B. Casein, $1\frac{1}{4}$ lbs.
C. Fat, $\frac{1}{2}$ lb.
D. Starch, $1\frac{1}{4}$ lbs.
E. Starch, $1\frac{3}{4}$ lbs.
F. Fat, $\frac{1}{2}$ lb.
G. Starch, $2\frac{1}{4}$ lbs.
H. Starch, $1\frac{3}{4}$ lbs.; fat, $\frac{1}{5}$ lb.
I. Fat, 1 lb.
J. ————

In addition to the above, a constant basal ration (see p. 212) was fed throughout the entire experimental period.

Discussion of the Results.

It is clear that the percentage of, and total milk fat can be increased, within limits, by increasing the ration. Thus an increase in the starch diet in goat 1 (periods D and E) from $1\frac{1}{4}$ to $1\frac{3}{4}$ lbs. produced considerable increases in milk fat. There is a limit to the capability of the animal in this direction, and a maximum milk fat yield is reached above which it is impossible to go. In goat 1 the feeding of $2\frac{1}{4}$ lbs. of starch gave no better yield than the feeding of $1\frac{3}{4}$ lbs. (periods E and G). It is evident that the feeding of a ration constituted like that given in period G, no matter in what quantity it is consumed, does not enable the lactating animal to give the maximum return in milk fat. There is some necessary constituent supplied in sub-minimal quantity, and we shall see later that that material is fat. The results from goat 1 (periods B, C, and D) and from goat 3 (periods A and B, and periods B and D) show that for the production of milk fat, starch and protein, i.e. protein in excess of minimum requirements, replace one another in equal quantities. The comparative value is not so clear in the case of fat and starch. In goat 1 (periods E and F) the substitution of fat for three and a half times its weight of starch caused an increase in milk fat yield, and in the same goat (periods F and G) the replacement of fat by four and a half times its weight of starch caused a decrease in milk fat yield. Similarly in goat 2 (periods A and B) fat in the ration gave a better yield of milk fat than two and a half times its weight of starch, and in the same goat (periods E and F) fat gave a better result than three times its weight of starch. Similar conclusions can be drawn from goat 3. $1\frac{1}{4}$ lbs. of starch are inferior to $\frac{1}{2}$ lb. of fat (periods C and D). The same relation seems to hold between fat and protein, $\frac{1}{2}$ lb. of fat being considerably superior to $1\frac{1}{4}$ lbs. of casein for milk fat production—fig. 3 (periods B and C). When, however, we consider the substitution of carbohydrate by fat, in rations already containing a fair proportion of fat, we get quite different results. From the results from goat 1 (periods H and I) it is seen that 1 lb. of fat is very slightly superior for milk fat production to $1\frac{1}{4}$ lbs. of starch, plus $\frac{2}{5}$ lb. of fat—that is, $1\frac{1}{4}$ lbs. of starch is almost equal for that purpose to $\frac{3}{5}$ lb. of fat, or $2\frac{1}{12}$ lbs. of starch to 1 lb. of fat. In the same way it is determined from goat 3 (periods H and I) that $1\frac{3}{4}$ lbs. of starch almost serves the same purpose as $\frac{4}{5}$ lb. of fat, or $2\frac{3}{16}$ lbs. of starch to 1 lb. of fat. A very slight increase in the starch figure would render the results similar, so that it is justifiable to nominate the figure $2\frac{1}{4}$ for starch as being equivalent to 1 of fat. Since starch and protein replace one another in equal parts, it might be reasonably concluded that fat and protein replace one another in the proportion of 1 to $2\frac{1}{4}$ also. Though fat in the ration is necessary to induce the maximum activity of the mammary gland for milk fat secretion, a small proportion of fat is as efficacious in this direction as a large quantity. Thus in goat 1 (periods G and H) $\frac{2}{5}$ lb. of fat, and in goat 3 (periods G and H) $\frac{1}{5}$ lb. of fat per day, in addition to the fat contained in the basal ration, was sufficient to yield maximum results. Apparently less than that quantity would suffice for the purpose of inducing maximum yield of milk fat, for in goat 2 (periods E and H) we see that the addition of even $\frac{1}{16}$ lb. of fat per day increased the percentage of milk fat to a level higher than was reached by that animal at any other period of her lactation; but whether a higher level could be attained by feeding more fat, say 1 lb. per day, as in goats 1 and 3 (period I), was not determined. The foregoing results establish a definite relationship between proteins and carbohydrates for milk fat production. It is shown in the case of fat that a certain quantity must be

present in the ration in order that the maximum milk fat percentage may be reached: beyond that level fat and carbohydrate, and presumably fat and protein, replace one another in definite proportions. In connexion with the special role of fat in the food of a lactating animal, the foregoing results are in exact agreement with the findings of Morgen, Beger, and Fingerling (1904-05). Numerous conflicting statements as to the effect of food fat on the yield of milk fat are found in the literature. Morgan and his collaborators, working with goats and sheep, showed (*a*) that, when the fat in the ration of a milking animal amounts to less than 0·5 gram daily per kilogram of animal weight, an addition of fat to the ration, as a substitute for carbohydrate, has a marked effect in increasing the percentage of fat in the milk as well as the total milk yield; (*b*) that the milk yield and fat content of the milk continue to increase with the addition of fat to the ration up to one gram per kilogram of animal weight; (*c*) that further substitution of fat for carbohydrate has no effect except in unusual cases. The role of fat in the ration of a lactating animal having been definitely established, there remains to be worked out the practical problem of the extent to which fats should be economically fed to animals like the cow, whose mammary glands are regarded as machines, to be worked with maximum efficiency for the purpose of milk fat production. It is generally agreed, and the idea is borne out by the results of Jordan and his co-workers (1901), that the addition of large quantities of fat to the diet of the milking cow does not produce a permanent increase in the yield of milk fat. Nevertheless the cow, in all probability, requires, like the goat, a certain minimum of fat in the food to enable her milk fat producing organs to work with maximum efficiency.

Conclusions.

1. The milk fat yield of lactating animals can be increased, within limits, by feeding.

2. Protein in the food may replace carbohydrate for the production of milk fat: the protein, casein, when supplied in quantity in excess of the minimum requirements for body metabolism and milk yield, replaced starch in equal quantity for that purpose.

3. Fat may replace starch or protein, i.e. protein in excess of the minimum requirements of a lactating animal, in the proportion of 1 to 2¼: this proportion holds only for rations already containing a certain quantity of fat.

4. In a diet containing less than a certain quantity of fat a replacement of some of the carbohydrate by fat gives results which credit fat with a much higher value for milk fat production than that represented by the above proportion: in this investigation fat has been shown to be at least five times as valuable as carbohydrates when fed under such conditions.

5. If a diet is constituted so as to contain less than a certain quantity of fat, the maximum yield of milk fat will not be returned no matter how liberal the ration is in quantity.

6. Fat in the ration of a lactating animal stimulates the secretion of milk fat: this stimulative action required for maximum milk fat production is induced by a comparatively small quantity of fat; fat fed in excess of the requirements for this purpose has no special value, but simply replaces the carbohydrates in isodynamic proportion.

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No. 25.

THE UTILISATION OF MONOMETHYLANILINE IN THE PRODUCTION OF TETRYL.

By THOMAS JOSEPH NOLAN, D.Sc., F.I.C.,

AND

HENRY W. CLAPHAM,

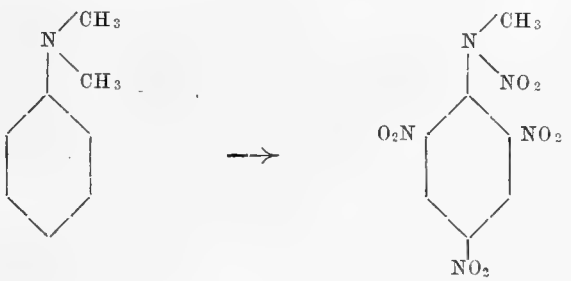
Nobel Research Laboratories, Ardeer.

(COMMUNICATED BY PROF. H. RYAN.)

(Read APRIL 24. Printed JULY 6, 1923.)

THE standard method for the production of tetryl in the explosives industry is based on the nitration of dimethylaniline. In works practice dimethylaniline is dissolved in a relatively large proportion of strong sulphuric acid, and the resulting solution run into strong nitric acid, which is maintained at a temperature usually not less than 50° C., and vigorously agitated; 2 : 4 : 6 trinitro phenyl methyl nitro-amine, the so-called tetryl, separates from the reaction mass as a light yellow crystalline product, which is then removed by filtration and washed free from acid by repeated boiling with water. On drying, the product is in a suitable condition for most purposes, but is very often crystallised before being taken into service.

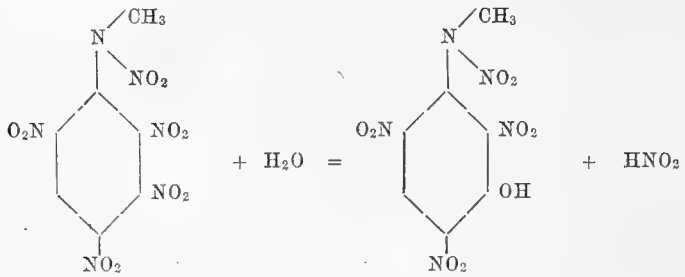
The utilisation of dimethylaniline as a raw material for the production of tetryl has, however, certain disadvantages; the reaction involves the complete oxidation of one of the methyl groups attached to the nitrogen atom, with a consequent loss of nitric acid.



In fact, in practice the usual consumption of nitric acid by this process is not less than seven molecules per molecule of dimethylaniline. The use of monomethylaniline is naturally suggested as a raw material, but its cost of production is at present considerably higher than that of dimethylaniline, which is readily obtained by the interaction of methyl alcohol and aniline at elevated temperatures and under pressure in the presence of sulphuric acid. However, with the development of a relatively cheap process for the production of monomethyl-

aniline, or even of a commercial grade containing a proportion of dimethylaniline, the possible utilisation of this material for the production of tetryl becomes of technological interest.

It has been stated by Knowles (J. Ind. and Eng. Chem., **12**, 1920, p. 246) that in the nitration of dimethylaniline the amount of metanitro tetryl produced is proportional to the methylaniline present in the raw material. While we are unable to agree with this statement, it is sufficient to point out that the mere production of metanitro tetryl gives rise to the possible subsequent development of m-oxy tetryl by hydrolysis,



and it has been established by Farmer (J.C.S., **117**, 1920, p. 1605) and by Hinselwood (J.C.S., **119**, 1921, p. 722) that this oxy-tetryl has a considerable influence in reducing the stability of tetryl.

The experiments recorded in this paper bear out the observation of Knowles (*loc. cit.*), as it is found that monomethylaniline on nitration with nitric acid in the presence of sulphuric acid gives rise to the production of considerable amounts of m-nitro tetryl, as shown by the recovery of oxy-tetryl from the waters used in washing the crude product of nitration of monomethylaniline. In our observations the nitration of monomethylaniline is most unattractive for large-scale operations. Unless agitation during nitration is very vigorous, there results the formation of sticky, toffee-like masses; if, however, very vigorous agitation is employed, the oily product which separates about half way through the nitration is broken up, and the nitration can be completed to give a readily handled granular product.

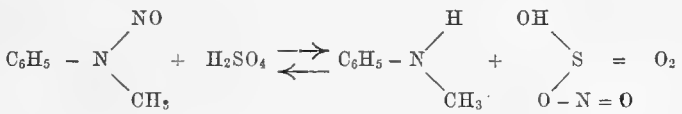
In view of the difference in orienting influence thus shown by the aminic groups in monomethyl and dimethylaniline, it seemed probable that derivatives of monomethylaniline in which the hydrogen of the aminic group was replaced by various radicles might be suitable for the production of tetryl practically free from m-nitro tetryl. In this connection it is apposite to refer to the observation of Ryan and Sweeney (Scientific Proc., R.D.S., vol. xvii, p. 157) that tetryl of a high degree of purity is produced by the action of oxides of nitrogen on *α α* methyl phenyl urea.

From the technological standpoint, the most suitable derivative of monomethylaniline for investigation is the nitrosamine, as this product is readily and simply obtained from pure monomethylaniline or a commercial product containing dimethylaniline; and in the event of a successful nitration, the spent acid from the process would present no unknown features in the subsequent denitration process; while the nitrous acid used in making the nitrosamine would presumably be thus to some extent recoverable in the form of nitric acid.

Our experiments show that methyl phenyl nitrosamine may be nitrated to give tetryl without the simultaneous production of any appreciable quantity of

m-nitro tetryl; the nitration of a sulphuric acid solution of methyl phenyl nitrosamine is distinguished by the ease and smoothness with which the reaction proceeds, there being no difficult temperature control, such as is experienced in the nitration of dimethylaniline. This is presumably in part due to the exothermic nature of the reaction between methylaniline and nitrous acid.

Our results further indicate that a satisfactory product cannot be effected by the nitration of a sulphuric acid solution of methylaniline containing sufficient nitrous acid to form the nitroso compound; in other words, the preliminary isolation of the nitroso compound is necessary. Our results would point to the fact that in sulphuric acid solution there is an equilibrium condition between nitrous acid (or nitrosyl sulphuric acid), methylaniline, and methyl phenyl nitrosamine, the equilibrium being preponderatingly in the latter direction.



Owing to the stable condition being that containing a preponderating amount of methyl phenyl nitrosamine, the latter when dissolved in sulphuric acid is dissociated only very slowly, and consequently can be nitrated to give a good quality of tetryl, provided that reaction is carried out soon after solution of the nitrosamine in sulphuric acid. When, however, the solution is allowed to stand a considerable time, an appreciable amount of dissociation occurs, with the result that the product gives rise on nitration to m-nitro tetryl, which is identified in the form of its derivative oxy-tetryl in the waters obtained on washing the product of nitration. Nitrosyl sulphuric acid acting on methylaniline in sulphuric acid solution, or methyl phenyl nitrosamine dissolved in sulphuric acid, gives rise to some p-nitroso methylaniline, but that this is not the cause of the production of m-nitro tetryl is quite clearly established by our experiments on the nitration of p-nitrosomethylaniline.

In view of the above, it was thought that the most satisfactory method of nitration of the nitrosamine might be to run it into a mixture of nitric acid and sulphuric acid; but under these conditions violent reaction ensued, accompanied by charring at the point of entry of the nitrosamine.

EXPERIMENT 1.—*Nitration of Monomethylaniline (pure).*

Monomethylaniline (30 g.) was run into 97 per cent. sulphuric acid (300 g.), with good mechanical stirring, the temperature being kept below 10° C. 92 per cent. nitric acid (111 g.) was warmed up to 45° C., and the sulphate solution slowly added, the temperature being maintained between 50° and 55° C. by means of external cooling. When about three-fourths of the sulphate solution had been added, nitro body separated as an oil, which, unless very vigorous stirring was employed, either became a plastic material or formed into large lumps. By vigorous agitation the material could, however, be obtained in a semi-granular, semi-crystalline form. After all the sulphate solution had been added, the mixture was maintained for one hour at 50-55° C. The mass was then cooled to 20° C. and filtered. The acid tetryl was added to about 1½ litres of ice cold water; it was then filtered, ground under water, filtered, washed, and finally given three four-hour washings with water heated by injected steam. In the first washing, when the temperature of the water reached 95° C., the whole

mass of tetryl became quite soft, and formed into lumps, but on continuing the washing it began to harden and break up. From the first wash waters 7·7 g. of a yellow solid was obtained on evaporation, m.p. 176-177° C., which, on crystallisation from benzene, gave almost colourless crystals, melting at 180-181° C. (uncorr.) with decomposition, and was identified as trinitro methyl nitro amido phenol (oxy-tetryl). A further 2·3 g. was obtained from the second wash waters, but none from the third.

The washed tetryl, which was very brown in colour, weighed 53 g., being 65·8 per cent. of the theoretical yield. The melting point of the product was 125·5°-126·5° C. (corr.). On crystallising from aqueous acetone the melting point of the product was 126-127° C. (corr.).

EXPERIMENT 2.—*Nitration of Methyl Phenyl Nitrosamine (pure).*

30 g. of the methyl phenyl nitrosamine, b.p. 128-130° C. at 15 mm., was added gradually to 97 per cent. sulphuric acid (300 g.), with cooling and efficient stirring, the temperature being maintained at 0° C. 92 per cent. nitric acid (84 g.) was warmed to 45° C., and the nitrosamine solution slowly added, the temperature being maintained at 50-55° C. by external cooling. It was noticed that the heat of reaction was much lower than had been the case with monomethylaniline—in fact, the easy running of the nitration was a distinct feature of the process. When about half of the sulphate solution had been added, the reacting mass became slightly turbid, and almost immediately hard crystals of tetryl began to separate. After all the sulphate solution had been added, the mixture was maintained at 50-55° C. for a further period of one hour by external heating. The whole was then allowed to cool to 20° C. and filtered. The acid tetryl was treated exactly as in Experiment 1. From the first wash water on evaporation 0·2 g. of a yellow, somewhat tarry solid was obtained, which on crystallisation from aqueous acetone melted at 119-121° C., showing it to be probably a crude tetryl. The second and third washings gave no weighable residue. The washed tetryl was cream in colour, and weighed 55 g., being 87 per cent. of the theoretical yield. The melting point of the product was 128·0-129·0° C. (corr.), which on crystallising from aqueous acetone melted at 129·0-129·5° C.

EXPERIMENT 3.—*Nitration of Crude Methyl Phenyl Nitrosamine.*

In view of the good results obtained with pure methyl phenyl nitrosamine, it was considered advisable to examine the behaviour of the crude product, such as would be obtained directly from the commercial grade methylaniline. A synthetic mixture of 79 per cent. methylaniline and 21 per cent. dimethylaniline was made up, dissolved in the requisite amount of dilute hydrochloric acid, and treated with just sufficient sodium nitrite solution to form the nitrosamine from the secondary base. The yield of crude nitrosamine was 90 per cent. of the theoretical. This product appeared to contain a little p-nitroso compound. The nitration was carried out with the same quantities and under the same conditions as in Experiment 2. The reaction proceeded exactly as in the previous instance. No weighable residue was obtained from any of the wash waters, and the tetryl, which was cream to straw-yellow in colour, weighed 55·3 g., being 87·3 per cent. of the theoretical yield. The melting point of the product was 127·5-128·5° C. (corr.), which on recrystallisation from aqueous acetone melted at 128-129° C.

Consequently the crude nitrosamine is a suitable material for the production of tetryl. This indicates the possibility of using the monomethylaniline in the

commercial article, through its nitrosamine, for the production of tetryl, and recovering dimethylaniline from the acid liquors, after separating off the nitrosamine, by neutralisation and subsequent steam distillation. It would probably, however, be more suitable after the formation of the nitrosamine to neutralise the residual solution of dimethylaniline hydrochloride, and add the recovered dimethylaniline to the nitrosamine for nitration of the mixture.

EXPERIMENT 4.—*Nitration of Methylaniline dissolved in sulphuric acid containing sufficient nitrous acid to form the nitrosamine.*

24 g. of monomethylaniline were dissolved in 120 g. of sulphuric acid 97 per cent., and to this solution, which had been cooled to 0° C., was added a solution of 28·5 g. of nitrosyl sulphuric acid dissolved in 120 g. of sulphuric acid. The mixture was nitrated in the manner described in previous experiments, using 84 g. of 92 per cent. nitric acid. The nitration proceeded quite normally. The nitro body as filtered off from the waste acid was very red in colour as compared with products from previous nitrations. In the first boiling the nitro body darkened considerably, and the colour of the resulting wash water was deep orange. On evaporation, 4·1 g. of crude oxy-tetryl was obtained. After the usual hot water washings, the nitro body was yellowish brown. The yield was 45·0 g., being 70 per cent. of the theoretical, and the product melted at 126-127° C. (corr.). Recrystallisation from aqueous acetone did not affect the melting point.

EXPERIMENT 5.—*Nitration of a sulphuric acid solution of pure methyl phenyl nitrosamine allowed to stand in a closed vessel for 24 hours before nitration.*

The methyl phenyl nitrosamine (30 g.) dissolved in 97 per cent. sulphuric acid (300 g.) was allowed to stand in a stoppered bottle at room temperature for 24 hours. It was then nitrated in the manner already described for the other experiments. It was noted during the nitration that the nitro body did not separate from the acid solution very quickly, and even then it tended to be somewhat sticky, vigorous agitation being necessary to bring it down in a workable condition. The same characteristics were noted with the product as had been observed in Experiment 4. The wash waters gave 4·7 g. of crude oxy-tetryl. The yield of tetryl obtained was 39 g., being 62 per cent. of the theoretical. Melting point, 126·0-127·0 C. (corr.), unchanged by recrystallising from aqueous acetone.

EXPERIMENT 6.—*Nitration of p-nitroso monomethylaniline.*

The pure base, m.p. 114-114·5° C. (20 g.), was dissolved in 97 per cent. sulphuric acid (200 g.), and nitrated as described in previous experiments, with 56 g. of 92 per cent. nitric acid.

The nitration proceeded quite smoothly, and was easily controlled. The nitro body, on filtering off from the waste acid, was deep red in colour; and after the usual hot-water washing was obtained as a brownish coloured product, with a m.p. of 125·5-126·5° C. (corr.). The yield obtained was 36 g., being 85·3 per cent. of the theoretical.

No oxy-tetryl was obtained from any of the wash waters. On recrystallising from aqueous acetone, the melting point was 129·0-129·5° C. (corr.).

We desire to thank Messrs. Nobel Industries, Ltd., for permission to publish these results.

NOBEL RESEARCH LABORATORIES, ARDEER.

No. 26.

EVIDENCE OF DISPLACEMENT OF CARBONIFEROUS STRATA, COUNTY SLIGO.

By ARTHUR E. CLARK, B.A.,
Trinity College, Dublin.

(COMMUNICATED BY MR. L. B. SMYTH.)

(Read April 24. Printed July 6, 1923.)

This paper is the result of a detailed survey of the district represented by Sheet 12, Co. Sligo, 6″ Ordnance Survey. It includes the village of Dromore West, on the western side, and Aughris on the east, including part of the northern coast of Co. Sligo, with a few miles of country inland.

The formation is lower Carboniferous, consisting of anticlinal strata of the Mountain Limestone Series. To the west the beds consist of shales, in some places dark, almost black, and very soft; in other places ochreous, harder, occasionally with harder and softer beds alternating.

To the east of these beds of shales there is an anticline of calcareous sandstones underlying the shales on the west, and alternating with dark micaceous flagstones. The boundary line between the sandstone series and the dark shales to the west is incorrectly marked on the Geological Survey maps. The N.-S. fault passing through Carrickpatrick is represented as passing through the shales—d^{2}′—instead of passing through the highest beds of the sandstone anticline—d^{1}. The true boundary line is marked on the map (fig. 1). A bed of oolite near the junction of shales and sandstone, which, according to the memoir, lies at the base of the shales and overlies the sandstone, really lies between two beds of sandstone.

The sandstone anticline is visible for about two miles; then the beds dip beneath greyish compact limestones, which rest conformably on the sandstone.

In addition to the sedimentary rocks described above, there are a number of greenish microcrystalline dolerite dykes, and the distribution and structure of these dykes form the basis of this research. Some of the dykes of the district are marked on the Geological Survey maps, but their positions are in some cases incorrect.

In this district, on the western side, the dykes are first seen on the Carrownrush shore at Pollbrean. They cross a small bay, and pass through land for a short distance; they then emerge at Oughmore, and proceed out to sea in the direction of Aughris Head, towards a point considerably north of Kilrusheighter (see map).

The Geological Survey mark only two dykes crossing the small bay at Pollbrean, and three going out to sea on the east. They represent the two dykes as converging, and the third as a short line to one side (see fig. 2, A).

At Pollbrean, however, there are really three dykes—the two marked, and a third, of a different type, between them, passing into an inlet in the land. It

is almost impossible to see the third dyke unless the loose boulders covering it are removed. These three dykes are of bluish-green microcrystalline dolerite, but the central dyke contains small white amygdaloids slightly tinged at the edges.

The dykes pass across the little bay and reach the land, and here the structure changes. The northern dyke becomes symmetrically multiple; the central dyke divides, catching up three feet of shale between the parts, and becomes unsymmetrically multiple. The three dykes emerge again at Oughmore, and run out to sea in the direction of Aughris Head. The dykes remain parallel, and their true course is shown in fig. 2, B.

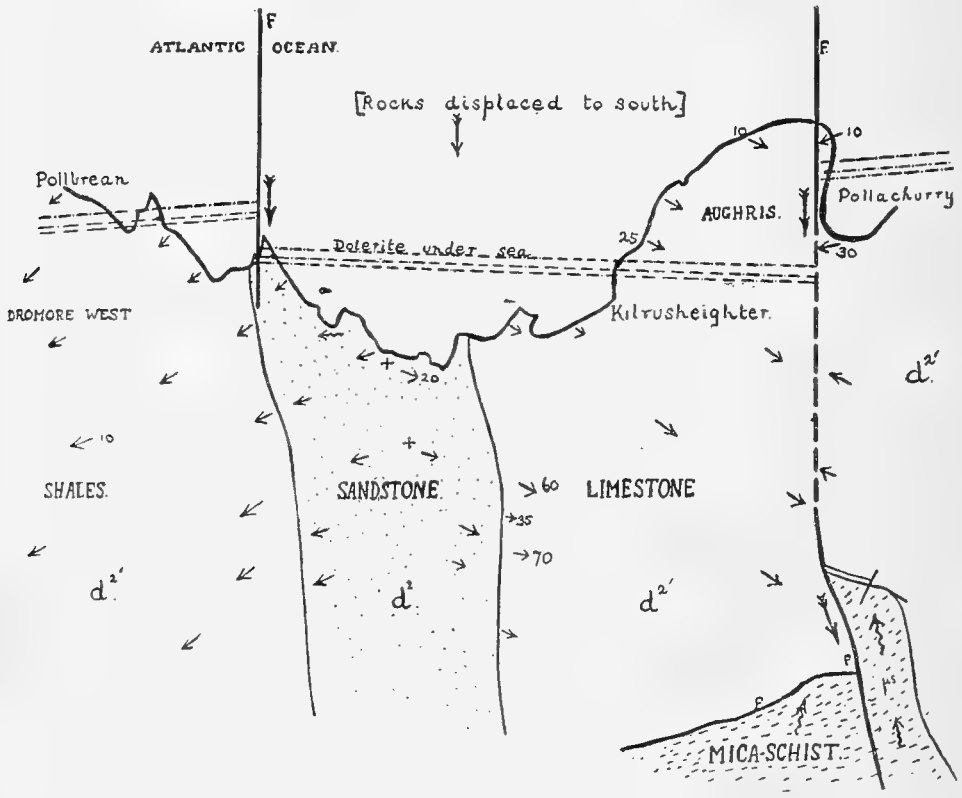


Fig 1.

South-east of these dykes, through a small headland similar to that mentioned above, are three more dykes, which on close examination are found to be exactly equivalent to the first set, though not in their course. The dykes retain the same relations towards each other, and each dyke has the same characteristics as its equivalent in the former set. Their course is shown in fig. 2, D, and differs slightly from that marked in the Geological Survey maps (fig. 2, C).

It will be observed that the dykes do not pass through the small headland of sandstones (marked shale by the Survey) at Carrickpatrick, but have been displaced between the land and these rocks. The dykes run out to sea towards a point a little north of Kilrusheighter sand dunes, and considerably south of the point where the Pollbrean dykes would enter the land if produced.

It is easy to find where the dykes in the Donaghintraine sandstones should appear on the Aughris shore if they run straight across to sea. A careful search in this region will reveal the three dykes again—or rather two of the actual dykes, and unmistakable evidence of the third. Of one dyke there is nothing left; but there is a deep channel in the rocks, the sides of which show the unmistakable "platy structure" in the limestone which usually accompanies a dyke, due to slight metamorphism. The other two are visible, and are marked by the Geological Survey. They are, of course, the three dykes seen in the sandstone.

The point at which the Pollbrean dykes should reach the Aughris shore was ascertained, but a most exhaustive search along the entire west coast of Aughris revealed not a trace of any dyke, except those just described, to the south.

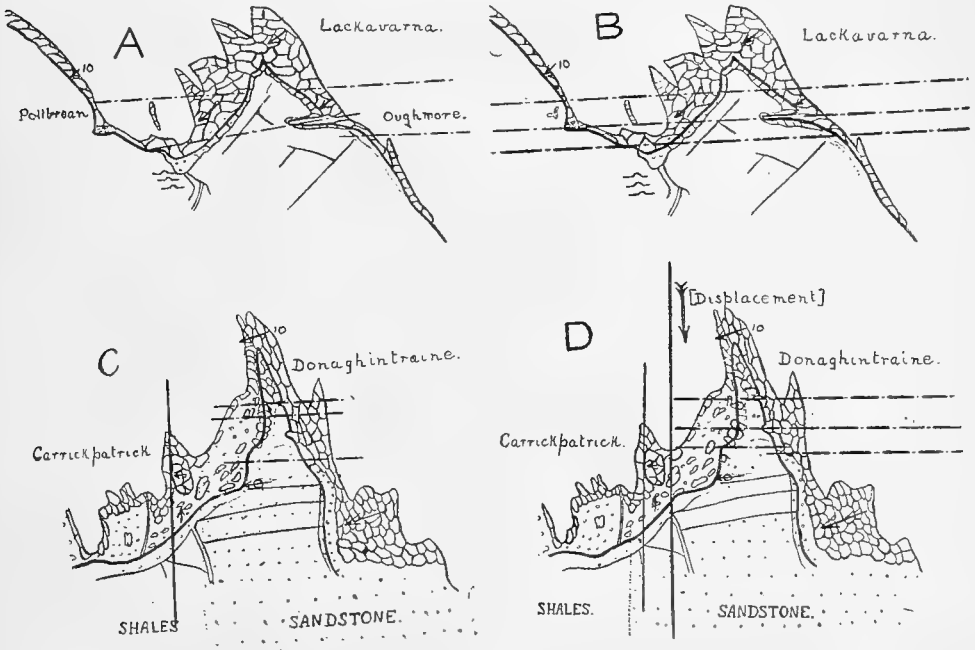


FIG. 2.

On the east coast of Aughris there is a narrow, deep inlet at Pollachurry. It is similar to that at Oughmore, but does not now contain water, and is overgrown with grass. The floor of this channel consists of a dyke of amygdaloidal dolerite. This dyke is in a perfect straight line with the central dyke at Pollbrean and Oughmore. The other dykes are also present; the northern dyke has diverged a short distance from the central, and the southern dyke has come closer. None of them are marked on any of the maps, or mentioned in the memoirs of the Geological Survey.

It is obvious that in every case the dykes are the same three; but where the Pollbrean dykes should have reached Aughris there is no trace of them. They have been displaced from their course between the east and west shores of Aughris, as well as in the sandstone beneath the sea shown above.

The first explanation of these facts which suggested itself was that these dykes, running in a definite direction, were suddenly deflected from their course

along a distance of about four miles by some subterranean agency, resuming their original course and direction again. Slight "jumps" are frequently observed in dykes in the "country" rock, but there appears to be no recorded case of displacement of dykes alone to such a great extent.

The second explanation is that the whole mass of rock-shales, sandstones, and limestones, about four miles in width from east to west, with the dykes contained in them, have been displaced *en bloc* to the south, subsequent to the intrusion of the igneous rock, thus deflecting the dykes from their course. A study of the principal faults in the district, and other considerations detailed below, favour the second view.

There are two small, unimportant faults in the shales on the west. Then there is a N.-S. fault between Carrickpatrick sandstones and the land at Donagh (see fig. 2, D). This is evidently a considerable fault under the sea, displacing the dykes about a quarter of a mile to the south.

The largest fault in the district is visible at Aughris Head. This is marked by the Survey as probably running obliquely inland, though the fault where visible is north and south. It would rather appear to be a very great N.-S. fault, not oblique, but almost parallel to the fault in the sandstones, and displacing the Pollachurry dykes to the south for a distance of about three-quarters of a mile.

Further considerations of the structure of the bay confirm the evidence that displacement of strata has taken place. The sandstone and micaceous "flagstones" at Donaghintraine are hard and compact, whereas the shales at Oughmore and Pollarone are very soft. The greater resistance to denudation shown by the sandstone is evident from a comparison between the weathering of the dykes in the two kinds of "country" rocks. In the sandstones the dykes form the floors of long deep channels in the rocks. In the shales, on the other hand, the dykes form durable walls. Yet the sandstones form the inner part of the bay, and the shales form one side.

A displacement of the sandstones to the extent of about a quarter of a mile would explain this curious feature. Thus it is probable that this displacement of the harder strata to the south was one of the agents in the formation of the bay.

There is a remarkable structure found further inland, south of Aughris Head. At the base of the Ox Mountains the Carboniferous rocks rest unconformably on metamorphic rocks—mica-schist in this region. At the junction between the two there is a fault running east and west, separating the Carboniferous rocks on the north from the mica-schist on the south. Directly south of the fault at Aughris Head previously described there is a large fault north and south, cutting the east and west fault. This separates Carboniferous rocks on the west from mica-schist on the east for a distance of about three-quarters of a mile towards the shore, and continues further with limestone on either side (fig. 1).

Thus immediately south of the fault at Aughris Head, which displaces the dolerite dykes three-quarters of a mile to the south, is this fault which appears to displace the limestone over the mica-schist for the same distance.

It is undoubtedly the same fault; and the whole mass of rock—four miles in width—must have been displaced to the south, and carried over the underlying mica-schist for about three-quarters of a mile on the east and a quarter of a mile on the west.

In conclusion, I wish to express my indebtedness to Mr. L. B. Smyth for his kind assistance and advice, and for undertaking the communication of this paper to the Society in my absence.

No. 27.

ON A PROBLEMATIC STRUCTURE IN THE *OLDHAMIA* ROCKS OF BRAY HEAD, CO. WICKLOW.

By LOUIS B. SMYTH, M.A., Sc.B.

(Plate XII.)

(Read May 29. Printed July 6, 1923.)

The rocks of Bray Head, Co. Wicklow, have long been well known as the original source of the fossils *Oldhamia antiqua* and *Oldhamia radiata*. The only other structures found in them, which may, with fair confidence, be regarded as due to organisms, are worm burrows. Besides these, a small number of markings have been claimed, but not generally accepted, as of organic origin.

The series has generally been attributed to the Cambrian formation, but its stratigraphical relations are somewhat obscure. A Pre-Cambrian, and even an Ordovician, age has been suggested.

This being the state of affairs, it seemed that any new structure from these rocks might prove of interest, and should be described.

A piece of green shale bearing certain peculiar markings (Pl. XII, fig. 1-3) was collected by Mr. T. W. Moran from a loose block on the railway south of Bray Head. The slab is split into two along the bedding, and, on the bedding plane thus exposed, is a group of markings, first pointed out by Professor Joly. These are darker green than the matrix, and rather inconspicuous. On wetting the surface, however, they become clearly differentiated. Any doubt as to the origin of the specimen from the Bray Head Series is set at rest by the presence, on the same bedding plane, of *Oldhamia antiqua* (Pl. XII, fig. 3).

Some of the spots are of a uniform green colour, but others (see Pl. XII, lower part of fig. 1) have a reticulate pattern, consisting of groups of yellow patches, separated by dark green lines, which form an irregular network. The yellow patches vary from light greenish yellow to a rusty yellow. In the latter case the patch is often depressed, as though its substance had crumbled away, the dark green boundary remaining as a slightly elevated rim.

The largest continuous patches, about four in number, are about 15 to 25 mm. long, have a very irregular and ragged shape, with, for the most part, angular outlines, and show the reticulate structure. Their appearance suggests that either they are aggregates of the smaller elements to be described, or they are disintegrating into such units. The smaller spots are mostly between 1 and 3 mm. in diameter, though there are some smaller and some larger. Many of the smaller spots are plain green, but others show the reticulate structure more or less clearly.

The most striking point about the spots is that the majority have angular outlines (Pl. XII, fig. 6). In a great many cases they are bounded by lines which are straight, or nearly so. Quite a number approximate to a parallelogram or rectangle in shape. In fact, after a careful study one gets the impression

that there is a distinct tendency towards a quadrilateral form in the isolated spots, and that some of the more irregular are aggregates of quadrilaterals. Only two or three were seen with a pentagonal shape, and no other outlines were observed definite enough to be given a name.

In a number of cases there are notches in the corners of quadrilaterals, with linear sides approximately parallel to the sides of the quadrilaterals.

On the same bedding plane are a specimen of *Oldhamia antiqua*, the cast of an arched worm burrow about 3 mm. in diameter, another horizontal one about 0·3 mm. in diameter, and a shallow funnel-shaped depression surrounded by a tumid elevation raised about 0·5 mm. above the general level, and 1 mm. above the bottom of the depression.

Horizontal and transverse sections were made through the spots. The latter (Pl. XII, figs. 4, 5) showed that the spots are tabular bodies, with a thickness varying little from 0·3 mm. The rock is very fine grained, with well-marked bedding, certain levels being marked by dark lines. The bodies in question lie upon one of the bedding planes so marked, and have their upper surfaces on the plane along which the slab was split.

The light green shale is apparently composed chiefly of flakes of sericite and chlorite and minute grains of quartz. The dark bands are due to an increase in chlorite. The bodies we are studying, when examined with the microscope in thin section, are seen to differ from the rest of the rock in that the flakes of sericite and chlorite are, on the whole, larger, the chlorite more abundant, and interstitial quartz more evident.

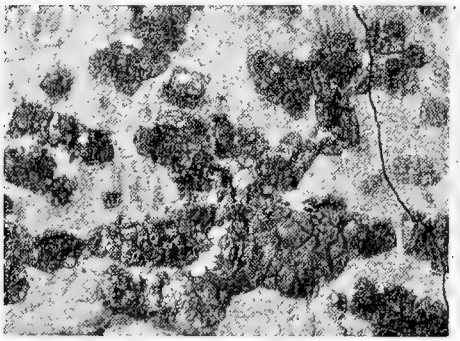
A horizontal section of a reticulate specimen shows a marginal accumulation of chlorite and an increase of colourless constituents forming the spots. In some of the transverse sections there is a light-coloured interior, with, apparently, much quartz, a chloritic layer bounding it above and below, as well as at the sides (Pl. XII, fig. 4 left, and fig. 5 right).

With regard to the origin of these curious markings, it is not easy to suggest a satisfactory explanation. It seems very unlikely that they are of organic origin. The possibility of their being crushed specimens of primitive cystids was considered. The tabular form, the angular outlines, and the apparent presence of a thick middle layer having a less firm structure than the rest, are points of resemblance. But the general quadrilateral, instead of pentagonal or hexagonal shape, the angular notches, and the complete absence of any characteristic structures, such as pores or marginal folds, seem to rule out this suggestion. The angular nature of the markings appears incompatible with the activities of worms.

Turning to inorganic agencies, concretionary action could not produce such sharp angles and straight edges. An inspection of some of the more regular outlines, with their occasional notches and "outworks" (Pl. XII, fig. 6), strongly suggests crystals. Fig. 7, Pl. XII, shows camera lucida drawings of the shapes of pseudomorphs of halite crystals in Triassic marl. Some of the shapes are remarkably like those we are considering.

It is suggested, therefore, that the spots originated as crystals embedded in the sediment. These subsequently became dissolved away, and the cavities filled by mud. If the crystals had been those of an iron-bearing mineral, the pseudomorphs might have contained a residue of iron, especially along their boundaries, which would help to account for the greater abundance of chlorite in the spots, and its segregation at the edges in some of them.

It seems unnecessary to assume that the original crystals were tabular, since pseudomorphs in mud of equidimensional crystals would presumably be squeezed flat by the compression of the sediment into shale. This would also



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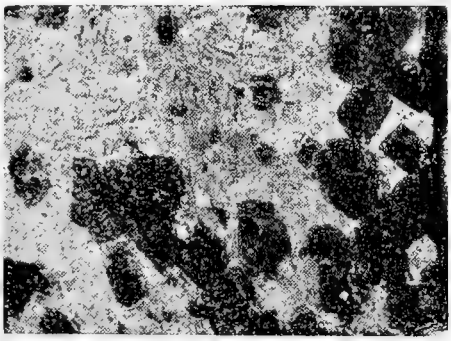

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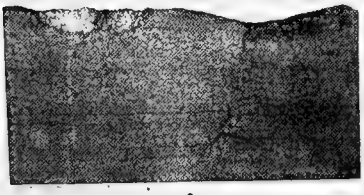

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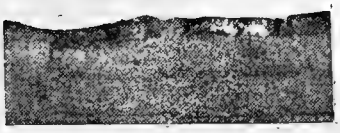

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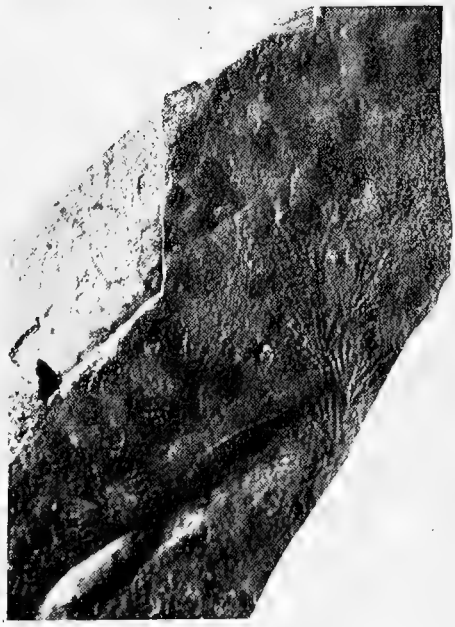

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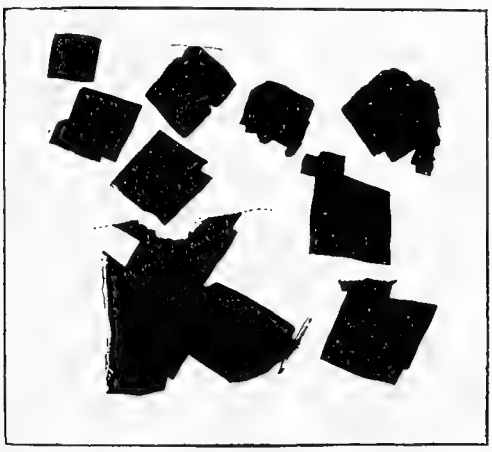

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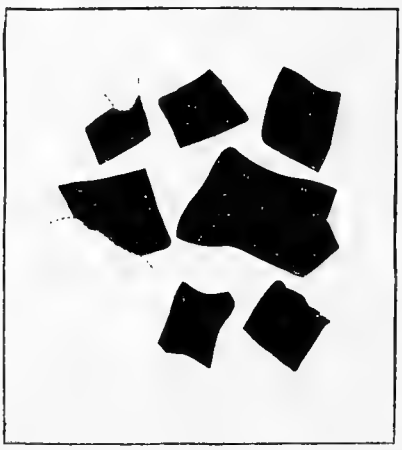

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SMYTH.

account for distortion of angles.[1] Pyrites suggests itself as containing iron, having a cubic form, and being common in argillaceous sediments. But, unless some orienting influence can be assumed, one would expect more varied shapes to result from the compression of cubes.

EXPLANATION OF PLATE XII.

Figs. 1 & 2.—Portions of the bedding plane of the shale, photographed wet. ×2·7.

Fig. 3.—A portion, photographed dry. *Oldhamia antiqua* on the right; worm burrow below. Note the slight differentiation of the marks when not wetted. ×1·7.

Figs. 4 & 5.—Transverse sections through the markings. Each figure contains two of the marks; the one on the right in fig. 4 is the same as that on the left in fig. 5. ×12.

Fig. 6.—Camera lucida drawings of some of the spots from various parts of the specimen. ×5.

Fig. 7.—Camera lucida drawings of pseudomorphs of halite in Triassic marl. ×1·8.

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[1] That such distortion may be remarkably small was shown by experiments with plasticine. Cubes of red plasticine were cut and embedded in green plasticine, the latter being carefully built up round the cubes in order to secure as perfect contact as possible. The whole was then strongly compressed between two parallel boards, and sections made in various directions.

No. 28.

THE HYDROGEN ION CONCENTRATION OF THE SOIL AND OF NATURAL WATERS IN RELATION TO THE DISTRIBUTION OF SNAILS.

By W. R. G. ATKINS, O.B.E., Sc.D., F.I.C.,

AND

M. V. LEBOUR, D.Sc.

[Read June 26. Printed July 27, 1923.]

It has been shown that the hydrogen ion concentration of the soil exercises a profound effect upon the distribution of plants and upon the growth of crops. Accordingly it seemed possible that similar effects might be observed in the case of animals, such as worms and snails.

With the object of exploring this possibility, snails were collected in diverse situations. In order to get an idea of the relative abundance of the members of each species, every snail found by careful searching within a small area, about four square feet, was collected without any sort of selection. In the table of results the actual numbers are given. It was considered that this was better than to give the number of situations at which each species was found. The numbers given must not, however, be considered as precise statistical values, but as an attempt to give a rough quantitative record. Many of the more acid sites examined were very poor in snails.

The hydrogen ion concentration of the soil was determined, for each situation in which snails were gathered, by means of the colorimetric method, as previously employed by one of us (Atkins, 1922) when studying plant distribution. The results are recorded, as is customary, in *pH* values. The symbol *pH* denotes the logarithm of the reciprocal of the number of grams of hydrogen ion per litre, viz., $pH = \log \frac{1}{H}$. Only a few observations relate to aquatic species.

In order to economise space, the *pH* values of typical soil situations and waters are recorded in Table 1. A detailed account of the determinations and the supposed causes of the reactions found is given in the paper already mentioned.

It may be mentioned that the R. Yealm, where examined, supports *Asellus aquaticus*, but not *Gammarus pulex*. The latter crustacean abounds, however, in the alkaline streams of the district. In water from these it can live and breed in small glass vessels, but when placed in the R. Yealm water it has invariably died.

Determinations of soil *pH* values are made to *pH* 0·1, but in tabulating the values obtained, the results are grouped to nearest *pH* 0·5. In doing this, *pH* 6·8-7·2 is called *pH* 7·0, and *pH* 7·3-7·7 is given as *pH* 7·5.

TABLE 1.

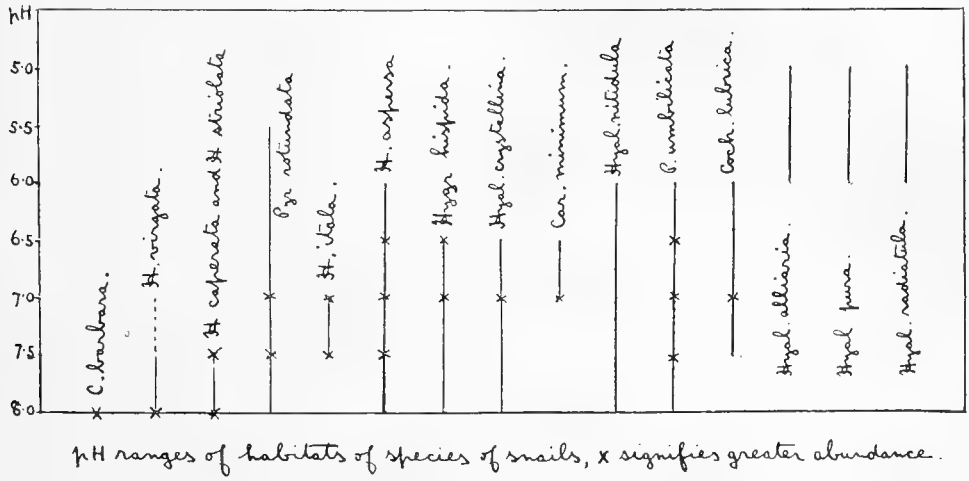
Solution.	*pH.*	Notes.
Peat, S. of Ireland bog	4·1	Saturated solution.
,, Dartmoor	4·6	,, ,,
,, Dublin Mts.	4·6	,, ,,
Disintegrating granite, Dartmoor	5·2	No vegetation.
Light soil, over slate	5·4	Bracken thrives.
Surface soil, copse, in alkaline district.	6·4	Surface is more acid than subsoil.
Clay soils, heavy	5·4–6·8	These become less heavy if limed.
Garden soil	6·5–7·5	Common values.
Limestone soil	7·6–8·2	Where limestone is not leached out.
Bog pool, Dartmoor	5·0	Typical amber water, tadpoles abundant.
Bog stream, Dartmoor	5·5	Light amber water, no recent rain.
Rain water	5·9	Acid from carbonic acid.
R. Yealm, Dartmoor	6·4–6·8	Examined where streams from sedimentary rocks flow in.
Stream, Devon	8·2	Calcium bicarbonate saturation value.
Sea water	8·2	Slight seasonal changes occur.
Small reservoir	7·6–9·2	Depending on season, most alkaline when photosynthesis is active.

TABLE 2.

	Number of specimens found at each *pH* value.							No. of situations.
pH values of soil.	5·0	5·5	6·0	6·5	7·0	7·5	8·0	
ZONITIDAE.								
Vitrina pellucida	0	0	0	1	3	1	3	5
Hyalinia crystallina	0	0	1	0	4	0	1	5
H. nitidula	0	0	1	4	3	4	8	13
H. fulva	5	0	0	0	0	0	0	1
H. pura	4	0	5	0	0	0	0	2
H. radiatula	4	0	1	0	0	0	0	2
H. alliaria	3	0	2	0	0	0	0	2
HELICIDAE.								
Pyramidula rupestris	0	0	0	0	1	0	3	2
P. rotundata	0	1	5	5	9	8	7	18
Helicella virgata	0	0	0	9	1	9	74	15
H. caperata	0	0	0	0	0	23	36	11
H. itala	0	0	0	0	many	v. many	0	2
Cochlicella barbara	0	0	0	0	0	0	8	1
Hygromia hispida	0	0	0	29	10	4	5	17
H. striolata	0	0	0	1	0	12	16	7
Vallonia pulchella	0	0	0	1	1	0	0	2
Helix aspersa	0	0	1	8	4	5	3	11
H. nemoralis	0	0	2	0	2	1	2	5
H. hortensis	0	0	2	2	1	0	0	3
STENOGYRIDAE.								
Cochlicopa lubrica	0	0	5	17	8	2	0	8
PUPIDAE.								
Pupa umbilicata	0	0	7	20	16	0	11	9
P. anglica	0	0	0	0	3	0	0	1
CLAUSILIIDAE.								
Balea perversa	0	0	1	0	0	0	2	2
Clausilia bidentata	0	0	0	0	5	7	0	5
SUCCINEIDAE.								
Succinea putris	0	0	0	0	4	0	0	1
AURICULIDAE.								
Carychium minimum	0	0	0	3	8	0	0	3
No. of species found (total 27)	4	4	13	15	20	16	14	

The results of Table 2 are shown graphically in figs. 1 and 2.

The record of *H. virgata,* 9 at *pH* 6·5, must be explained. One was found on acid soil near the sea, the situation being exposed to blown spray, so it must have been close to an alkaline region. The other eight were on bracken and nettles in a coastal situation where alkaline soil, as tested, was within fifty yards, probably less.



pH ranges of habitats of species of snails, x signifies greater abundance.

FIG. 1.

It must be pointed out that the bottom line of Table 2 records the number of species for each *pH* value, each species being assumed to occur for every value within its extreme range. Thus *H. nemoralis,* found at *pH* 6 and *pH* 7, is presumed to occur at *pH* 6·5 also.

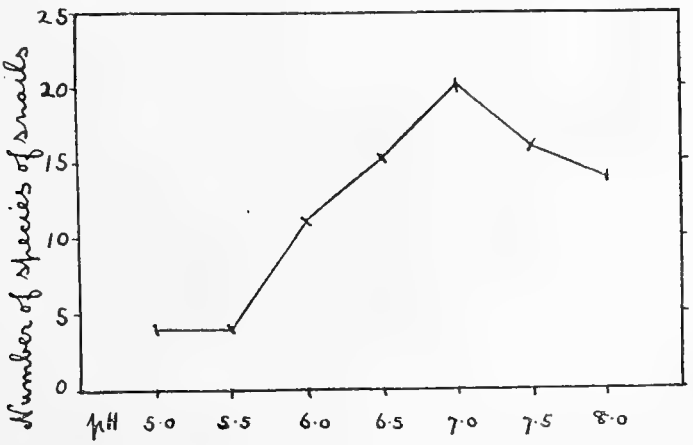


FIG. 2.

Viewed as a record of species of snails for the districts concerned, mainly the Plymouth district and the country round Bray Head, Co. Wicklow, the collection ranks as very imperfect. Sufficient work has, however, been done to show that the *pH* value of the soil is a factor which is important in regulating the distribution of snails in any district. Certain minute Hyalinias are

found on soil as acid as *pH* 4·8 over quartzite rock. Other Hyalinias are found at *pH* 8. The genus therefore covers a very wide range. Apparently species with hyaline shells can inhabit districts free from calcium salts, or where at least very minute amounts are found. On the other hand those with markedly calcareous shells, such as *Hellicella caperata* and *H. virgata,* appear to be strictly confined to regions which are predominantly alkaline.

Again, there are a number of species which are plentiful in a neutral or slightly acid habitat, though they are found in alkaline regions also; these include *Hygromia hispida, Pupa umbilicata,* and *Helix aspersa.* When one recalls the fact that snails possess powers of locomotion, and that within a narrow area considerable differences in reaction may be found, it would be unreasonable to expect very hard and fast rules as to distribution. For example, owing to more thorough leaching out of calcium carbonate, a gully is usually more acid than the land it drains, and, even on alkaline land, plant remains and leaching may together render the surface soil slightly acid. It is precisely on such soil that *H. hispida* abounds around grass roots.

The results for various species are shown graphically in fig. 1, as are also those for the numbers of species at different *pH* values in fig. 2.

The authors are indebted to Mr. F. W. R. Brambell, of Trinity College, Dublin, for collecting specimens on and near Bray Head, Co. Wicklow; also to Miss Worsnop for certain Plymouth specimens. Mr. J. W. Taylor, of Leeds, kindly identified the species collected by Mr. Brambell.

Not included in the list of Table 2 are the following:—*Limnaea truncatula* at *pH* 7, *L. stagnalis* in abundance, in a pond at Kew (it is absent from Devon and Cornwall), at *pH* 7. It must be added that the pond contained hard water, rich in calcium salts. This could become much more alkaline, about *pH* 9, through abstraction of carbonic acid by water plants. Other ponds near were more alkaline, being richer in water plants.

Bathyomphalus contortus also was abundant in a marshy pool at Plymouth, at *pH* 8. One specimen of *Valvata piscinalis* was obtained from the same situation, in which it may be remarked the large ciliate *Spirostomum ambiguum* was very plentiful. We are indebted to Mr. W. C. De Morgan for this identification.

It has not been possible to collect many of the aquatic species as yet, but it may be said that they are either very poorly represented, or altogether absent from the upper reaches of the R. Yealm and the more acid waters listed in Table 1.

As is well known, snails are more abundant in limestone regions than in most others. The hydrogen ion determinations put this observation on a quantitative basis; furthermore, many situations upon calcareous sandstones and shales are quite as alkaline as similar situations upon limestone. It may be mentioned that boulder clay, covering limestone rock, is often acid, as it is genetically unrelated to the underlying strata. Through leaching by rain most limestone regions are acid in parts, so a greater variety of soil reaction may be met with in them than in those where the rocks are granitic or quartzite. Thus Roebuck (1921) in his census records 97 species of land and fresh water mollusca from Co. Dublin, where limestone lowland and plutonic uplands are found. But the neighbouring Co. Wicklow, which is also maritime, has only 74 recorded species, Co. Wexford, on the other side of Wicklow, having 87. The absentees from Wicklow include *Limnaea stagnalis, Valvata piscinalis,* and *Bathyomphalus contortus,* though these are present in Wexford and Dublin. The records for these three species, as shown in this paper, are from alkaline water. Wicklow contains no extensive limestone areas, if indeed any limestone is to be found. The uplands consist of plutonic rocks, and most of the rest is made up of altered and unaltered Silurian strata, with the Bray Head Cambrian;

Wexford is in part similar, but contains, like Dublin, a tract of limestone. It seems probable that the absence of these three species from Wicklow is not due to want of complete investigation, as Stelfox (1912) records for Dublin, Wicklow, and Wexford, 96, 73, and 80 species for 1910, which have only been increased to 97, 74, and 87 in Roebuck's list nine years later. Moreover, *Bathyomphalus* (*Planorbis*) *contortus* is found in every other county in Ireland according to Stelfox. There is, however, a discrepancy as regards records for *V. piscinalis,* the species being listed by Stelfox as present in Wicklow, though not in Roebuck's list. It must at least be a rarity in Co. Wicklow.

One of the writers collected Mollusca at Dalbeattie, Kirkcudbrightshire, where the rock is chiefly grey granite. Very few occurred on the granite, but about two and a half miles from Dalbeattie, on the Palnackie road, near Munches, where the rock was basalt, the snails were strikingly numerous. The following is a list of those taken from near the road where the basalt begins :—

Helix nemoralis juv.
Arianta arbustorum juv.
Pyramidula rotundata, many.
Acanthinula aculeata, common among fallen leaves.
A. lamellata, common among fallen leaves.
Punctum pygmaeum, common among fallen leaves.
Hyalinia excavata, many.
H. cellaria, fairly common.
H. crystallina, common.
H. nitidula, fairly common.
H. alliaria, common.
H. pura, common.
Euconulus fulvus, common.
Vitrina pellucida, a few.
Pupa umbilicata, common.
Sphyradium edentula, very common.
Sphyradium edentula v. substriata, common.
Cochlicopa lubrica, common.
Balea perversa, on walls, common.
Acme lineata, one, among leaves.
Carychium minimum, very common.

Also the following slugs were present :—*Arion hortensis, Agriolimax agrestis,* common, *A. laevis,* common.

It may be pointed out that soil over granite is normally markedly acid, since this rock gives off inappreciable amounts of alkali to neutralize plant remains. With basalt it is, however, otherwise, for it has been found that while a weathered basalt boiled with distilled water gives usually a slightly acid solution, yet a fresh basalt surface may quickly yield sufficient alkali to give a reaction of *pH* 8·0. Out of eight basalts tested, on further boiling one remained acid, *pH* 5·4, six were at *pH* 7·0–7·3, and one at *pH* 8·3. It is highly probable therefore that the situation where snails were plentiful over basalt was markedly less acid than the granite region. It was most probably slightly acid or even almost neutral. The species found notably in the alkaline region were absent from the basalt, viz., *H. virgata, H. caperata, H. itala,* and *Hygromia striolata. H. hispida* was also absent, but species of Hyalinia, *H. pura, H.* (*Euconulus*) *fulva* and *H. alliaria,* known to occur even on highly acid soil, were present.

While the authors feel convinced that the acidity or alkalinity of a situation is an important, and often a dominant, factor in limiting the distribution of

species of snails, they are fully aware that other factors may also be important or dominant. The amount of salt in the soil is tentatively put forward as one of these for land species near salt marshes, as the concentration of salt obviously is for water mollusca. Furthermore, the dry or wet nature of a habitat is also of much importance, though hard to express quantitatively. This factor and the relation between various plant associations and snails have been studied recently by Kendall (1921, 1922). The plant associations are themselves regulated by the soil reaction. Salisbury and Tansley also (1921) have pointed out that the molluscan fauna of certain woods on the Wenlock limestone are intermediate in character between the plentiful fauna of a calcareous beech wood, and the restricted list of species obtainable from an acid oak wood (Salisbury, 1918).

In order to examine possible factors limiting the distribution of snails, other than the hydrogen ion concentration, a detailed study was made of three localities, similar in soil reaction and general situation. From these large numbers of snails were collected, and since the sites were calcareous it was possible to get the specimens in a small area. For each site the *pH* value was determined, also the salt content as shown by the electrical conductivity of a mixture of one part of air-dry soil with five of water. The soil was sieved, and the portion passing the hundred mesh to the inch was used in the determinations. The mixtures were shaken at intervals for three hours on a rotating wheel, by which time it was judged that all the readily soluble salts had gone into solution. On standing, however, the conductivity increased and attained an equilibrium value, probably due to the solution of calcium carbonate as bicarbonate, occasioned by bacterial production of carbonic acid. The conductivity was measured at 0°C., and the apparatus was standardized against one hundredth normal potassium chloride. The snail population is given in percentages, the total number collected being also shown.

The sites were as follows :—

A. Plymouth Hoe, grassy bank, near laboratory, gentle slope, south aspect.

B. Plymouth Hoe, grassy bank, near sea, steep slope, south aspect.

C. Fort Stamford, near Plymouth Sound, east side, grassy and stony bank, with moss and red valerian (*Centranthus ruber*), at east end, south aspect.

D. Oreston Quarry, near Plymouth, on estuary of R. Plym, limestone rubble and grassy banks, aspects south and east.

	A.	B.	C.	D.
Helicella caperata	8 per cent.	2 per cent.	72 per cent.	54 per cent
H. virgata	79 „	51 „	18 „	0 „
Pupa umbilicata	6 „	23 „	1 „	10 „
Helix aspersa.	2 „	1 „	4 „	2 „
Helix nemoralis	0 „	0 „	0 „	1 „
H. pulchella	0 „	0 „	0 „	1 „
Hygromia hispida	0 „	1 „	2 „	3 „
H. striolata	0 „	0 „	2 „	12 „
Pyramidula rotundata	1 „	0 „	0 „	5 „
Hyalinia nitdula	4 „	0 „	0 „	7 „
H. crystallina	0 „	0 „	0 „	2 „
Cochlicopa lubrica	0 „	0 „	1 „	3 „
Vitrina pellucida	0 „	0 „	0 „	1 „
Cochlicella barbara	0 „	22 „	0 „	0 „
Total count	117	348	150	159
Species	6	6	7	12
pH	7·8	7·5-8·0	7·5-7·7	7·7-8·0
C × 10^5, 3 hours	12	12	13	11
C × 10^5, 11 days	37	31	31	27

Site A appeared to be the best soil, growing good grass with leguminous plants, and conductivity measurements indicate that it was less washed out than were the other soils. The three-hour values show that none of the soils have more than small amounts of salts, the figures found being less than one hundredth part of that for sea-water. So the soils from B are not markedly salt, as it was thought they might be. Chlorides are, however, present in B in larger traces than in the others, and give an opalescence with silver nitrate though inland soils often give nearly as much.

It is evident that though the *pH* values are all between *pH* 7·5 and 8·0 there is no definite sequence in them. All the sites may be considered as more or less uniformly of slight alkalinity.

With regard to exposure to wind, B is most exposed, followed by A, C, and D. The distribution of the snails is strikingly different, for example, in B and C, where the physico-chemical values are almost identical. Again, though D has twelve species out of the total fourteen, *H. virgata* and *C. barbara* are absent, in spite of the fact that they constitute 73 per cent. of the population of B and *H. virgata* 79 per cent. of A. There appears to be no specially marked difference in temperature or moisture in the sites, all being dry and very warm, A probably retaining moisture a little better than the others. Of all the characteristics of the sites perhaps the force of the wind is the most noticeable difference, though it is not easy to see why on this score Oreston should have no *H. virgata*.

According to the "Age and Area" theory of Willis the extent of the distribution of a species depends upon the interval of time since its introduction into the region under consideration. This conception is obviously modified by the existence of ecological factors inimical to any particular species. On the other hand ecological factors alone must be incapable of explaining the distribution of newly arrived species. On turning to the map given in Roebuck's census it is seen that *Cochlicella barbara* is widely distributed in Ireland, with inland as well as coastal records. In England, Wales, and Scotland, however, it is only found as a maritime species on the west coasts, and along parts of the south of England. These facts point to its having a south-western origin—from Europe or by Atlantic drift possibly. The area colonised by *Theba cantiana* is to be accounted for similarly in all probability, for it occupies the east of England, extending some way down the south coast and up as far as Northumberland, where it has been found by one of us. This species is absent from western England, Wales, Scotland, and Ireland, and may be considered to have an eastern origin.

SUMMARY.

1. The hydrogen ion concentration of the soil is a factor limiting the distribution of snails.

2. Snails are more numerous at *pH* 7-8 than they are elsewhere.

3. The number of species of snails found in the districts studied increases from *pH* 5 four species, to *pH* 7 twenty species, falling at *pH* 8 to fourteen out of the total twenty-seven species found.

4. Snails with hyaline shells may be found in any portion of the range, but those with calcareous shells are limited to the more alkaline end. Granite and quartzite regions have few species, basaltic districts have a more numerous fauna, and in limestone areas both species and numbers of individuals give high values.

5. There remain over a number of puzzling cases in which, within an area of two square miles, certain species are altogether absent from one locality, though abundant in others, in spite of similarity in *pH* value, salt content (as shown by electrical conductivity measurements of soil extracts), and aspect. A difference only in exposure to wind could be noted.

6. The distribution of some species within the British Isles is probably explained by the " Age and Area " theory of Willis rather than by a limitation through unfavourable ecological factors. *Cochlicella barbara* appears to have a western, and *Theba cantiana* an eastern, origin.

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No. 29.

IMPROVED METHODS OF EVAPORATION IN THE LABORATORY.

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Owing to the frequency with which the operation occurs in chemical work, it is desirable that the methods of evaporation used in the laboratory should be as efficient as possible. On the industrial scale evaporation has been brought to a high degree of efficiency, largely owing to the fact that in this case it is not necessary to distinguish between true evaporation and ebullition. On the laboratory scale, however, the problem is complicated by the requirement that the liquid must vaporise without ebullition in order to avoid loss by spirting, and this has led to the usual method of heating the liquid to a low temperature on a water bath, and allowing the evaporation to proceed slowly over long periods of time.

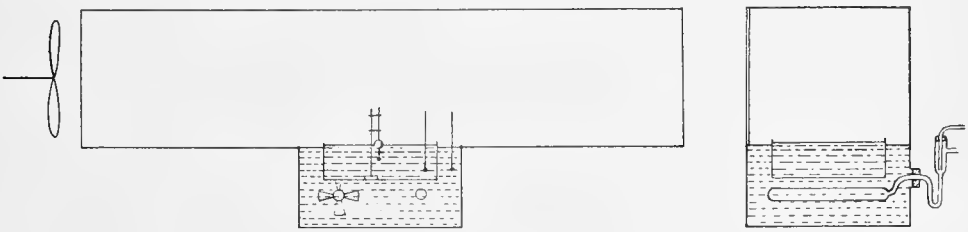

Fig. 1.

The object of the work here described was to determine experimentally to what extent this quiet evaporation could be hastened by allowing it to take place under the most favourable conditions, and whether the saving in time effected would justify the use of the more complicated apparatus which might be required.

For practical purposes the determining factors in the vaporisation of liquids are (1) the rate of heat transmission to the liquid (which is determined by the temperature of the source of heat and the conductivity of the containing vessel), and (2) the rate at which the vapour is removed (which may be fixed by diffusion, a current of air, or by the removal of atmospheric pressure). The ratio between the two rates determines the temperature of the liquid, and also whether vaporisation takes place with or without ebullition. From the point of view of quiet evaporation the removal of the vapour is the more important of the two, since the rate at which the vapour can escape from the free surface of an adequately heated liquid is the determining factor in preventing the temperature rising to the boiling point. The most favourable conditions for rapid, quiet evaporation are therefore that the rate of removal of the vapour should be as

great as possible, and the heat supply so adjusted that it keeps the temperature of the liquid just at its boiling point without any danger of superheating. The first part of this work was therefore directed towards finding how far these conditions could be realised for evaporation in open dishes.

In order to observe the rate of evaporation up to the boiling point, it was necessary to use an oil-bath for heating the dishes. The bath was made of copper, in the form of a rectangular box, 10″ square and 5″ deep, and was built into the lower surface of a copper wind-tunnel, of which the front could be closed by glass plates. As shown in fig. 1, the whole was designed so that the dish containing the water was almost completely immersed in the oil, and the water surface formed a continuation of the bottom of the tunnel. The cross-section of the tunnel was 10″ by 8″, and it projected 14″ on each side of the bath. The bath could accommodate dishes up to 8″ diameter, and was provided with a stirrer, a thermo-regulator, and a thermometer. For the lower temperatures one bunsen burner was used for heating, but for the higher temperatures two were necessary.

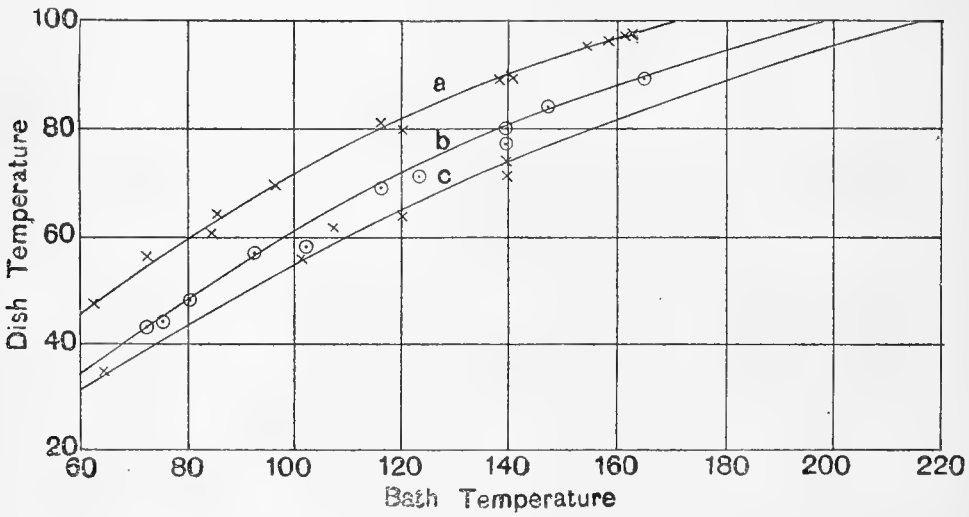

Fig. 2.

The dishes used were glass crystallising dishes, with straight sides, and the rate of evaporation was measured by the fall in level of the dish. The level was observed by providing a small glass float drawn out to a fine tip and kept in a vertical position by a small stand made of brass rod, so as to rise and fall with the surface of the liquid, the position of the tip of the float being read by means of a cathetometer. This method allowed of a great number of observations being taken in a short time, so that the mean rate of evaporation could be obtained from a graph of the observations.

At the left-hand end of the wind-tunnel an aluminium fan, 8″ diameter, was fixed, which could be driven by an $\frac{1}{8}$ H.P. motor. By this means air-currents up to 1,000 feet per minute could be produced, the speeds being measured by a small anemometer.

With this apparatus the rate of evaporation of distilled water was measured over a range of 30° to 98° C. in still air, and from 30° to 70° C. in air currents.

As the oil was maintained at a steady temperature by the thermo-regulator, it was possible to note the difference of temperature between the oil-bath and the water in the dish during the evaporation. This is an important point, as it is an indication of the rate at which heat is being supplied to the liquid. As the loss of heat becomes greater at higher temperatures, this difference naturally increases rapidly with increasing temperature.

The temperature of the water for different bath temperatures is shown in fig. 2, where it will be seen that a bath temperature of 100° C. gives a water temperature of about 70° C. in still air (curve a), about 60° C. in a moderate draught (curve b), and 54° C. in a strong draught (curve c). These temperatures, therefore, represent the maximum obtainable under the given conditions with a water bath as source of heat. However, by using a higher bath temperature it is possible to maintain the water at, or near, its boiling point, and thus obtain the great advantage of the higher vapour pressure. The graph shows that to maintain water at 100° C. in still air the bath must be at 170° C., in a moderate air current it must be at 197° C., and in a strong draught at 215° C. This graph thus shows the approximate temperature at which any given bath should be maintained in order that water may evaporate at a certain temperature under the given conditions.

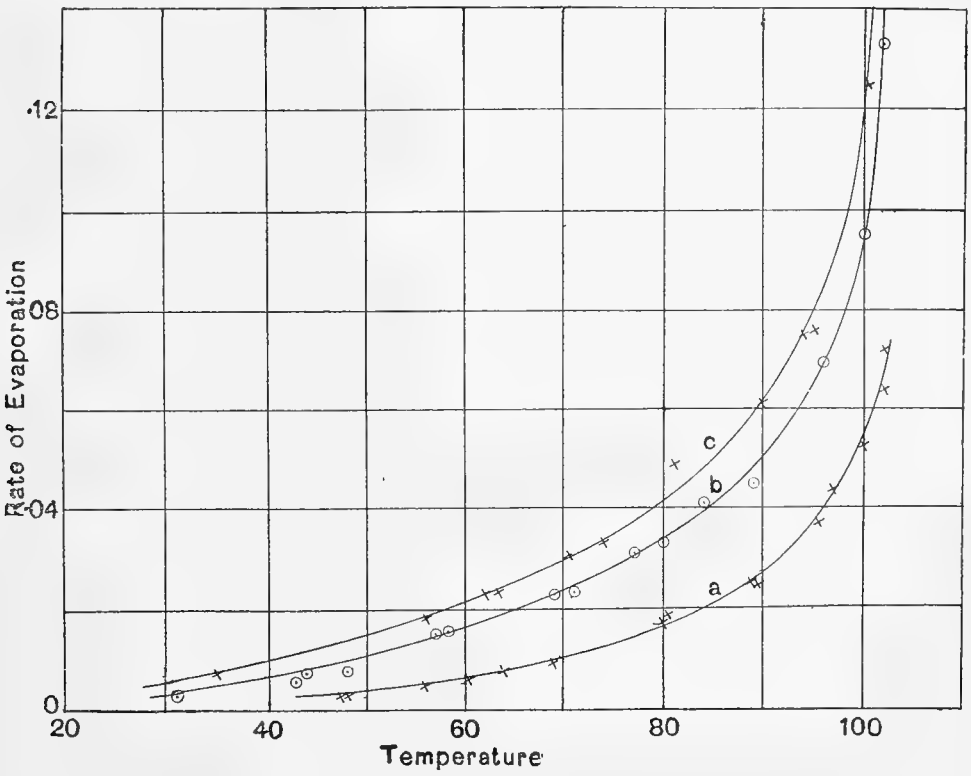

FIG. 3.

Owing to the difficulty of maintaining the higher temperatures with the oil-bath, the evaporation from 70° to 100° C. in air currents was measured in a slightly different way. An aluminium dish was supported on a powerful ring burner in close proximity to the fan, so that the air could be blown across its

surface. The burner was lighted, the fan started, and the burner regulated until the water came to the steady temperature required, when the observations of the float were made as before. By this means the temperature of the water could be maintained at the boiling point even in a draught of 1,000 feet per minute.

The results of these experiments are shown plotted as a graph in fig. 3. The effect of the vapour pressure is shown by the upward sweep of the curve in the region 70° to 100° C. Thus the increase in rate of evaporation due to the rise from 90° to 100° is equal to that due to the rise from 30° to 90°, so that, roughly speaking, each degree rise in the higher region is six times as effective as a degree in the lower region.

This is further emphasised in the graph fig. 4, where the rate of evaporation is plotted against vapour pressure. It will be seen that the rate is proportional to the vapour pressure up to 90° C., but above this it increases more rapidly.

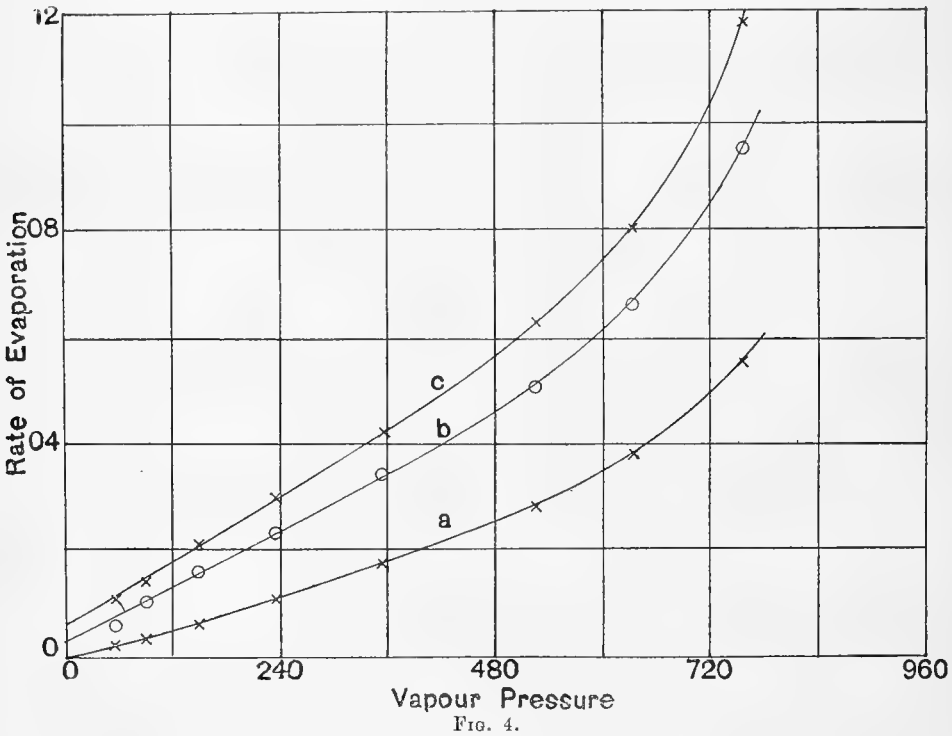

Fig. 4.

The region 90° to 100° is therefore particularly effective in producing rapid evaporation, and wherever possible the water should be kept within this temperature range.

It is also interesting to note how the results for evaporation in still air compare with figures calculated by means of an approximate formula put forward by Hinchley,[1] namely, rate of evaporation in kilograms per sq. metre per hour from water surface = $\left(\frac{P_e - P_d}{50}\right)^{1\cdot2}$ where P_e = vapour pressure of the liquid in mm. of mercury P_d = pressure of the water vapour in the air.

[1] "The General Problem of Evaporation," J. W. Hinchley. Jour. Soc. Chem. Ind., **41**, 242 T., 1922.

The two series of values agree fairly closely up to 90°, but above this the experimental values are higher than the calculated. From both these facts it appears that, as the boiling point is approached, the rate of evaporation increases more rapidly than it would if it were proportional to the vapour pressure. This may be due to the fact that at the higher temperatures the convection currents in the vapour over the surface of the liquid become much more rapid, thus leading to a more effective removal of the vapour.

The effect of a current of air in hastening evaporation is also shown by the two upper curves (b) and (c) in fig. 3. The effect is most marked at low dish temperatures, and falls off as the temperature rises. Thus at 50° C. the rate is 2·8 times as great in a current of 500 feet per minute, and 3·8 times in 1,000 feet per minute; at 80° C. the figures are 2·0 and 2·5 respectively, while at 100° C. they are 1·7 and 2·2. These figures also show that, while at low temperatures the rate is approximately proportional to the velocity of the current, at high temperatures the moderate current is almost as effective as the higher speed.

The relatively high efficiency of the air current at low temperatures is probably due to its action in preventing the vapour from accumulating over the surface of the liquid, as it is liable to do, owing to the weakness of the convection currents. On the other hand, the falling-off in efficiency of the current at high temperatures is probably due to the fact that the draught removes the heat from the surface layers faster than it can be supplied by conduction from the body of the liquid; this results in a surface cooling, which slightly reduces the rate of evaporation. When the air is heated before being led over the surface of the liquid this surface cooling is avoided, and slightly higher rates of evaporation are obtained. Thus Aldrich[1] has described an apparatus for evaporating solutions at 20° to 30°, in which an average rate of evaporation of ·01 c.c. per sq. cm. per minute is obtained in a draught of warm air, while the rate obtained with the draught at room temperature is about ·007.

In most of the formulae put forward for rate of evaporation in a draught the factor connecting it with evaporation in still air is assumed to be a constant, but this probably arises from the fact that the experimental results were mostly obtained at low temperatures. Thus Leonard Hill found that the evaporation from a muslin surface was doubled in a wind velocity of ·55 metre per second (107 feet per minute); and Carrier arrived at a value[2] of 1·17 metre per second (228 feet per minute) for a similar doubling. The present experiments show that the factor cannot be regarded as constant over the range 40° to 100° C.

From a consideration of these results the best conditions for evaporation from open dishes can be laid down. The rate of evaporation on the water bath (dish temperature 70° C.), as at present in use, is approximately ·01 c.c. per minute per sq. cm. Raising the temperature of the water to 95° C. raises the rate to ·04, without leading to any increased risk of loss, while, if the water be kept at the same temperature in a draught, the rates increase to ·07 for a draught of 500 feet per minute, and to ·087 for 1,000 feet per minute.

In order, therefore, to cut down the time required for evaporation to one-seventh of its present value it is merely necessary to make arrangements to keep the water at 95° C. in a moderate current of air.[3] This may be done by means

[1] J. Biol. Chem., **23**, 255, 1915.

[2] See Hinchley, *loc. cit.*

[3] It is important to note that it is not sufficient to heat the water to 95° C. and then start the draught, as the cooling effect of the air then practically neutralises its efficacy in removing the vapour; the heat supply must be sufficient to *maintain* the liquid at 95° C. in the draught.

of a thermostatically controlled air, oil, or sand bath provided with a suitable fan. With such an arrangement an evaporation which would take a day with existing apparatus could be completed in an hour, so that the saving in time would amply repay any extra trouble in setting up the bath in the first instance.

The main difficulty in evaporation, as will be seen from the foregoing, is to supply the heat to the liquid sufficiently rapidly to produce rapid evaporation and yet prevent ebullition. The latter is to be avoided on two grounds: (1) its very existence shows that the liquid is being superheated locally; and (2) it may lead to loss of the liquid by spirting.

When liquid is heated in a vessel with only the convection currents to keep it mixed, the bottom of the vessel may become heated much above the boiling point of the liquid, and, since the convection currents are unable to mix the liquid sufficiently rapidly, ebullition of more or less violence occurs if the heat is supplied at more than a certain rate.

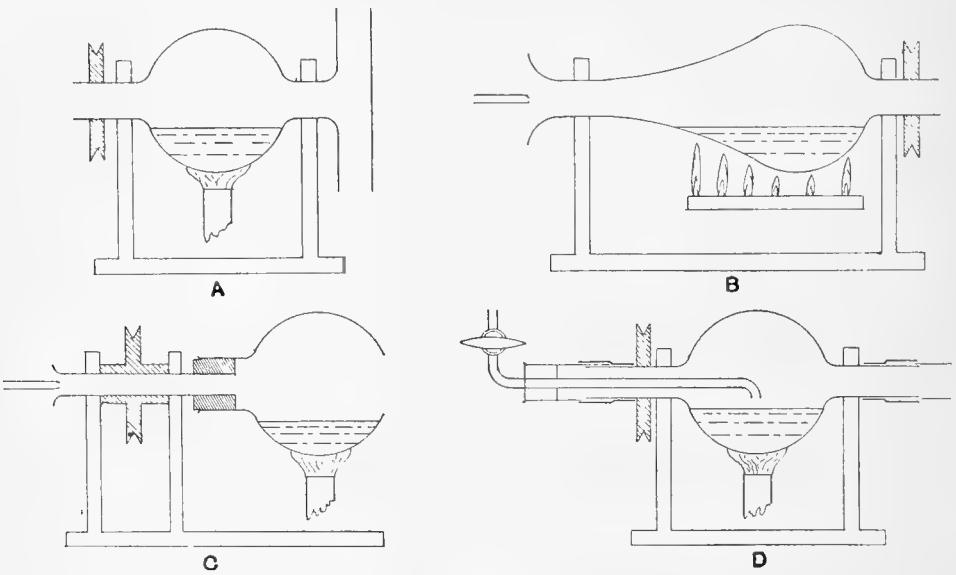

Fig. 5.

It is well known that this tendency to superheating of the liquid is diminished by stirring or shaking, but this leaves the liquid still in contact with the highly heated bottom of the vessel. Any method which would keep the liquid mixed and at the same time prevent the heating of the containing vessel to a temperature greatly in excess of the boiling point of the liquid would provide almost ideal conditions for rapid evaporation.

Such a method is incorporated in the design of the evaporator described below. In its simplest form this consists of a glass bulb containing the liquid, and kept in constant slow rotation while it is heated by a gas burner. Owing to the adhesion of the water to the glass, it is drawn up on the side of the bulb which is rising, and a continuous thin film is spread over the upper part of the bulb. This ensures very thorough mixing of the liquid, and also materially increases the surface of liquid from which free evaporation can take place. At

the same time, the rotation of the bulb over the burner prevents any portion of it getting superheated; and, in fact, the temperature of the liquid can be regulated very exactly by the height of the flame used. With a plain bulb of this type water may be rapidly boiled away with very slight formation of bubbles, and consequently no loss by spirting.

If a current of air be blown through the bulb, a similar effect to that noted in the experiments recorded in the first part of this paper is produced—namely, that the liquid may be much more strongly heated without the formation of bubbles. The air current may be produced in two ways: either by making use of the rotation of the bulb to eject the mixture of air and vapour centrifugally, or by blowing in air directly by means of a filter-pump or bellows. Fig. 5, A shows the modification designed to use the first method, consisting of the addition of a wide bore T-piece fused to one end of the bulb. When the bulb is revolved at a moderate speed (about 150 r.p.m.), the mixture of air and vapour is thrown out through the arms of the T by centrifugal force, and the air is consequently drawn in through the other end. In this way the escaping vapour forms its own blower, and materially hastens the evaporation. Fig. 5, B shows the modification suitable for use with an outside current of air. The air-jet is placed in the bell-mouth of the tube, so that it draws in with the primary air a considerable volume of secondary air, thus producing a current which completely removes the vapour as it is formed.

The latter method can be further amplified by supplying to the jet any particular gas in which the liquid being evaporated may be most stable. Thus easily oxidisable substances can be evaporated in an atmosphere of coal-gas or hydrogen, while readily hydrolysable chlorides may be evaporated in hydrogen chloride. When the gas used is scarce or expensive, arrangements can be made for circulating it continuously through the bulb, so that a limited volume would suffice for a large volume of liquid.

When the liquid has to be evaporated to dryness and the solid recovered, as in the determination of silica in analytical work or in the recrystallisation of salts, a form of apparatus with a very wide opening at one end is used. This is shown in fig. 5, C, and in its simplest form may consist of a round-bottom flask of which the bottom has been softened and blown out, the neck cut off short, and the whole mounted on a piece of glass tubing by means of a rubber stopper. This allows the bulb to be revolved and a current of air blown through at the same time, while the solid left behind can be scraped or washed out at the conclusion of the evaporation. Incidentally it may be noted that, owing to the action of the rotating bulb, solutions never become supersaturated, but the salt crystallises out in very fine small crystals, even at the high temperature, as the liquid becomes concentrated. In this way no difficulty is experienced in crystallising such difficult substances as potassium hydroxide and ferric chloride.

Owing to the complete absence of superheating, the apparatus is particularly suited for use in evaporating at reduced pressure, as it obviates the violent bumping which usually gives such trouble at low pressures.

A form which has been found effective for this purpose is shown in fig. 5, D. The revolving bulb is connected to the fixed end tubes by means of carefully selected pieces of black rubber tubing, of such a size that when slightly moistened they allow the bulb to revolve freely. When the whole is properly adjusted, the power required to revolve the bulb is not large, even when the atmospheric pressure is acting on the rubber joints, a small electric motor of 1/20 H.P. being quite sufficient.

The following rates of evaporation have been obtained with revolving bulb evaporators of different types:—

Bulb with one end open to air (type C)	...	·142 c.c. per sq. cm. per minute.
Bulb with one end open to air, with air-jet	...	·237 „ „ „ „
Bulb with centrifugal cross-arm (type A)	...	·170 „ „ „ „

The rates of evaporation are calculated on the area of the water surface when at rest.

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No. 30.

A RAPID GASOMETRIC METHOD OF ESTIMATING DISSOLVED OXYGEN AND NITROGEN IN WATER.

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Introduction.

The transient milkiness of water in which potash is dissolving, due to minute bubbles of dissolved air, suggested the possibility of utilising this phenomenon for the estimation of dissolved gas. Consultation of the literature[1] encouraged this view, since it appeared that the solubility of a gas in dilute solutions is generally considerably less than in pure water. While little was known regarding the solubility of gas in concentrated solutions, it appeared probable that it should be nearly zero in very concentrated solutions of certain electrolytes. It was proposed, therefore, to work out a method whereby the water should be brought in contact with the solid electrolyte under such conditions that the displaced gas could be readily collected and measured. Preliminary attempts made to displace the dissolved oxygen and nitrogen from tap-water showed that the gas evolution was fairly complete, though rather slow at ordinary pressure. With a view to hastening the process, it was considered advisable to arrange that the gas should be liberated under a greatly diminished pressure.

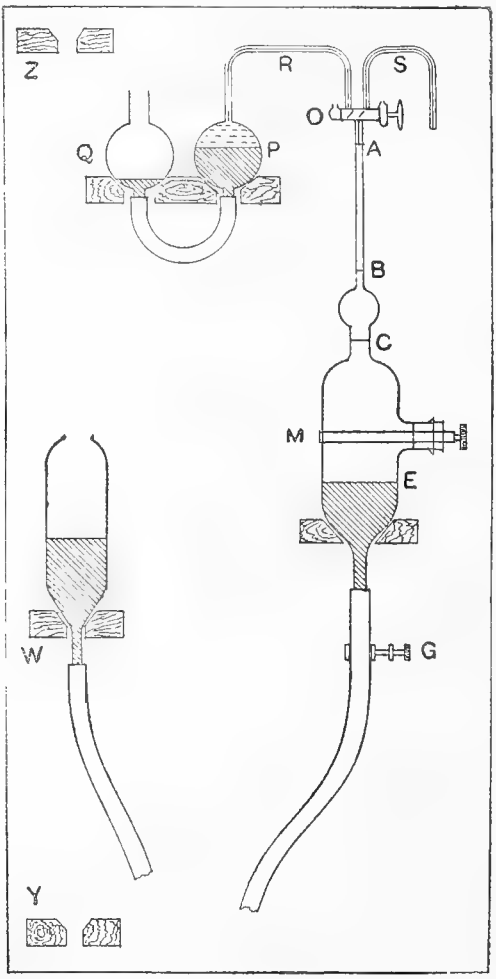
The choice of a solute was the first point to be considered. Caustic potash had been shown by the preliminary experiments to be very good in most respects. Owing to its high cost, however, attempts were made to find a cheaper substance which would be equally effective. Such materials as were not available in a compact form suitable for testing were prepared for use in our apparatus by fusion, and subsequent solidification. Caustic soda was as good as potash, except for a tendency to crystallize and block the capillaries during manipulation. Magnesium chloride, and calcium nitrate in large quantity expelled practically all the dissolved gas, but at a very slow rate. Fused potassium chloride expelled about 95 per cent. of the dissolved gas, but twenty to thirty minutes were required for this evolution, as against one to two minutes when using potash. Sodium sulphate, citric acid, calcium chloride, sodium carbonate, sodium thiosulphate, zinc chloride, and alum proved unsatisfactory. The rate of evolution of gas when using potash or soda is very remarkably greater than that which follows the use of any other of these materials. Glycerol, and strong sulphuric acid in large amounts give a rapid and good, but possibly not quite complete, evolution of gas. It was finally decided to adopt potash as the solute in the absence of a satisfactory substitute; but we recognise that we have not investigated the possibilities of substitutes as fully as we would desire. Thus it has

[1] e.g., G. Geffcken Z. physik. Chem. 49, 271, 1907.

been shown[1] that a litre of a normal solution of ammonium chloride only contains 0·07 c.c. of dissolved oxygen, and, if this substance can readily be prepared in a suitable form, it would probably prove satisfactory. On the other hand, ammonium chloride dissolves with absorption of heat, while potash evolves heat on solution, and this might militate against ammonium chloride as compared with potash, since heat evolution undoubtedly greatly accelerates the displacement of gas.

Description of Apparatus.

The final form of apparatus used is shown in the figure. *O* is a capillary 3-way mercury-sealed tap, joined to a tube *AB* of 2·5 to 3 mm. bore. This is



joined to a bulb of 20 to 30 c.c. capacity, connected by a short tube of about 1 cm. bore to a vessel of the shape shown, about 9 cm. long by 4 cm. in diameter.

[1] MacArthur, J. Phys. Chem., **20**, 495, 1916.

The side tube *E*, for introducing solid potash into the apparatus, is of 2 cm. bore, and is carefully shaped so that a rubber cork will fit it perfectly. Above the 3-way tap one of the capillaries is connected to an absorption pipette containing alkaline pyrogallol, while the other is bent sideways and downwards, and projects in front of the tap. *W*, *Y*, and *Z* are rests for the mercury reservoir on the wooden stand to which the apparatus is fastened. *Y* was at such a height that when the reservoir was placed on it the mercury level fell to *E*, leaving a vacuum in the upper part of the apparatus.

Marks were etched on the glass at *A*, *B*, and *C*, and the volumes *OA*, *OB*, and *OC* were determined by weighing their contents of mercury.

Experimental Procedure.

The mercury level is brought to rest at *E*, and secured in this position by a screw clip at *G*. Then the potash sticks, broken up into pieces 1 to 1½ inches long, are introduced. The potash used was Merck's (85 to 86 per cent. KOH). The same amount can be introduced into the apparatus for each estimation by using the same length of stick, but in nearly all our experiments a quantity sufficient to ensure saturation was used. As a general rule it may be taken that 1 gram of potash should be used for each cubic centimetre of water sample, although possibly less than this may suffice. The cork is replaced and secured by a metal clip *M* passing around the bulb. The mercury level is then raised until the capillary *S* is full of mercury, whereupon the tap is closed. On placing the mercury reservoir in the bottom rest *Y* the mercury falls to the vicinity of *E*, leaving the potash sticks exposed and in a partial vacuum. When the reservoir is raised, a bubble collects in *OB*, and is expelled from the apparatus *via S*. This operation is repeated until no more air can be detected in the apparatus. Three exhaustions usually suffice.

The apparatus and capillary *S* being full of mercury, the sample to be tested is drawn in through *S* until the mercury level reaches the mark *C*. By this means a definite volume of water *OC* is measured. Having closed the tap, the reservoir is placed in *Y*, whereupon the water sample falls on the potash sticks, and the dissolved gas is expelled with great vigour in one to two minutes. When boiling ceases, the mercury level is made the same inside and outside the apparatus, and the bubble is measured by making its top correspond with *O*, and measuring the distance of its bottom from *A*. The height of the potash column is also measured.

The bubble and a little of the potash are then forced into *P*, and the rest of the potash is removed through *S*. After gently rocking the stand for one to two minutes, the bubble is drawn back to *OB* and re-measured. This operation is repeated to ensure complete absorption of oxygen. Slightly over five minutes are required for the total gas determination, while the oxygen determination increases the time by about ten minutes. From the measurements it is easy to calculate the volume of gas per 100 c.c. of sample.

The alkaline pyrogallol reagent is best prepared by dissolving 5 grams of pyrogallol in 30 c.c. of water, and saturating with potash in the apparatus, in the same way as a water sample. The reagent can then be driven into the pipette without contact with the air.

Before starting a new determination it is essential to wash all traces of pyrogallol from *OC*. It is also advisable to wash the tap with water and acid after a series of experiments.

Discussion of Results.

The new method was standardised at first by comparing the results obtained by it with those obtained by Winkler's iodometric method, using distilled water saturated in a thermostat under definite conditions. At a later date it was tested by comparing the results of analyses obtained by its use and by boiling out. Many series of experiments, with modifications of apparatus and procedure, were performed, and the results are given in the accompanying table. Each figure represents the mean of three determinations.

Results in c.c. gas at N.T.P. per 100 c.c. water. Each figure average of 3 determinations.

TOTAL GAS.			OXYGEN.			
New Method.	Calculation from Tables.	Boiling Out.	New Method.	Winkler Determination.	Calculation from Tables.	Boiling Out.
1·92	1·76	—	0·590	0·613	0·611	—
1·90	1·73	—	0·604	0·621	0·604	—
1·83	1·72	—	0·562	0·580	0·595	—
1·81	1·69	—	0·579	0·597	0·590	—
1·86	1·73	—	0·591	0·605	0·594	—
1·84	1·75	—	0·589	0·593	0·600	—
1·87	1·76	—	0·606	0·620	0·603	—
1·86	1·75	—	0·595	0·595	0·604	—
Mean 1·86	Mean 1·74	—	Mean 0·590	Mean 0·603	Mean 0·600	—
2·34	2·27	2·304	0·750	—	0·800	0·808
2·25	2·15	2·15	0·732	—	0·750	0·747

It is seen that the total oxygen and nitrogen by the new method is consistently greater than the standard values. On the other hand, the figure for oxygen recorded by the new method is always less than that given by the standards. The following appear to be the possible sources of error:—

(*a*) *Gas contained in potash sticks.*—The magnitude of this error was determined by performing blank experiments with air free water and different amounts of potash in the apparatus. Minute bubbles were evolved in all cases, and measured in the fine capillary tubing of the tap. The size of the bubble was proportional to the quantity of potash dissolved. When 26·4 c.c. of water were saturated with potash the bubble measured 0·01 to 0·015 c.c. This would only account for about 0·06 c.c. of the difference of 0·12 c.c. between the experimental and calculated values for total gas in the first part of the table. It would, however, just suffice to account for the difference in results in the second part.

(*b*) *Defective oxygen absorption.*—At first the low value for the oxygen figure was put down to imperfect evolution of oxygen. When, however, this

figure continued low in further series of experiments, and, moreover, the total gas figures remained higher than the tabular value, defective oxygen absorption appeared to be the cause. The literature revealed a widespread dissatisfaction with alkaline pyrogallol as an oxygen absorbent for very accurate work, and very varied recipes are given by different authors. In our work the difficulty inherent in alkaline pyrogallol is no doubt greatly aggravated by the minute size of the bubble to be tested. The formula we finally adopted approximates to that recommended by Anderson.[1] It is, nevertheless, not wholly reliable, as is amply proved by the unsatisfactory results recorded in the second last series in the table, obtained when using a freshly prepared sample of this reagent. It was further noticed that the reagent does not work very well on the first absorption of a series if it has remained idle for some time. This difficulty, which is mentioned by other workers, can be overcome by absorbing an unknown volume of oxygen from air before beginning a series of determinations.

Chromous chloride was tried as an alternative absorbent, but had to be abandoned owing to the precipitation which occurred when the acid solution met with a trace of potash.

(*c*) *Incomplete evolution of gas.*—The facts discussed under (*a*) indicate that it is probable that the error introduced by this cause is negligible.

Conclusions.

The work so far completed shows that the use of a very soluble substance, such as potash, to expel dissolved gases has many advantages. The value of direct gasometric measurements is very generally recognised, and volumetric methods are only resorted to in order to avoid the tedious process of boiling out the gases and measuring them. This method gives all the advantages of the gasometric method, and the further important one of rapidity.

The modifications which must be introduced into the simple Winkler process, for instance, when dealing with heavily polluted liquids, or in the presence of chlorides, not only complicate the manipulation, but add very greatly to the time required. On the other hand, there is no reason to suppose that the action of the potash would be affected by the ordinary impurities in a water, and an estimation could be done as easily as with distilled water.

Where the quantity of the sample is limited, the method only requires 20 to 30 c.c. of the water, as compared with the 250 c.c. used in the volumetric processes.

The disadvantage (from a commercial point of view) of the cost of the potash may quite possibly be removed by a more minute search for a suitable substance than the present authors have had opportunity to make. The method certainly works much better than its simplicity would lead one to expect, and it is hoped that it may be possible to develop it still further in the future.

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[1] Journ. Ind. Eng. Chem., 1915, p. 587.

No. 31.

LIGNEOUS ZONATION AND DIE-BACK IN THE LIME (*CITRUS MEDICA*, VAR. *ACIDA*) IN THE WEST INDIES.

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(Plates XIII--XVI.)

(Read May 29. Printed August 28, 1923.)

Introduction.

Though the Lime thrives on the whole in the more humid of the Lesser Antilles, yet its cultivation in those islands which are subjected to periods of pronounced desiccation is fraught with considerable hazard. A progressive dying back of the terminal shoots is very prone to occur in habitats in which the aridity of the plant's environment is liable to fluctuate markedly.

Nowell's (8) view is that the young trees become established and do well for about ten years, attaining to a good size and bearing normal crops. Then, in a uniform field of this nature, the trees most exposed to the desiccating blast of the Trade Winds begin to show signs of die-back, which sooner or later extends widely, and in two or three years may involve the whole field, or may leave for a time groups of less affected trees here and there.

The loss of dominance of the apical buds and the replacement of the mother shoots by laterals become especially accentuated. The terminal part of the mother shoot beyond the daughter shoot ceases to grow, sheds its leaves, and dies. For a time the losses incurred in this way may be balanced by the production of new shoots. Partial recovery may even occur, provided the water balance of the plant (6) becomes stabilized, but normally the decline, when once initiated, continues. The tree dies branch by branch; a process of self-pruning, which is accelerated, as a rule, by the presence of scale insects and the infestation of the dying shoots by Diplodia, etc.

Etiology.

Nowell (8) states that the underlying cause of the decline appears to be one of insufficiency and irregularity of the water supply, and that the duration and completeness of the dry season seem to have more effect that the intensity of the wet season. He also points out that a heavy crop of fruit, especially in dry weather, frequently leads to a loss of branches. Spasmodic applications of manure or of cultivation are generally attended by similar results.

Plan of Work.

As the writer recently (April, 1921) had occasion to visit a number of Lime estates in the islands of Montserrat and Dominica, a collection of shoots was made from trees showing the die-back condition and from those in a more

healthy state. It is probably unnecessary to point out that nearly every stage between trees in perfect health and those showing the most pronounced dying back of the branches is to be encountered. The collection of shoots was made with a view to an examination of the differentiation of the xylem cylinder. It was argued that if pronounced fluctuations in the water balance of the plant (6) were the cause of the affection, these fluctuations might be registered by different types of zonation in the woody cylinder.

Periodicity of Growth.

Before describing the results of this examination it seems desirable to say something concerning the periodicity of growth and its relation to moisture conditions. Temperature fluctuates so little in these islands in the course of the year that it does not obtrude itself as a factor. Reference to fig. 1 will

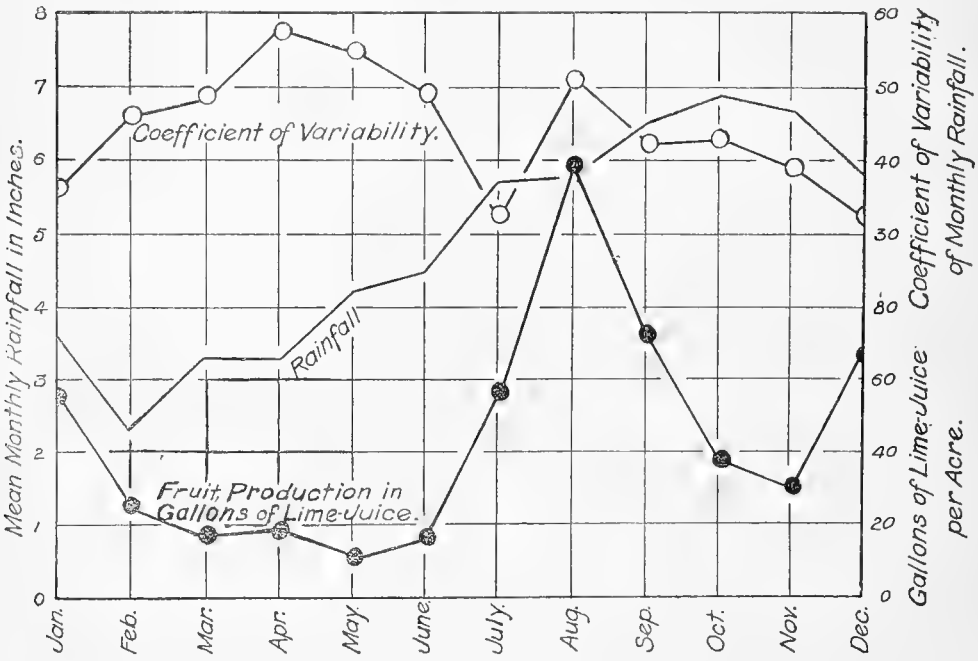

Fig. 1.

show that there are two periods in the course of the year at which the rate of fruit production becomes markedly accelerated. In other words, there are two crops, both of which are gathered in the wet season. Inasmuch, however, as the fruit requires some five months to mature, the earlier portion of the first crop may develop under rather arid conditions. The large values for the coefficients of variability in rainfall from February till June, when considered in relation to the meagre rainfall at this time, will serve to indicate how frequently spells of drought occur.

Vegetative growth (leaf production and shoot elongation) commences some time after the maturation of the fruit, and is generally most active between January and August—that is to say, in the dry season. It will thus be seen that the period of maximum vegetative activity is also the time at which spells of drought are especially liable to occur.

FIG. 2.

FIG. 3.

MASON.

In a word, vegetative activity takes place in the dry months of the year, and the maturation of fruit in the wet. The aridity fluctuates rapidly and markedly in the course of the dry season, while the vegetative meristems are most active. There is generally a bare sufficiency of soil moisture and frequently a pronounced shortage at this season, while the desiccating power of the aerial environment becomes particularly accentuated as a result of the vigour with which the Trade Winds blow about this time.

The Zonation of the Xylem Cylinder.

The different types of zonation presented by the wood of the shoots from the various habitats will be now considered. As the habitats and the condition of the trees supplying the shoots, which are dealt with in this paper, have been recently described by Hardy (2) in the course of his "Studies in West Indian Soils," it is possible to consider the zonation in relation to his description, which is reproduced below in italics. The sections, which were prepared by polishing, are enlarged approximately nine diameters.

THE MULCHED PLOT.—ROSEAU LIME EXPERIMENT STATION (FIG. 2, PLATE XIII).

Lime trees completely free from die-back.

The mulched plot, which supports a vigorous and healthy crop of lime trees, is well drained, and is composed of soil in good tilth and of considerable depth. It is adequately sheltered from winds by the proximity of lime trees of neighbouring plots, and by a dense artificial wind-belt that lines the nearest hill-crest.

Mean Annual Rainfall—80 inches.

The environment is on the whole conducive to the maintenance of the water balance of the plant throughout the year, though rather dry periods may occur between February and May. The presence of the mulch does much, however, to stabilize the soil moisture conditions.

Xylem Cylinder.

Growth zones can be distinguished, though they are not very marked. They are indicated by circumferential areas, in which the large vessels are more densely grouped. Somewhat ill-defined tangentially disposed bands of parenchyma tend to occur *within* these areas.

This section does not suggest that the autonomy of the plant was markedly influenced by the adversities of the environment. The tendency for the vessels to be grouped is presumably associated with periods of more rapid leaf-production. The parenchyma bands, it is conceived, may indicate intermittent checks in the activity of the cambium as a result of the production of considerable tension (1) in the water-tracts of the plant; in other words, they record intermittent periods at which the aridity of the environment (4) became pronounced.

OLVESTON VALLEY, MONTSERRAT (FIG. 3, PLATE XIII).

Lime trees free from die-back.

The soil in this valley near the sea is typical of the valley soils of the northern part of Montserrat. It is rocky but deep, especially in the pockets between the boulders. The situation is sheltered, and the soil at the time of sampling (April, 1921) *was moist to the touch. The weeds growing between the lime trees were tall and vigorous.*

Mean Annual Rainfall—60 inches.

The environment in this valley is less conducive to the maintenance of the water balance of the plant than in the Lime Experiment Station at Roseau, Dominica; the absence of a surface mulch is especially important.

Xylem Cylinder.

The zonation is here very marked. The large vessels are rather densely grouped circumferentially conferring the appearance of "spring wood," *within* which there occur bands of tangential parenchyma; these bands, it will be seen, tend to be grouped radially. It will be evident that there was a rather definite periodicity in the activity of the cambium. The presence of "spring wood" may indicate that leaf-production was vigorous over a rather limited period; that, in horticultural parlance, there was a flush of growth. It will be remembered that growth is generally most active during the dry season. The presence of the radially grouped parenchyma bands may be due to intermittent periods of desiccation about this time.

O'Garra's Hillslope, Montserrat (fig. 4, Plate XIV).

Lime trees free from die-back.

The steep talus slope above O'Garra's is dissected longitudinally by deep ghauts, bordered by steep round-backed ridges. In the ghauts a certain amount of shelter from winds is experienced, but the slopes of the divides are exposed and dry. The lime plot examined occurs in one of these sheltered ghauts, at an elevation of some 250 feet. The soil is a brown deep loam, and is characterized by the presence over its surface of a very well-marked layer of volcanic cinders and small stones. They form a loose stone-mulch on the soil, and, when removed, expose a plexus of lime rootlets, which ramify through a moist soil.

Mean Annual Rainfall—60 inches.

Xylem Cylinder.

A rather definite periodicity in the activity of the cambium is indicated. The parenchyma bands, it will be observed, again are radially grouped, but they tend to be distributed in the "autumn" rather than in the "spring" wood. This is intelligible, if it be assumed that leaf-production was deferred until the close of the dry season, and that the tangential parenchyma resulted from intermittent desiccation of the cambium during these months.

Grove Botanic Station, Montserrat (fig. 5, Plate XIV).

Lime trees free from die-back.

The soil is very uniform in texture, deep, and uncompacted. A heavy mulch of cane grass, cut from a neighbouring plot, covered the soil surface. In consequence of this, and partly also because of the sheltered position, the soil at the time of sampling (April, 1921) *was moist to the touch. Well-grown specimens of the leguminous shrub,* Gliricidia maculata, *occurred between the lime trees.*

Mean Annual Rainfall—60 inches.

Xylem Cylinder.

The differentiation into zones simulating "spring" and "autumn" wood is here scarcely perceptible. The zonation is mainly due to the parenchyma bands, which are on the whole distributed at regular intervals radially; this is more

FIG. 4.

FIG. 5.

MASON.

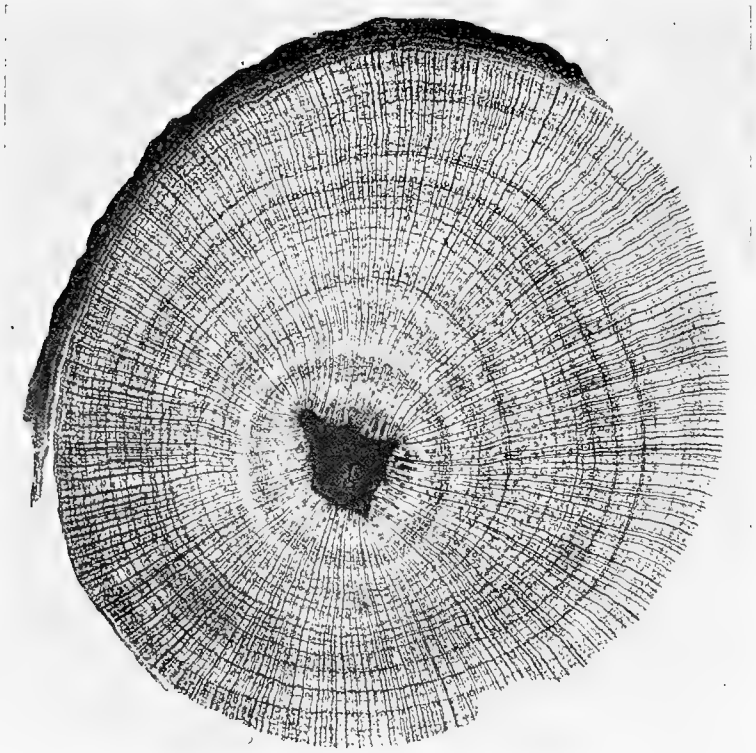

FIG. 6.

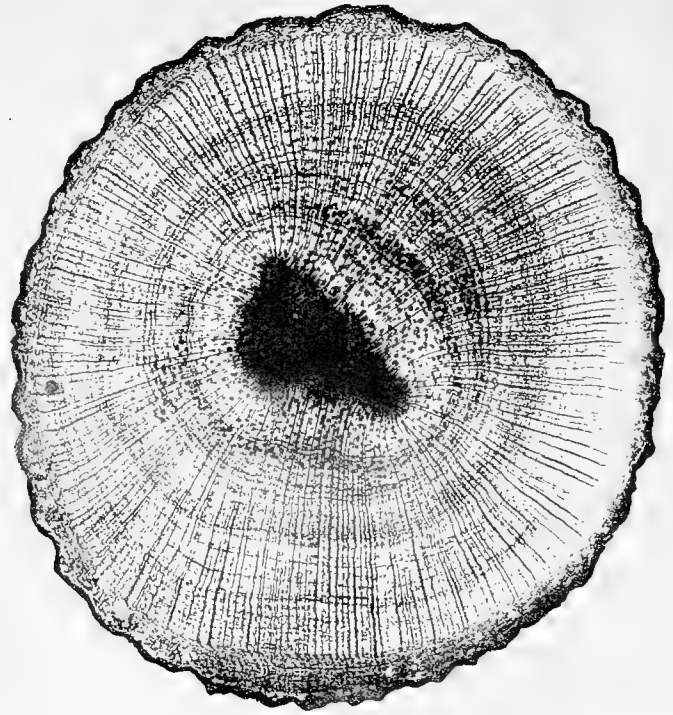

FIG. 7

MASON.

marked in the right-hand side of the section. These bands are again grouped, and are not especially marked. It would seem that they originate as a result of regularly recurring periods of desiccation. A certain similarity between this section and that from the Lime Experiment Station at Roseau, Dominica, can be traced in the radial distribution of the vessels; the vessels are, however, distinctly smaller in diameter, and, moreover, the parenchyma bands better defined. Both characters may be due to a more pronounced aridity in the environment (cf. 6).

BELLE FIELD, GROVE ESTATE, MONTSERRAT (FIG. 6, PLATE XV).

Lime trees exhibiting die-back.

The soil is of the talus slope type, free from large stones, and in good tilth. The situation is exposed to winds, and the existing artificial wind-belts appear to be inadequate. It was here that certain cultural and spraying experiments, designed with a view to suppressing attacks of scale insects, were inaugurated in 1915.

Mean Annual Rainfall—58 inches.

Xylem Cylinder.

This section resembles that just described from the Grove Botanic Station, Montserrat, in the diameter and radial distribution of the vessels. It differs, however, in one respect. The parenchyma bands are more sharply delimited, and do not occur in groups along the radii. If it be again assumed that these bands of tangential parenchyma originate during periods of pronounced desiccation, it may possibly be inferred from their more irregular distribution that the activity of the cambium was checked at irregular intervals in the course of the year, and not alone for a short period during the dry season. It is also necessary to assume that these checks in growth were of greater duration than in the case of the Grove Botanic Station.

It will be observed that Hardy states that the situation is exposed to winds. It is quite conceivable, therefore—for the conductivity of the wood is evidently small—that growth may have been checked at any period in the course of the year, even though the water-supplying power (5) (7) of the soil was adequate.

OLVESTON, MONTSERRAT (FIG. 7, PLATE XV).

Lime trees exhibiting die-back.

This locality is situated on the gently sloping side of an exposed low hill near the sea-coast. The soil is very shallow and rocky. The lime trees have been planted chiefly in pockets of soil. At the time of sampling (April, 1921) *the soil was very dry and compact.*

Mean Annual Rainfall—60 inches.

Xylem Cylinder.

In this section there is little or no regularity exhibited in the zonation. It is possible to infer only one thing, namely, that the activity of the cambium was repeatedly interrupted, presumably as a result of irregularly recurring periods of desiccation, due, no doubt, to the exposed position of the field.

SALTOUN, 9-ACRE PLOT, DOMINICA (FIG. 8, PLATE XVI).

Lime trees exhibiting die-back.

The soil at Saltoun is shallow, and overlies sheet rock. The subsoil is a

yellow clay. The drainage is mainly superficial, the land sloping in the direction of a natural water-course. As a result of a very high rainfall, the soil has been subjected to leaching and washing.

*It is markedly acidic in reaction (*pH *4·7), and is almost continuously water-logged.*

Mean Annual Rainfall—200 inches.

Xylem Cylinder.

The zonation here is essentially similar to that in the former section. The growth of the cambium has evidently received repeated checks. The conductivity of the wood is small. The soil in which the trees were rooted was indubitably physiologically dry for the greater part of the year as a result of a low oxygen-supplying power, and, possibly, the presence of toxic substances, and not, of course, from an excess of soluble salts. It is probable that the numerous irregularly distributed parenchyma bands are due to the drying blast of the Trade Winds.

O'Garra's Coastal Region, Montserrat (fig. 9, Plate XVI.)

Lime trees exhibiting die-back.

This locality is situated on low ground near the extremity of a steep talus slope. The soil is somewhat shallow and stony, with pockets between the rocks. It was dry and compacted at the time of sampling (April, 1921), *and its weed flora was withered. Under drainage appears not to be good, for the presence of nut grass* (Cyperus rotundus) *and* Commelina nudiflora, *growing amongst the xerophytic short-lived annuals that make the natural plant association, indicates that the land is subjected to periodic water-logging.*

Mean Annual Rainfall—43 inches.

Xylem Cylinder.

There is here a very marked differentiation into zones simulating "spring" and "autumn" wood. It will be observed how very sharply these zones are delimited from one another. It will be evident that the rate of growth must have been checked with great rapidity. This is possibly the result of the periodic water-logging to which Hardy refers.

Conclusions.

The interpretation of the zonation exhibited by the woody cylinder of the shoot of an arborescent evergreen, particularly in a tropical climate, is evidently a task beset with no little hazard. As yet, it would seem, the problem has never been approached experimentally. It is but natural that this should be so, for the prosecution of work of this nature would call for resources and equipment at present possessed by no tropical, and few northern, botanical departments.

It has been generally assumed by palæontologists that rings of growth in the stems of fossil plants indicate the existence of a Temperate Zone climate and their absence a more equable one. In the woody cylinder of the shoot from the Lime Experiment Station of Dominica, where the moisture conditions are more generally favourable for growth than in any of the other habitats visited in the course of this survey, zonation is scarcely appreciable. It is indicated merely by circumferential areas, where the large vessels are somewhat more densely distributed than elsewhere, and within which ill-defined tangential bands of parenchyma occur. This grouping of the vessels, which culminates in some

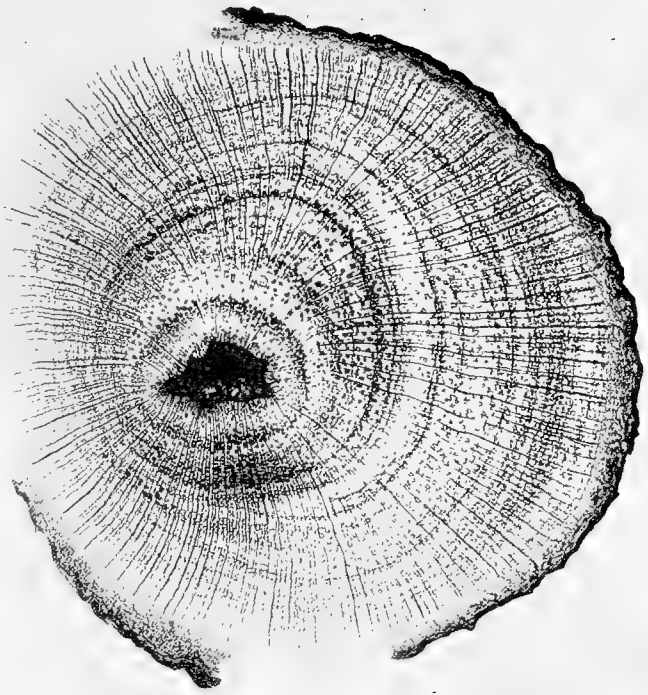

FIG. 8.

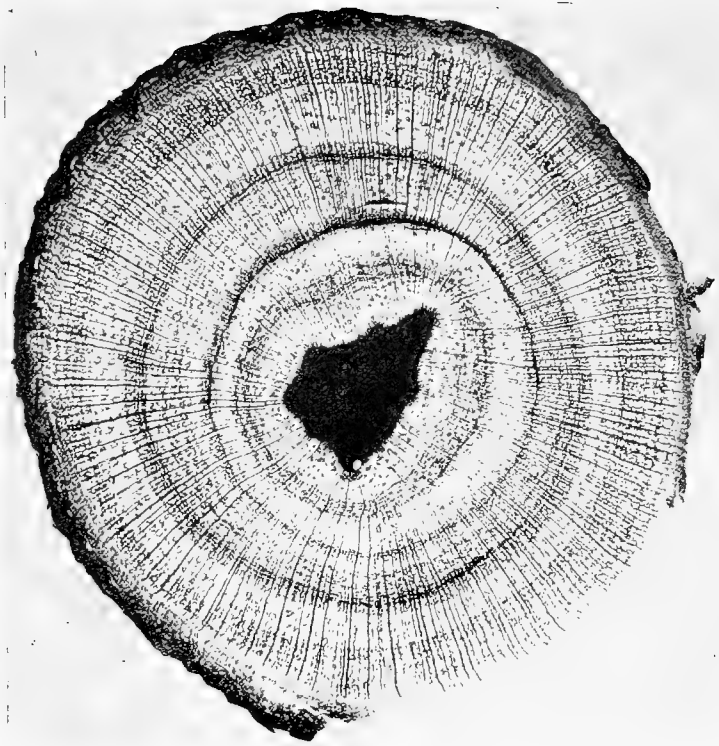

FIG. 9.

MASON.

of the sections in areas presenting all the characters of "spring wood," is evidently not especially associated with the absence of the die-back condition, for it is particularly marked in the wood from the coastal region of O'Garra's, where the trees were badly affected. Inasmuch as nothing definite is known concerning the factors which determine the anatomical differences between "spring" and "autumn" wood, even in temperate regions, it would be obviously unprofitable to attempt to discuss this matter further.

It will be also clear that the diameter of the vessels is subject to considerable variability, and that, though it is generally greater in healthy trees than in those showing the die-back condition, yet no significant relationship is to be traced.

It was suggested in the preceding section that the parenchyma bands originated during periods of desiccation. Jeffrey (3) has pointed out that the impulse towards production of terminal parenchyma was probably supplied in the past by climatic cooling. It would seem that the impulse needed for the production of tangential parenchyma may also be provided by desiccation of the cambium. It is not improbable, of course, that the production of parenchyma as a result of climatic cooling is in the last analysis effected by desiccation.

It will be evident that, if the interpretation of the zonation adopted is correct, namely, that the zones of vessels correspond to periods during which the plant's water-supply was adequate, and that the bands of parenchyma were conditioned by desiccation of the cambium, their frequent association must indicate very rapid changes in the aridity of the environment. Rather sudden fluctuations do, in fact, characterize the dry season. There is generally at this time a bare sufficiency of moisture for growth. It may be suggested that the production of tangential parenchyma is due to autogenic changes in the activity of the cambium, or that it is possibly related to the circumvasal parenchyma, but there would seem to be no foundation for such a view.

It will be recalled that the parenchyma bands were generally more pronounced and more irregular in their radial distribution in those habitats in which the aridity of the environment fluctuated most markedly. It is in these habitats that the die-back condition is most conspicuous. It would seem that the rapid and repeated desiccation of the meristems, which is recorded in the case of the cambium by tangential parenchyma, results in the premature loss of dominance of the apical over the lateral buds, as a result of which daughter shoots grow out and replace the mother shoot. A repeated dying back of shoots of this nature is characteristic of trees affected with die-back.

The growth of the Lime in the West Indian islands of St. Vincent and Carriacou is of some interest in this connexion. The islands lie only some fifty miles apart, but differ very markedly in two respects. The annual rainfall in St. Vincent is about 100 inches, and the soil is shallow and porous, drying out with great rapidity whenever a couple of weeks pass without rain. The annual precipitation in Carriacou, on the other hand, is only about 40 inches, but the soil is very retentive, and dries out slowly. The consequence is that in the dry season St. Vincent undergoes rapid though normally short-lived spells of drought, whereas in Carriacou drought conditions are initiated relatively slowly as a result of the retentive nature of its soil, but are frequently prolonged. Now the Lime cannot be grown in St. Vincent, though repeated attempts have been made to do so, while it thrives in Carriacou, where the aridity is many times greater. It would seem that this plant can tolerate considerable aridity and still thrive, but that it is unable to withstand a rapid desiccation of its tissues. The writer is unaware of any other hypothesis that so well accords

with the actual distribution of Lime cultivation in the West Indies. That the tree can thrive under a wide range of relatively static edaphic conditions (hydrogen ion concentration, etc.) has been convincingly shown by Hardy (2). There can be little doubt that the rate, rather than the amount or duration of desiccation is in many cases of extreme importance in determining the distribution of plants. It is possible that this is one reason why certain plants will grow vigorously in clay soils and fail completely where the colloid content is small.

In conclusion, the writer wishes to express his indebtedness to Mr. Robson, in Montserrat, and Mr. Keys, in Dominica, for making observations on the periodicity in the growth of the Lime tree.

Summary.

1. In this paper the results of an examination of the ligneous zonation of a number of Lime shoots from trees growing in different parts of the West Indian islands of Dominica and Montserrat are considered. Half of the shoots were collected from trees affected with die-back.

2. The zonation in the woody cylinders from healthy trees indicates a rather definite periodicity in the activity of the cambium. Tangential bands of parenchyma are generally distributed within the more porous zone of vessels.

3. Inasmuch as the Lime tree in these islands makes its vegetative growth during the dry season, it was inferred that the zones of vessels registered the production of leaves at this period, while the water-supply was adequate, and that the tangential parenchyma which is distributed within these zones originated at periods of relatively great aridity. Rapid fluctuations in the aridity of the environment, which the association of the porous vessels and the circumferential parenchyma indicates, are characteristic of the climate in the dry season.

4. The wood of shoots from trees affected with die-back exhibited considerable irregularity in the distribution of the parenchyma bands. All the sections suggested that the cambium had been exposed to sudden checks in its activity.

5. It was tentatively concluded that an important factor in causing the dying back of the shoots was rapid and repeated desiccation of the meristems. In the terminal meristem this resulted in a premature loss of the dominance of the apical bud, and its replacement by daughter shoots, which in turn suffered the same fate; and in the cambium by the production of tangential parenchyma.

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[*Continued on p. 3 of cover.*

No. 32.

ON THE EXTRACTION OF SAP FROM LIVING LEAVES BY MEANS OF COMPRESSED AIR.

By HENRY H. DIXON, Sc.D., F.R.S.,
Professor of Botany in the University of Dublin,

AND

NIGEL G. BALL, M.A.,
Assistant to the Professor of Botany in the University of Dublin.

[Read November 27. Printed December 7, 1923.]

It has already been pointed out (1 and 2) that there are many reasons which favour the suggestion that the xylem provides the conducting channel for the transport of organic substances in plants.

According to this theory a backward current of water containing dissolved carbohydrates, etc., must pass, either continually or intermittently, from the leaves to places of growth and storage.

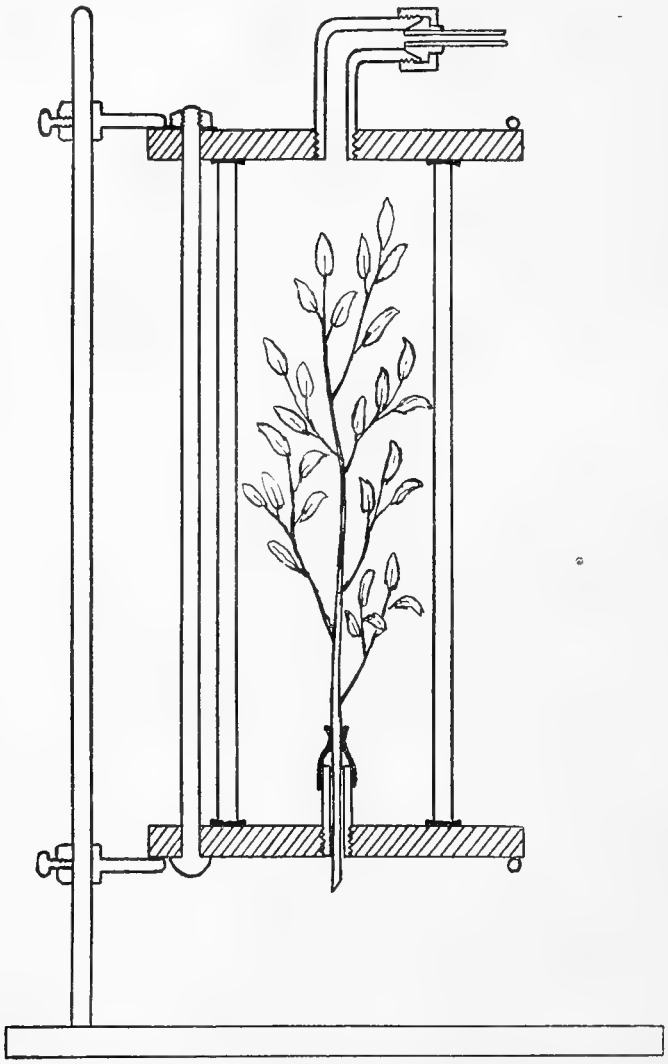
By the application of a method which was used by one of us (3) a long time ago for the direct measurement of osmotic pressure in plants, it was found possible to cause a backward current to flow in the xylem from the leaves towards the lower parts of the plant. It seemed desirable, therefore, to test whether this current, although produced artificially, would be utilised by the plant for the purpose of transporting carbohydrates.

The apparatus used is represented in the diagram. It consisted of a thick-walled cylinder of specially annealed glass about 40 cm. long by 12 cm. external diameter. (Owing to the bursting of this cylinder it was subsequently replaced by a similar one made of mild steel.) The ends of this cylinder were closed by strong castings secured by three long bolts, the joints between the cylinder and the castings being made air-tight by means of leather washers impregnated with wax. The cut end of the branch was passed through a tubulure in the lower casting, the joint being secured by thick rubber tubing wired on and covered with glue. Compressed air, supplied through a lead tube of narrow bore, was admitted at the top of the cylinder. In order to obtain the necessary pressure a pump was used in the earlier experiments, but later this was replaced by a cylinder of compressed air. The pressures were measured by means of a Bourdon pressure gauge.

The experiments were carried out as follows:—a small branch was fitted with its cut end protruding through the lower casting. The cylinder was then assembled and bolted up tightly, the waxed-leather washers being previously rendered soft and limber by heating. The compressed air was then admitted. At a pressure of from 5 to 8 atmospheres drops began to appear at the cut end. By gradually increasing the pressure to a maximum of 20 atmospheres something more than 3·5 c.c. of liquid could be obtained from a small branch.

July, 1922.

Branches of *Sambucus nigra* and of *Tilia americana* were subjected to pressures up to 15 atmospheres. From 1 to 3 c.c. of liquid was obtained from each branch; but when boiled with Benedict's solution this liquid, whether inverted with hydrochloric acid or not, showed complete absence of sugars. Some of the branches were kept in the dark for 24 hours before the experiment, but this did not affect the result.



September, 1922.

Branches of *Tilia americana* were again subjected to pressure. The liquid obtained gave a very slight reduction with Benedict's solution. The amount of reduction was not increased by previous inversion of the sample with hydrochloric acid.

In one experiment a branch weighing 42 g. was exposed to 20 atmospheres for 20 minutes, i.e. until no more liquid was obtained. The amount of liquid yielded was 2·25 c.c. The pressure was then reduced and a few c.c. of toluene was introduced into the cylinder. After 1½ hours a pressure of 20 atmospheres was again applied and continued until no more liquid was obtained. The amount yielded this time was 6·5 c.c. This liquid was brown in colour and, after inversion with invertase, was titrated with Benedict's solution. About 5 per cent. of sugar was found to be present. The branch was then removed and dried. Its dry weight was found to be 17 g. The amount of liquid extracted represented therefore about 35 per cent. of the total amount of water in the branch.

June, 1923.

An experiment with *Tilia americana* gave a similar result to that obtained in September, 1922.

It seemed possible that the liquid, which was obtained before the cells were rendered permeable with toluene, represented only that which was present in the wood at the time of the experiment, being forced out by the sap pressed from the leaf cells. To obtain this latter with as little dilution as possible the following arrangement was made:—Three small branches supporting together 18 large and 4 small leaves were fixed in the lower casting and enclosed together in the apparatus. From these about 3 c.c. were extracted under a pressure of 20 atmospheres. The volume of the wood, excluding the negligible amount in the veins of the leaves, was estimated by making cross-sections of the twigs at various levels, and found to be about 2 c.c. About 50 per cent. of this volume would be represented by the walls of the tracheae; so that about 2 c.c. of the liquid obtained must have come from the cells of the leaves. The first few drops of the liquid which were collected showed the presence of a trace of reducing sugars, due probably to contamination from the cut surface of the twigs; but the remainder gave no reduction with Benedict's solution either before or after inversion with hydrochloric acid.

It seems clearly established by these experiments that, even when the cells of the leaves contain considerable quantities of sugar, no part of this sugar can be driven by external pressure from the tracheae of the supporting branch. This may be due to the continued impermeability of the leaf cells under the conditions of the experiment, or possibly the cells in contact with the conducting channels at lower levels extract what sugars pass into these channels from the leaves, and prevent the carbohydrates appearing at the cut surface of the stem.

In order that a backward transport of organic substances from the leaves to the stem may take place through the wood, two conditions must be fulfilled:—(1) The cells, owing to internal or external causes, must be rendered permeable; (2) a backward flow must take place through the tracheae. It is probable that the second condition would follow as a result of the first, since then any tension existing in the tracheae would be no longer resisted by the osmotic pressure of the leaf cells. The experiments described above seem to show, however, that the first condition is not of necessity a result of the second.

At the present state of our knowledge it would be useless to speculate on the underlying causes which, under normal conditions, effect changes in the permeability of plant cells. No matter what theory regarding the mechanism of transport be held, it seems to be necessary to postulate the occurrence, either gradually or suddenly, of such changes. It is probable that further progress towards the elucidation of the problem of the transport of organic substances

in plants can only be made when the conditions governing the permeability of plant cells are better understood.

SUMMARY.

Branches of *Tilia americana* and *Sambucus nigra* were enclosed in a strong cylinder in such a way that their cut ends protruded. Compressed air at pressures up to 20 atmospheres was admitted into the cylinder, and the liquid which exuded from the cut end of the branch was collected. This liquid was found to be completely, or almost completely, free from sugars. Experiments carried out in early and late summer gave similar results. After the leaf cells had been made permeable by means of toluene vapour the sugar in the expressed sap amounted to about 5 per cent.

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No. 33.

SOME EXPERIMENTS ON THE CONVECTION OF HEAT IN VERTICAL WATER COLUMNS.

By H. H. POOLE, Sc.D.

[Read November 27. Printed December 7, 1923.]

Introduction.

In view of the importance of radioactive heating in modern geological theories, considerable interest attaches to the question as to how far the estimated upward flow of heat through the earth's crust accords with that to be expected from the known radioactivities of the surface materials. The figure obtained by multiplying the thermal conductivity of dry rock by the average temperature gradient just below the surface is surprisingly small. The question has recently been discussed by the writer elsewhere.[1] It would appear that there are several factors tending to reduce the gradient near the surface, one of these being the effect of underground water, which may bring heat to the surface either by means of thermal springs rising from considerable depths or by convection currents in "stagnant" water in pores, pockets, or fissures in the rocks, thus adding to the effective thermal conductivity of water-logged strata.

An account is here given of some experiments on the convection of heat in vertical water columns, made with a view to estimating, if possible, the probable magnitude of the heat flow in stagnant water. As might be expected, the effect appears to be extremely complex, depending greatly on the size, and especially on the diameter, of the column.

Experimental Details.

Fig. 1 represents in section the general outlines of the apparatus used, the scale being 1/5. The column of water under test was contained in a glass tube *A*, open at both ends, connecting a copper vessel *B*, about 6 cm. deep by 4 cm. in diameter, with an open upper vessel *C*, the cork stoppers through which *A* passed being rendered water-tight with paraffin wax. The outer cylindrical surface of *B* was uniformly covered with a heating winding of silk-covered 28 S.W.G. Nichrome wire, wound directly on the vessel, and covered with molten paraffin wax. This winding extended from near the bottom of the vessel to within 1 cm. of the lower surface of the cork. Inside the vessel were placed 24 copper rods, each 5 cm. by 0·4 cm., 16 of them being approximately vertical, reaching from the top to the bottom of the water space, near the wall of the vessel, and the remaining 8 crossing it diagonally at an angle of about 45° to the vertical. This arrangement of rods, which is not shown in the figure, left a conical region just below *A* unobstructed, while ensuring that the water in it was at an approximately uniform temperature. *C* was kept cool by a stream of tap-water

[1] Phil. Mag., September, 1923.

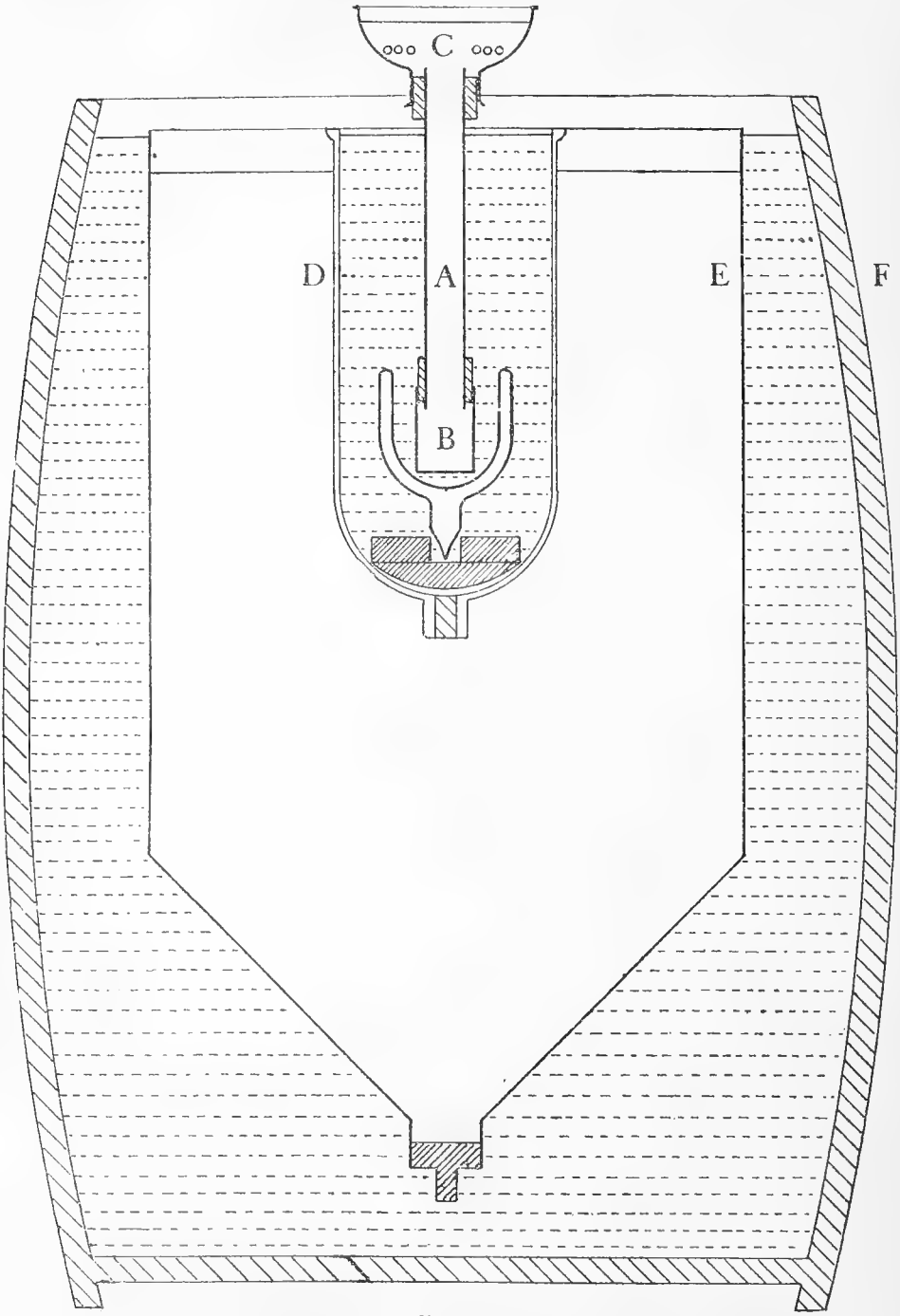

Fig. 1.

flowing through a flat metal worm immersed in it. In most of the experiments, the water, before entering the worm, passed through a coil of metal tubing immersed in the lower part of the large water-bath *E*.

A and *B* were enclosed in an inverted bell-jar *D*, the intervening space being filled with granulated cork, and *B* further screened by a Dewar vessel, as shown in the figure. Metal ballast in the bottom and a few small weights on top of the cork kept *D* floating vertically in the water-bath, which was enclosed in a barrel *F*, the intervening space being filled with granulated cork. The worm tube in *C* was independently supported.

The difference of temperature between the ends of *A*, ranging from a small fraction of a degree to about 10 degrees, was read by a thermo-couple, whose junctions were enclosed in thin glass tubes passing through the annular corks into *B* and *C* respectively, so that the junctions were level with and close to the ends of *A*. The temperatures of *C* and *E* were read by a pair of accurate mercury thermometers, one being independently supported with its bulb close to the upper thermo-junction, and the other floating in *E* with its bulb rather below the level of *B*. No stirring was used, as it was desirable to work with the water in A as quiescent as possible, but even so the water in *E* was generally at a very nearly uniform and constant temperature throughout a test.

This arrangement was almost entirely made up of apparatus which chanced to be available. A thermostat would obviously have been an improvement, but a rather complicated system of temperature control would have been necessary, as it was desirable to work below 20° C., and considerable quantities of heat had to be got rid of. Even if *C* and *E* were maintained at constant temperatures, the mean temperature of the water in *A* would vary with the heat supplied to its base, so, to keep the latter temperature constant, *C* should be cooled as the power supply was increased. In view of the essentially rough character of the tests such a complication did not appear to be justified.

The thermo-couple consisted of 5 pairs of iron and constantan wires, the total resistance being 8·5 ohms, and was connected through a Morse telegraph key to a galvanometer of resistance about 800 ohms, the key being arranged so that the galvanometer was normally short-circuited. The couple was inserted in the circuit by depressing the key, which was enclosed in a wooden box and operated by a long vulcanite handle to reduce thermal effects. The zero was re-set, when necessary, by moving the galvanometer lamp, the scale being fixed. In this way thermal effects at the galvanometer were almost completely eliminated. It was found that leakage from the battery supplying the heating current might cause a shift of a few divisions in the zero, but did not appreciably affect the deflection produced by depressing the key. This was tested by reversing the heating current, and also by momentarily disconnecting the battery. In the later experiments the zero shift was entirely eliminated by earthing one end of the heating winding, which was electrically connected to the vessel *B*. Identical results were obtained whether this earth connexion was made or not.

The couple and galvanometer were calibrated throughout the scale of 500 divisions by comparison with the two mercury thermometers, these, in turn, being compared with a standard. The deflection per degree varied from 32·9 scale divisions for small deflections to 32·0 for large ones, and was sensibly independent of the mean temperature of the couple over the range (10° C. to 25° C.) covered.

The current and the P.D. across the heating coil were both measured, and it was found that over a large part of the range of power used, the resistance

of the coil remained sensibly constant. The power supplied ranged from 0·04 watt to 25 watts.

Two sets of tests were carried out with the heating and cooling vessels filled with water, but the connecting tube, or tubes, corked and empty. First the initial rate of rise of temperature was noted when a known power was turned on, so that the effective thermal capacity of the heating vessel and its contents could be found. This allowed a small correction to be applied, when necessary, for any slight changes of temperature during a convection test. Secondly, by noting the final temperature attained by the heating vessel for a given power, it was found that the loss of heat through the cork and the vacuum vessel was proportional to the difference of temperature between the heating vessel and the surrounding water-bath. A correction for this loss was thus made. This was also small, except in cases of low power supply with a narrow tube, when it became very serious.

The stopper was then removed from the top of the tube, which was filled with water, and the convection tests started. The usual procedure was to turn on a certain current, start the cooling water flowing in the worms, and leave the apparatus for several hours, until a steady temperature had been attained. A series of readings of the voltmeter, ammeter, galvanometer, and thermometers over a period of 8 to 10 minutes was then made. From these the rate of flow of heat up the tube for a given temperature difference was found. In allowing for the effect of small variations in temperature the effective thermal capacity of the heating vessel and contents was taken as that already found with the convection tube empty plus half the volume of the tube.

The most satisfactory results were those obtained towards the middle of the power range. For small powers the correction for heat loss becomes very important and somewhat uncertain, as a very appreciable part of the heat flowing up the lower part of the convection tube must escape laterally through the cork jacket. On the other hand, near the top of the range the convection currents sometimes became unsteady, causing the temperature difference indicated by the couple to vary rapidly over a range of perhaps 20 per cent.

Five sets of tests were carried out with single columns of various diameters and lengths, and three sets with double columns, consisting of two parallel glass tubes of as nearly as possible the same dimensions, with their ends at the same level. In these cases circulation almost certainly occurred, warm water rising in one tube and cool water descending in the other. The tubes were separated by about 5 mm. of cork, which would form a fairly efficient thermal insulation.

The last pair of tubes tested being 2·8 cm. in diameter, necessitated the use of larger heating and cooling vessels, a larger Dewar vessel, and a larger floating vessel. In this case the heating coil was wound in several sections in parallel, the joint resistance being 9 ohms, and the power range from 0·4 watt to 66 watts.

As it was not possible to carry out all the tests with the same mean temperature of the convecting column, some experiments were made with heated water flowing through the worms in the water-bath and the cooling vessel. In this way the average temperature of the convecting water was raised without increasing the temperature gradient in it. The tests were in general less satisfactory than those with cold circulating water, as the temperatures were more variable. With a single column the rather surprising result was obtained that the heat flow for a given gradient did not vary very much for a range of mean temperature from 13·5° to 23·5° C. There was some

evidence of a maximum at about 17° C. followed by a fall for higher temperatures. With double columns the results were much more consistent, the heat flow for a given gradient being approximately proportional to the excess of the mean temperature above 4° C.

We should expect, at first sight, that the flow of heat should increase with the temperature at about this rate, owing to the increase in the coefficient of expansion and the decrease in the viscosity. The contrary results obtained with the single column are probably to be ascribed to the increased mixing of the ascending and descending currents with rise of temperature. In the case of the double tube this mixing cannot occur.

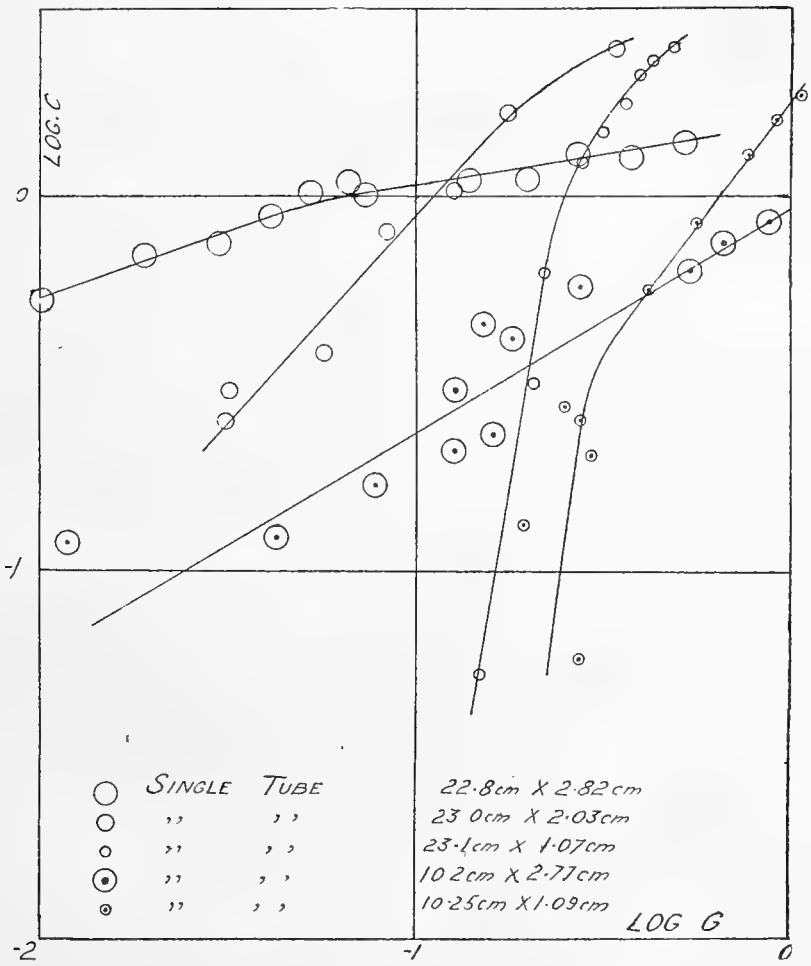

FIG. 2.

Results.

The results for single columns are plotted in fig. 2, and those for double columns in fig. 3, the lengths and diameters being shown on the figures. Since it was desired to estimate the probable magnitude of the effect of convection

on the thermal conductivity of the earth's crust, it appeared to be most convenient to state the results in terms of a quantity analogous to the conductivity of a solid. This quantity, which may be called the "convectivity," is denoted by the letter C in the figures, and is defined as the heat flow per sq. cm. of total horizontal area per sec. divided by the gradient G. As, however, the extreme range of C in the plotted results is from 5×10^{-2} to over 10, and that of G from 5×10^{-3} to over 1, all the results could not conveniently be plotted directly on one scale, so their logarithms are plotted instead.

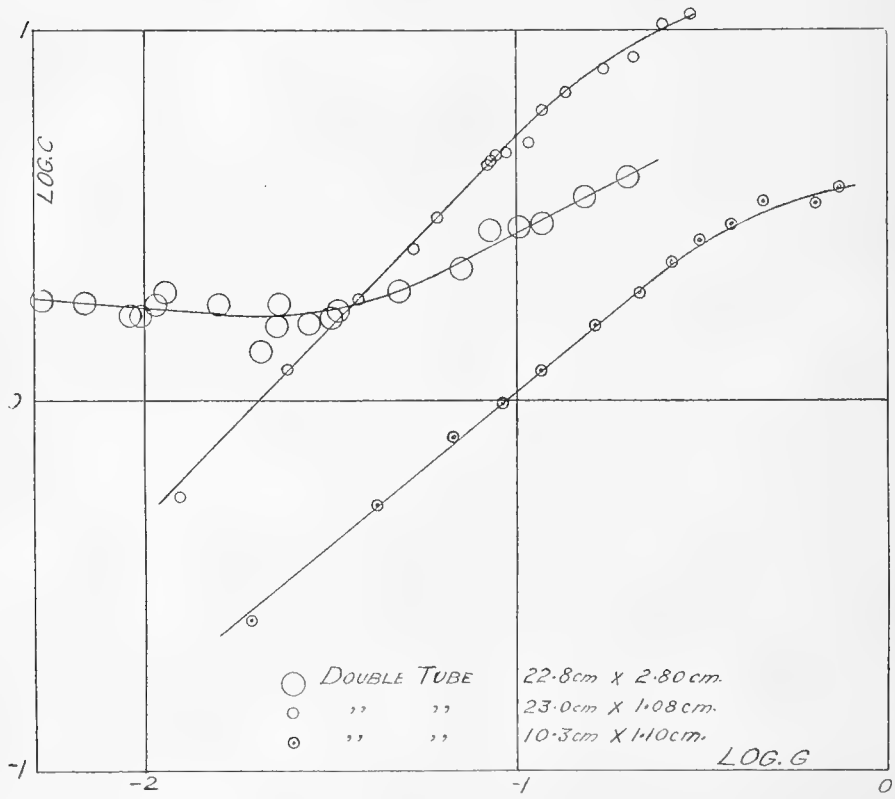

FIG. 3.

In the case of the results shown in fig. 2 no correction has been made for the mean temperature of the water column, as the effect of variations of a few degrees at about air temperature does not seem to be very important for single columns. The general average of the mean temperatures for these results may be taken as about 15° C., the temperature generally increasing with increase of gradient.

The results for the double columns shown in fig. 3 have all been reduced to a mean temperature of 17° C. by assuming that the convectivity is proportional to the excess of the mean temperature above 4° C. As unavoidable small differences occurred in the dimensions of the glass tubes, the figures given are, in each case, the means for the pair.

It will be observed that the convectivity generally rises rapidly with rise in gradient, the effect becoming more and more marked as the diameter is

reduced. The length of the column has also a great effect on the convectivity for a given gradient; in fact, the flow of heat up a tube of given diameter, for a given temperature difference between the ends, was generally about the same for short and long tubes, in spite of the fact that with the former the gradient was much greater.

Since the gradient in the earth's crust is about 3×10^{-4} we have to extrapolate the results down to a value for $\log G$ of $-3{\cdot}52$. It is evident that no reliance could be placed on the actual figures thus found, but they should furnish some idea of the magnitude of the convection effect in cavities of various sizes in the earth's crust. If we take the curves for the three long tubes, each about 23 cm. in length, which gave much more consistent results than the shorter ones, and assume that their slopes remain unchanged for small gradients, we obtain for the convectivity at earth gradient, $1{\cdot}6 \times 10^{-1}$, $1{\cdot}5 \times 10^{-3}$, and 4×10^{-18}, for single columns, 2·8, 2·0, and 1·1 cm. in diameter, respectively. For a double column each 2·8 cm. in diameter the corresponding figure is 2·4, and for a pair 1·1 cm. in diameter, $1{\cdot}4 \times 10^{-2}$. The shorter columns would, in each case, yield lower results.

The enormous importance of area of cross-section, especially in the case of single columns, is at once apparent. It would seem that for single columns less than 2 cm. in diameter, and for double ones less than 1 cm., the effect of convection at earth gradient should be negligible. Thus we should expect that, in the upward flow of heat through a water-logged porous rock, convection would play a very small part. The presence of water in the pores would doubtless greatly reduce their thermal resistance and prevent the rock from behaving as a very bad conductor of heat like dry pumice, but there is no reason to suppose that it would raise the effective thermal conductivity even as high as that of an otherwise similar rock devoid of pores.

On the other hand, the presence of water-filled fissures a couple of centimetres across, or more, should add very considerably to the upward flow of heat, especially if their shapes were such as to favour continuous circulation. The results obtained with the largest pair of tubes give a value of the convectivity some 400 times as great as the conductivity of common rocks. We might expect that a similar circulation would occur in a single fissure if its extension in both directions in its plane were considerable compared with its width.

The evidence of these experiments is, then, in favour of the view that convection in stagnant water in the earth's crust is of importance only in regions where fissures of appreciable size exist in the strata.

In conclusion I wish to express my gratitude to Prof. W. E. Thrift, F.T.C.D., for the laboratory facilities which he so kindly granted to me in Trinity College, Dublin.

Summary.

Experiments are described on the convection of heat in single and also in double vertical water columns.

The results show that the flow of heat in most cases increases much more rapidly than the temperature gradient. The smaller the column the more rapid is the rise of heat flow with rise of gradient.

It is concluded that, for the small gradient existing in the earth the effect of convection in water-logged porous rocks would be negligible. Where, however, water-filled fissures occur, we should expect an appreciable increase in the vertical flow of heat.

No. 34.

ON THE SUPPOSED HOMOLOGY OF THE GOLGI ELEMENTS OF THE MAMMALIAN NERVE CELL, AND THE NEBENKERN BATONETTES OF THE GENITAL CELLS OF INVERTEBRATES.

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AND

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(Plate XVII.)

[Read November 27. Printed December 10, 1923.]

I.—Introduction and Summary of Previous Work.

In the year 1898 Camillo Golgi (5) demonstrated, for the first time, the "apparato reticulare interno" in the nerve ganglion cells of the spinal cord. About the same time, but quite independently, Veratti achieved a similar result. Since then much work has been done on the Golgi apparatus of the somatic cells of vertebrates, and it has, in consequence, been demonstrated in all the cells that have been carefully examined by competent observers. Weigl, Hirschler, Gatenby, and others have applied the silver and osmium methods to the germ cells of invertebrates, and have demonstrated in them an apparatus which can be followed through the various stages of maturation, fertilization, and development. This apparatus is, in all probability, an integral part of every animal cell at some period of its life. The apparatus of the reproductive cells of the invertebrates, the "nebenkern" of some older writers, is considered by many to be strictly homologous to the Golgi apparatus of the vertebrate ganglion cells. In this paper we have endeavoured to state the evidence, already known, bearing on this question, and to put forward some more which we have gleaned by a careful study of the ganglion and other cells of Helix. We venture to believe that the whole body of evidence is sufficient to prove beyond doubt the homology of the "Golgi" bodies found in the various animal cells already mentioned.

II.—Methods and Technique.

The following work was carried out on *Helix aspersa*. As it was carried out in winter, the specimens employed had to be roused from their hibernation by immersing them in warm water. When a specimen was required for work, its head was cut off with a razor, without the use of any anæsthetic, and the cephalic ganglion was quickly excised and transferred immediately to a capsule of the fixative.

Da Fano's Golgi apparatus technique was employed with success. The best results were obtained with this method by fixing for two hours, washing quickly in aq. dist., and transferring to 1·5 per cent. silver nitrate solution. After being kept in the dark for two days the specimens were quickly washed in aq. dist.

and transferred to Cajal's reducer for two days. They were then again quickly washed and embedded in paraffin. The method of Cajal was also employed with success.

Champy-Kull fixation followed by Champy-Kull staining or iron-hæmatoxylin was very valuable, as was also Flemming-without-acetic-acid and iron-hæmatoxylin technique.

Good results were obtained by the Mann-Kopsch method. The material was fixed in the osmo-sublimate solution for one hour and impregnated with 4 per cent. OsO_4 for 14 days. Subsequently some of the sections were extracted with turpentine for some days, and stained with Altmann's acid-fuchsin.

Material was also fixed in 10 per cent. neutral formalin for two days, or in 90 per cent. alcohol for one day, both methods being followed by iron-hæmatoxylin staining. Petrunkewitsch was used, followed by toluidin blue, warmed on the slide.

The live ganglion cells were carefully examined in their own lymph, and also stained with Soudan III. in 70 per cent. alcohol, Dahlia 0·75 per cent. in 0·75 per cent. salt sol., Bismark brown in 1 per cent. acetic acid, Janus green 1 in 10,000 (approximately) and 1 per cent. osmic acid.

III.—General Description of the Ganglion Cells.

Neurones at every stage of development can be found, at the same time, in the cephalic ganglion of the adult Helix. The smallest of these measure little over 7 μ in diameter, while the largest attain a size of over 110 μ.

In preparations of the living ganglion cell, mounted in its own lymph, the axon and numerous branched dendrons are well seen. The cytoplasm of the body of the living cell appears coarsely granular. On the average the diameter of the nucleus is about two-thirds of that of the whole cell; this proportion remains approximately constant no matter what the actual size of the cell may be. In all the successful silver preparations it was observed that the majority of the nuclei did not take the silver, while other nuclei were deeply impregnated, the chromatin granules showing up well. The nucleoli failed to take the silver even in the most deeply impregnated nuclei.

IV.—The Golgi Apparatus.

The silver methods of Da Fano and Cajal and the Mann-Kopsch osmium technique impregnate well the Golgi apparatus in the nerve cells of Helix.

In the smallest neurones observed, the Golgi apparatus was in the extra-centric juxta-nuclear position (Pl. XVII, fig. 1). At this stage it consists of a large number of curved rods, lying on an archoplasmic sphere. This apparatus, at a slightly later stage, divides, the resulting portions tending to pass around the nucleus (fig. 2). The breaking up and scattering continues, the apparatus passing through a stage (fig. 3) consisting of a number of archoplasmic discs, each surrounded by three or four rods. At the end of this process all the rods, each with a portion of archoplasm filling its concave side, are separated and scattered completely around the nucleus. After this no further change takes place, but with the growth of the cell they greatly increase in number (figs. 4 and 5). In a large neurone the number of Golgi rods, some of which are straight, others curved or ring-shaped, is enormous. They form a zone around the nucleus, and pass out some distance into the dendrons, but are not found in the periphery of the cell. They are not found in the axon or axon-hillock, and are much fewer and markedly smaller in the region between the hillock and the nucleus (fig. 5).

In material fixed in Champy-Kull, Flemming-without-acetic-acid, Petrunkewitsch, 10 per cent. formalin, or 90 per cent. alcohol, the Golgi rods appear as unstained ghosts. On account of the highly granular nature of the cytoplasm, it is impossible, in the living cell, to identify the Golgi rods with certainty, but highly refractive bodies, which are probably they, and resemble the nebenkern batonettes of the living spermatocyte, can sometimes be distinguished. Attempts were made, by centrifuging the ganglia in their own lymph, to segregate the various cytoplasmic inclusions into distinct regions of the cell. It was hoped that by this method the Golgi rods could be made to occupy a definite region, and could, in consequence, be more easily identified "intra vitam." The apparatus, however, remained scattered around the nucleus in Da Fano preparations, which had been fixed immediately after centrifuging for half an hour at 4,000 revolutions per minute, occupying exactly the same position as in the normal cell.[1]

V.—The Mitochondria.

The mitochondria appear as numerous minute golden specks scattered through the cytoplasm after the silver methods of Da Fano and Cajal. They appear brown in Mann-Kopsch preparations, and dark grey in material fixed in Champy-Kull or Flemming-without-acetic-acid, and stained in iron-hæmatoxylin. The mitochondria are therefore not unique, but closely resemble those described from the ganglion cells of many vertebrates (Cowdry).

VI.—The Nissl and other Granules.

In order to see if the tigroid substance could be identified in the neurones of Helix, some material fixed in Petrunkewitsch, and stained by Scott's hæmatoxylin-eosin method (see Bolles Lee, 1922), or in toluidine blue, was studied. No marked granulation was present, but in all the cells fine flocculent granules, taking the blue stain deeply in each case, were scattered all through the cytoplasm of the cell body, and were in places more definite and better developed. It is probable that these basophil granules do represent the tigroid body of the neurones of vertebrates.

In the live cell a number of granules of uniform size are easily seen (fig. 7). They are scattered through the cytoplasm, but may be more or less aggregated in places. These granules are present in every neurone, and appear to be a definite constituent of these cells. They have a strong affinity for Soudan III, and also take Janus green and Neutral red. They do not stain perceptibly in Bismark brown, Dahlia, or 1 per cent. osmic acid. They do not appear after fixation in Da Fano's, Cajal's, Mann-Kopsch's, or Petrunkewitsch's fluids, or in 90 per cent. alcohol, or 10 per cent. formalin. They are preserved in Champy-Kull's fixation or Flemming's fluid without acetic acid, and stain well if these methods are followed by the iron-hæmatoxylin technique (fig. 6). They take the Fuchsin strongly after Champy-Kull staining. On centrifuging they collect at the top of the cell. It is difficult to state the nature of these granules from the above results; they are possibly lecithin or an allied substance.

VII.—Argentophil Zones.

A dark perinuclear zone is present in many of the neurones of Helix prepared by the silver methods of Da Fano and Cajal. This zone is approximately coincident with the distribution of the Golgi rods, and is narrower and less dense

[1] We would like to take this opportunity of thanking Professor H. H. Dixon, of Trinity College, for his kindness in allowing us to use the centrifuge in the School of Botany.

at the end of the nucleus near the axon-hillock (fig. 5), where, as already remarked, the rods are few and unusually small. This perinuclear zone is not seen "intra vitam," nor is it shown by any of the other techniques employed. The zoning is similar to that described by one of the authors (J. B. G.) in the oocytes of Saccocirrus prepared by Da Fano's method.

In many of the neurones, in which the Golgi apparatus is in process of fragmentation and spreading around the nucleus, dark zones, similar to those around the nuclei of the larger cells, can be observed around each rod or group of rods (figs. 2–4). This appears to indicate that the Golgi rods, not the nucleus, as suggested in the case of Saccocirrus, are connected with their formation.

In material fixed in Petrunkewitsch's fluid and stained by Scott's hæmatoxylin-cosin method (see Bolles Lee, 1922), the cytoplasm of the neurone is seen to be mainly basophil, with the exception of the axon, which is oxyphil. In medium-sized neurones, however, a juxta-nuclear oxyphil cloud appears. It seems possible that this cloud is connected with the argentophil clouds described in silver preparation, but this question is more fully dealt with by one of the authors in a separate paper (F. W. R. B., 2).

VIII.—Holmgren's Canals.

Canals, similar to those originally described by Holmgren, are present in the neurones of Helix, and were observed in many of our preparations. They are of considerable length and sometimes branched (fig. 8), thus in no way resembling the Golgi rods, as seen in our most thoroughly impregnated material. We are not prepared, in this case, to affirm or deny the extra-cellular connection of these canals, but in some neurones they were so related to processes of the sub-capsular cells that they might be interpreted as continuous with them.

IX.—On the Visibility of the Golgi Apparatus of Genital Cells "intra vitam."

If the ovotestis of Helix be teased out "intra vitam" and examined in its own lymph without staining, the nebenkern batonettes of the sperm cells of all stages can be seen clearly. The apparatus is also visible while in the eccentric position in the young oocytes, but, as it scatters in growth, gradually it becomes indistinguishable. These observations support those from fixed material, in which the nebenkern elements alter in chemical constitution during dispersal through the cytoplasm. This chemical change is accompanied by a change in refractivity, which tends to make them invisible "intra vitam," although they are still freely demonstrable by the osmic and silver nitrate techniques.

The fact that in most cells the Golgi elements are invisible "intra vitam" has been urged as evidence that in fixed preparations the so-called Golgi apparatus is merely an artifact. That this argument is not convincing may easily be shown, for, in the first place, the micro-chemical methods used for demonstrating the Golgi apparatus reveal the latter both in cells in which the Golgi elements are visible "intra vitam" (e.g. snail spermatocytes), and in cells in which the Golgi elements are not visible "intra vitam" (e.g. snail neurones).

Moreover, we have instanced the case of snail oocytes where, in the young cells, the apparatus is visible "intra vitam," but, as the yolk begins to form, the apparatus undergoes certain chemical changes, and is then no longer visible, but is still easily demonstrable after fixation.

X.—Discussion.

From the time of its discovery the Golgi apparatus of the nerve cell of mammals was recognised as a branching network, each element tending to

anastomose with its neighbour. Many observers who have worked almost exclusively on mammalian material, when shown the peculiar banana-shaped batonettes of mollusc, annelid, insect, and other invertebrate cells, have expressed doubts as to the homology of the Golgi apparatus of their material with these structures in the cells of invertebrates.

The evidence for the view that the two sets of structures are truly homologous falls into the following groups :—

1. In the neurones of invertebrates the Golgi apparatus techniques reveal an apparatus formed mainly of isolated rods and batonettes. This apparatus occupies the same position in both young and old neurones as does the true Golgi apparatus in young and old mammalian nerve cells.

2. The micro-chemical evidence shows that the silver nitrate techniques of Golgi, Cajal, and Da Fano consistently reveal both the dendriform apparatus of the mammals and the batonette or isolated elements of the lower animals.

3. The osmium techniques of Kopsch, Mann-Kopsch, and Sjovall likewise consistently impregnate these two sets of bodies in the vertebrate and the invertebrate animals.

4. The embryological evidence shows that the so-called Golgi bodies of eggs, during segmentation and histogenesis, are divided or sorted out among the blastomeres and their tissue derivatives, and that finally these egg Golgi bodies form the Golgi apparatus in the cells of the nervous system. Thus, cytologically the Golgi bodies of the egg have been traced from the small eccentric batonettes of the primitive germ cells, and embryologically their derivatives in the egg have been traced into the nerve cells of the new organism. The chain is therefore complete.

5. In many vertebrate animals (e.g. the frog) the Golgi apparatus of the nerve cell is formed of elements intermediate in shape and appearance between the banana-shaped genital element of invertebrates and the tree-like or net-like elements of the mammalian neurone.

It must then be admitted, especially in view of the embryological evidence (Hirschler and Gatenby, 3 and 6), that there is a complete continuity between the genital Golgi element and the nerve Golgi element. Some observers have expressed doubts as to the reliability of the various silver nitrate techniques; our evidence, however, does not depend solely on similarity of micro-chemical reaction. On the other hand, we recognise fully that the techniques of osmic acid and silver nitrate often reveal wide differences in micro-chemical reactions between the batonettes of invertebrate genital cells and the Golgi apparatus of mammalian cells: this is not a serious matter, however, because equally wide micro-chemical differences exist not only between the Golgi elements of different animals, but also between the Golgi elements of different tissues of the same animal.

We believe that the evidence adduced in this paper is sufficient to prove that the batonettes or nebenkern rods of such an animal as Helix, the snail, are homologous with the remarkable Golgi inner network of the mammalian neurone.

SUMMARY.

1. The Golgi apparatus in the smallest neurones of Helix was in the perinuclear extra-centric position, surrounding an archoplasmic sphere. In larger neurones it becomes dispersed around the nucleus and the individual elements become much more numerous.

2. Basophil granules, probably representing the tigroid body, and also lecithin (?) granules, are described in the neurones.

3. In silver preparations dark zones are found around the Golgi elements. These probably represent a product of its activity.

4. Long and sometimes branched Holmgren canals were found in the neurones. They were separate and distinct from the Golgi elements. There was some evidence for considering them processes of the subcapsular cells, but not sufficient to come to any definite conclusion.

5. From the position occupied by the apparatus in nerve and germ cell, from its similarity of micro-chemical reaction in both, and from embryological evidence, it is believed that the nebenkern batonettes of the invertebrate germ-cells are homologous to the Golgi network of the mammalian neurone.

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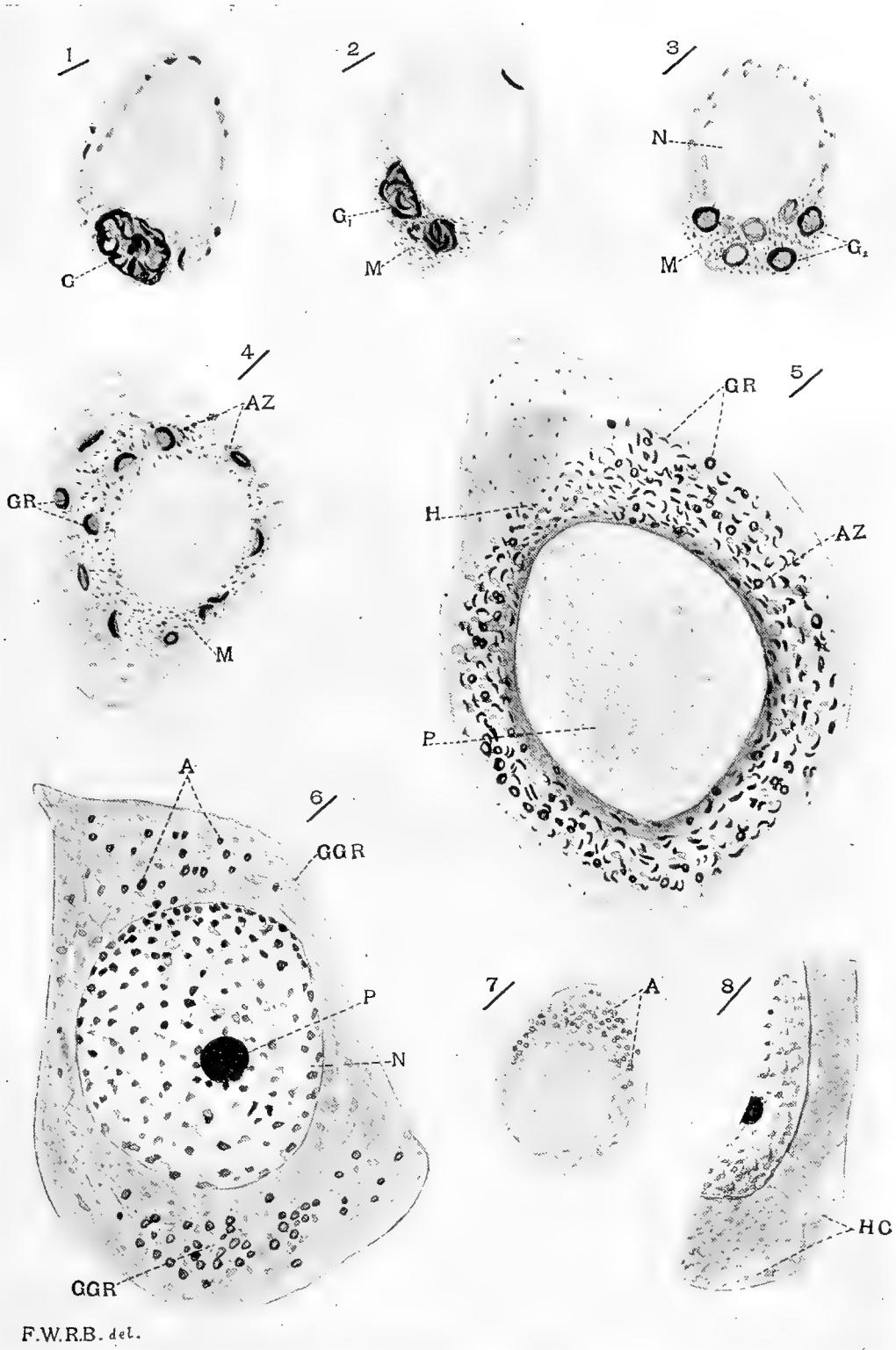
Description of Plate XVII.

Key to lettering:—Golgi apparatus in extra-centric position (G.), and in subsequent stages of breaking up (G_1 and G_2), Golgi Rods (G.R.), Ghosts of Golgi Rods (G.G.R.), Argentophil zone surrounding Golgi elements (A.Z.), Mitochondria (M.), Holmgren's canals (H.C.), Granules (Lecithin?) (A.), Nucleus (N.), Plasmasome (P.), Region of Axon hillock (H.).

These drawings were made with the help of a camera lucida.

Fig.

1. Small neurone with Golgi apparatus in perinuclear extra-centric position. Da Fano preparation. × 4000.
2. Small neurone with Golgi apparatus beginning to spread. Da Fano preparation. × 4000.
3. Further stage in spreading out of Golgi apparatus. Da Fano preparation. × 4000.
4. Medium-sized neurone with Golgi apparatus spread around nucleus. Da Fano preparation. × 2500.
5. Large neurone. Golgi rods small and scarce in region of Axon hillock (H.). In this and the preceding figure the argentophil zones around the rods are shown. Da Fano preparation. × 600.
6. Large neurone showing lecithin granules (A) and ghosts (G.G.R.) where the Golgi rods have been dissolved away. Champy-iron-haematoxylin preparation. × 1300.
7. Medium-sized neurone showing lecithin granules. From living cell.
8. Portion of large neurone showing Holmgren's canals in cytoplasm. × 1300.



BRAMBELL AND GATENBY.

No. 35.

PHOTOTROPIC MOVEMENTS OF LEAVES.—THE FUNCTIONS OF THE LAMINA AND THE PETIOLE WITH REGARD TO THE PERCEPTION OF THE STIMULUS.

By NIGEL G. BALL, M.A.,
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(Read December 18. Printed December 28, 1923.)

In spite of a considerable amount of work which has been done from time to time, the various factors involved in the orientation of leaves in response to light are by no means clearly understood. This applies especially to the question as to how far the lamina exerts a directing influence on the petiole, and so far unanimity on this point has not been attained.

Darwin (1) first showed that in experiments on leaves of *Tropaeolum majus* and *Ranunculus ficaria*, in which the blades were covered with black paper, the petioles became curved towards the light as completely as those of unprotected leaves. Vöchting (2), on the other hand, concluded that in the case of *Malva verticillata* the movements of the petiole are controlled by the lamina. Darwin's experiments on Tropaeolum were later confirmed by Rothert (3), who considered that illumination of the lamina was without influence on the bending of the petiole, and Krabbe (4) obtained similar results with Fuchsia and Phaseolus.

Haberlandt (5) repeated the experiments of Darwin and Rothert on Tropaeolum, but found that the correct diaphototropic position was not reached as completely or as accurately by the leaves covered with black paper as by those which were uncovered. He therefore concluded that while the main movement of the leaves of Tropaeolum was effected owing to the phototropic response of the petiole, the finer adjustments were controlled by the lamina. In the case of *Begonia discolor* and *Monstera deliciosa* he found that even when the petioles were covered with tinfoil the leaves were able to reach the correct phototropic position, and concluded that in these plants the lamina is the sole percipient organ, and that it transmits the stimulus to the petiole. Working with Phaseolus, however, he confirmed Krabbe's (4) results, and found that leaves in which the lamina was covered with black paper reached the correct phototropic position as rapidly and as completely as uncovered leaves.

More recently Wager (6) concluded that the "perception of light is located not in the leaf-blade but in the leaf-stalk."

As a result of the experiments which have been carried out by Boysen-Jensen (7), Paál (8), and others, on the transmission of the phototropic stimulus in seedlings, phototropic reactions have lately acquired a new interest, and further work on the movements of leaves seemed desirable.

In the first experiments leaves of *Oxalis macra* Small were used. A number of freshly-plucked leaves were fixed with the ends of the petioles in water in small glass jars. The petioles passed loosely through holes in corks fitting the necks of the jars, and were firmly fixed in these holes by means of

plasticine. These jars were placed in front of a window and suitably shaded, so that they were illuminated from one side only. In some cases the leaves were left intact, and in others the leaflets were cut off where they joined the petiole. The amount of bending was deduced by taking measurements of the horizontal displacement of the top of the petiole.

As a result of a number of experiments, it was found that the petioles from which the leaflets had been removed responded to phototropic stimuli as readily as those of intact leaves. The power of response of the decapitated petioles, however, was lost after a period of one to two days. An experiment in which both the growth in length and the amount of bending in the intact and decapitated petioles were measured, showed that the loss of the power of response in the decapitated petioles could be correlated with cessation of growth. In all cases the region of greatest growth and bending was about 4–6 cm. from the top of the petiole.

In order to obtain evidence regarding the vexed question as to whether phototropic stimuli can be perceived by the lamina and transmitted to the petiole, experiments were carried out on *Sparmannia africana*. This plant was found to be very suitable for the purpose as the leaves readily set themselves in the optimum position relative to incident light. The larger leaves are, on an average, about 15 cm. in length by 12 cm. broad, being supported on petioles about 12 cm. in length.

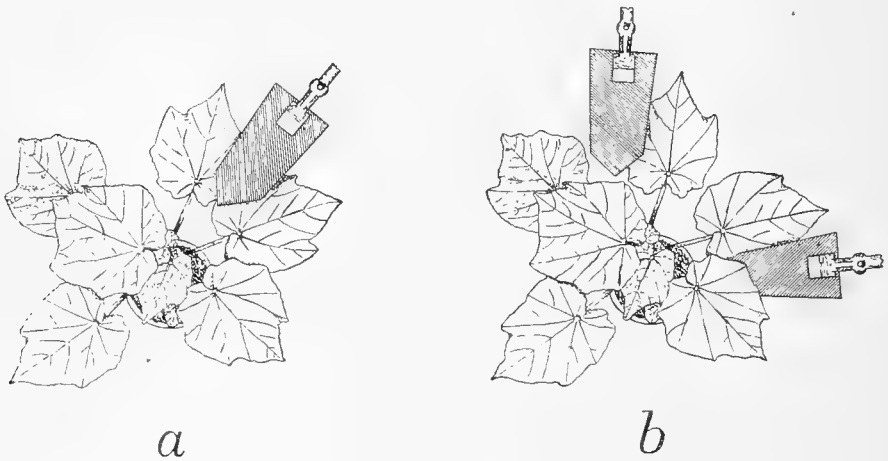

Fig. 1.

Plant of *Sparmannia africana*, viewed from above, showing the methods adopted in shading the leaves.

Two plants were chosen which were growing in pots and had been exposed to light from above in a greenhouse, with the result that the leaf-blades were all horizontal. In each plant a pair of similar leaves lying close together in the same horizontal plane was selected. A piece of cardboard was supported by means of a clamp attached to a retort stand just above, but not touching, the adjacent edges of the two leaves in the manner shown in fig. 1, *a*. The pots containing the plants were standing on the floor of a greenhouse, and were surrounded by tall screens so that they were illuminated from above only. Under these circumstances the shades affected small portions only of the leaf-blades, and were without influence on the light falling on the petioles.

The distance between the tops of the two petioles and also the distance between the two leaf-tips were measured from day to day and the means of these two distances are plotted in fig. 2, which shows the results obtained from the two plants (Expts. I and II), both experiments being carried on simultaneously. At the end of five days the shades were removed and replaced by similar shades which covered the outer edges of the pairs of leaves, as shown in fig. 1, *b*. The experiments were continued for a period of twenty days, the shades being changed at intervals of five days. The curves in fig. 2 show clearly that when the inner edges of the pairs of leaves were shaded the leaves moved apart, and when the outer edges were shaded they came closer together. The slight irregularities in the curves seem to be due to inequalities in the temperature of the surroundings, which varied from day to day during the course of the experiments. The effect due to this cause was particularly marked during the eighth day of the experiments, when, owing to failure of the heating arrangements in the greenhouse, the temperature fell considerably, with the result that movement of the leaves practically ceased.

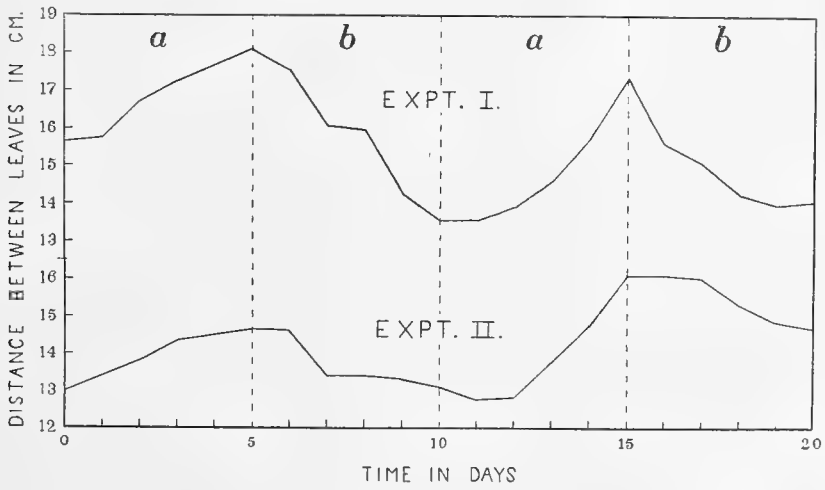

FIG. 2.

Curves showing movements of the leaves caused by shading portions of the leaf-blades. During the periods marked *a* the inner edges of the pairs of leaves were shaded, and during those marked *b* the outer edges were shaded. (Cf. fig. 1, *a* and *b*, respectively.)

The region of each petiole in which the greatest curvature took place was about 1 cm. from the basal end. During the last period of five days represented on the curves, the angles between the petioles were measured, and in one plant the angle between the pair of petioles decreased by 13° and in the other by 10°.

In order to confirm these results a modification of the experiments was carried out on another plant of the same species. As before the leaves were horizontal, the plant being illuminated from above only. Strips of tinfoil were attached to three of the leaves so as to cover one border of the lamina to a distance of about 2 cm. from the edge, the breadth of the lamina being about 10–12 cm. The tinfoil was attached by folding in the edges on the under side of the leaves. In each of the three leaves a slow horizontal movement

of the lamina took place in such a direction that, if the shade had not been attached, the lamina would have moved away from it. The mean horizontal movement of the three leaf-tips after 7 days was 5·5 cm. The tinfoil shades were then removed and placed over the opposite edges of the leaves, with the result that the three leaves moved in a direction contrary to the previous movement, the mean movement of the leaf-tips after 7 days being 4·1 cm. and after 14 days 8·4 cm. During the experiment the stem of the plant was tied to a stake, and the shades were arranged so that the three leaves should not all move in the same direction.

The fact that shading the lamina does influence the movements of the petiole appears to be definitely proved. The transmission of the stimulus from the edge of the lamina to the basal region of the petiole, a distance in this case of about 15 cm., raises some interesting problems which it is hoped to investigate in the near future.

These experiments afford an explanation of the method by which the mosaic arrangement of the leaves of a plant is attained. Should one leaf overlap part of another and thereby shade it from the light, the shaded leaf will tend to move until its edge is withdrawn from the shade of its neighbour. It is difficult to understand how this result could be achieved if the petiole were the sole percipient organ. For, if this were the case, after the petiole had come into position where it was freely illuminated, further movement of the leaf would cease, even if portion of the lamina were still in shade.

Furthermore, in the experiments described above, it is a difference in the light intensity in the shaded and unshaded parts of the leaf which causes the movement. That phototropic movements result as a response to the direction of the light rays and not merely to differences in their intensity has been the opinion of many plant physiologists. This view mainly seems to have been responsible for the different conclusions at which they have arrived as to the directing influence of the lamina on the petiole. For example, in the experiments on *Eranthis hiemalis* described by Wager (6), leaves were exposed to oblique illumination, but in such a way that the intensity of the light over the whole of the lamina was the same. Under these circumstances the petioles which were kept in the dark showed no phototropic curvature. When, however, the blades of the leaves were darkened and the petioles exposed to lateral illumination the conditions were different, since the two sides of the petioles were exposed to different light intensities, with the result that curvature took place. Such experiments, while proving that the lamina is not responsive to change in the direction of the incident light, leave unaffected the question as to its power of responding to differences in light intensity on its various parts.

If a leaf in which the lamina is quite flat is exposed to oblique illumination the whole surface will still be subjected to a uniform light intensity, and no directive influence can be exerted on the movements of the petiole. If, however, the lamina is somewhat curved the light intensity will not be uniform on all parts of the surface, and movements of the petiole may be expected.

Although under appropriate conditions a certain directive influence can be exerted by the lamina on the movements of the petiole, the main cause of these movements normally appears to lie in the perception of differences in light intensity by the petiole itself. That the petioles of leaves are positively phototropic has often been demonstrated, and that Sparmannia is not an exception in this respect is shown by the following experiments.

A plant was exposed to lateral illumination, the blade of one leaf, in

which the petiole was at right angles to the incident light, being enclosed in a bag of black paper in such a way that the petiole was quite uncovered. The petiole bent towards the light as much as that of a similar uncovered leaf on the opposite side of the stem, the region where the greatest bending took place being close to the base of the petiole. Similar results were obtained when the uppermost 3 cm. of the petiole (as well as the lamina) was covered with black paper.

In Sparmannia the motor regions of the petiole lie close to the upper and lower extremities. If the petiole is split longitudinally into several parts, which are then placed in water, the upper and lower ends of these become sharply curved owing to the swelling of the cortical cells while the middle portions remain approximately straight.

The uppermost 5–10 mm. of the petiole is usually nearly at right angles to the lamina, curvature taking place just below this region. It seems probable that the orthotropic sensitiveness of this part assists in the final adjustment of the lamina at right angles to the incident light. By illuminating the uppermost part of the petiole by direct light and the remainder from the opposite direction by means of a mirror it was found possible to cause the upper and lower motor regions to bend in opposite directions.

The view that phototropic curvatures are due to differences in light intensity, and not merely to the direction of the light rays, is very strongly supported by the work of Blaauw (9) and Buder (10 and 11), and the experiments described above show that this is also the cause of the movements of the leaves of Sparmannia. Such a view appears to admit of a simple explanation of the method by which leaves respond to phototropic stimuli.

It seems to have been conclusively shown (7 and 8), in the case of the coleoptiles of Avena and other similar plants, that the phototropic stimulus is conducted by means of a diffusible substance or hormone. Provisionally, we may regard such a hormone as being produced in any part of the lamina or petiole, the amount produced depending directly on the intensity of the light. This hormone will pass along the petiole in a basipetal direction. If the two sides of the petiole are unequally illuminated, inequalities in the concentration of the hormone on the two sides will result, and the petiole will tend to bend until it points towards the light. Owing to mechanical reasons the response of the petiole may be confined to certain regions where the tissues are more pliable and the magnitude of the response may be limited largely by epinasty or geotropism.

Should the lamina be uniformly illuminated, even though the light be directed obliquely on to its surface, the hormone produced in it will be distributed evenly to both sides of the petiole and no bending of the latter will result due to this cause. Shading of portion of the lamina will, however, disturb the equilibrium, more of the hormone being distributed to one side of the petiole than to the other. The petiole will therefore tend to curve in a direction parallel to the surface of the lamina.

It would appear, therefore, that both lamina and petiole have similar potentialities with regard to the perception of phototropic stimuli, that is the perception of differences in light intensity. Owing, however, to the morphological arrangement of the parts of a leaf, the petiole will be exposed more often than the lamina to differences in light intensity. The movements by which a leaf is turned towards the light will therefore normally be initiated by the perception of the stimulus by the petiole itself, but, under certain conditions, the bending of the latter may partially or entirely be controlled by the lamina.

Summary.

The petiole of *Oxalis macra* is strongly phototropic, even after the lamina has been removed. In this case, however, the power of response is lost after one or two days.

When a leaf of *Sparmannia africana* is shaded in such a way that portion of the lamina is exposed to a reduced light intensity, as compared with that falling on the remainder of the surface, a slow bending of the petiole takes place. The direction of motion is away from the shade and is parallel to the surface of the lamina.

The region where the greatest bending takes place is near the base of the petiole, and the stimulus is transmitted through a distance of about 15 cm.

The petiole itself is also phototropic, and this fact enables the leaf to adjust itself correctly to the light when exposed to oblique illumination. The lamina can only be expected to respond to oblique illumination when, owing to curvature of its surface, the intensity of the light acting on it is not uniform.

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No. 36.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON PHENYLBENZYLETHER.

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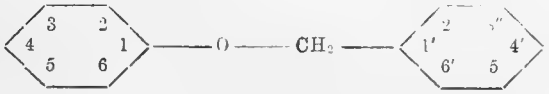
Introduction.

In a previous communication [Proc. R.D.S., xvii, N.S., No. 17] it has been shown by H. Ryan and J. L. O'Donovan that phenylbenzylurethane is difficult to nitrate, but that it can nevertheless be nitrated both in its phenyl and in its benzyl radicle without decomposition of the urethane. Thus at the ordinary temperature nitrogen peroxide converted it into a trinitrophenylbenzylurethane. It was also converted by nitric acid at more or less high concentrations into a tetranitro, and probably also a pentanitro, derivative. Secondary reactions, resulting in the formation of nitrobenzoic acid and nitrophenylurethanes, also occurred. But, in regard to ease of nitration at low temperatures and concentrations, the substance was very inferior to diphenylnitrosamine, and it seemed of interest therefore to find how the somewhat analogous oxy-compound, phenylbenzylether, would behave under similar conditions.

The action of nitric acid on 4-nitro-, 4′-nitro-, 2·4-dinitro-, and 2·4′-dinitrophenylbenzylether[1] has been already investigated by G. Kumpf [Lieb. Ann. d. Chem. cciv, 1884, p. 96], but under conditions which are not evident from his communication. In his experiments he obtained 2·4·4′-trinitro-, 4·4′-dinitro-, and probably 4·2′-dinitrophenylbenzylether. In general in our experiments, which were for the most part carried out at the laboratory temperature and at low concentrations of the interacting substances, the same products were obtained.

Nitrogen peroxide reacted rapidly with a four per cent. solution of the ether in carbon tetrachloride forming 4-nitrophenylbenzylether, which was then very slowly converted into its 4·4′-dinitro derivative. During the prolonged action of the peroxide secondary reactions also occurred, and these gave rise to the formation of tarry bodies and a substance soluble in alkali. The main

[1] The nomenclature adopted in this communication is based on the following formula:—



product of the action of nitrogen peroxide vapour on the solid ether was 4-nitrophenylbenzylether. Small amounts of benzoic acid and tarry matter were also formed. Similar results were obtained with solutions of nitrogen sesquioxide and the ether.

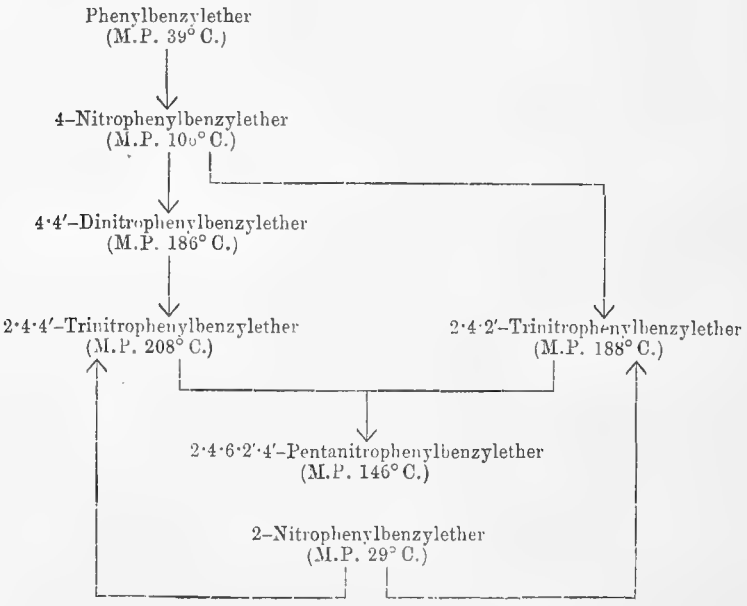
In dilute solution, and at the ordinary temperature, the action of nitric acid on the ether was greatly affected by the nature of the solvent. When dissolved in ordinary ether phenylbenzylether appeared to be unaffected by dilute solutions of nitric acid in this solvent. Similar results were obtained in glacial acetic acid solutions of the substances, but when the action in the latter solvent was very prolonged only decomposition products of the ether were recovered. On the other hand, the substances interacted fairly easily in carbon tetrachloride solution forming successively 4-nitro- and 4·4′-dinitrophenylbenzylether.

Concentrated nitric acid, even in the cold, acted energetically on phenylbenzylether, yielding forty to fifty per cent. of its 4-nitro derivative together with tarry and oily matter.

From 4-nitro- and from 2-nitrophenylbenzylether by the direct action of fuming nitric acid we obtained 2·4·4′- and 2·4·2′-trinitrophenylbenzylether.

A mixture of concentrated sulphuric and nitric acids converted both the trinitro compounds into the same pentanitro derivative, which was probably 2·4·6·2′·4′-pentanitrophenylbenzylether. When the latter compound was heated with a mixture of fuming sulphuric and nitric acids a white crystalline substance was obtained. This melted at 138-140° C., gave a reddish solution in dilute aqueous alkali, and appeared to be a decomposition product of the nitrated ether.

The results obtained by us in the nitration of phenylbenzylether are shown in the following diagram :—



1. *Action of Nitrogen Peroxide on Phenylbenzylether.*

(*a*) *In Carbon Tetrachloride Solution.*—A current of nitrogen peroxide, generated by heating lead nitrate, was passed for half an hour into a solution of 2 g. of phenylbenzylether in 50 g. of carbon tetrachloride, and the solution, which had a dark-brownish-red colour, was allowed to remain in a stoppered flask at the room temperature for two days.

On distilling off the carbon tetrachloride a brownish-red oily residue was obtained, which solidified on cooling. The solid was washed with dilute aqueous potash, and recrystallised from alcohol, from which it separated in brownish-white crystals, melting at 105·5–106° C.

A mixture of it with *p*-nitrophenylbenzylether, obtained by the action of benzyl chloride on the sodium derivative of *p*-nitrophenol, melted at the same temperature, the two substances being therefore identical.

Under these conditions nitrogen peroxide acts on phenylbenzylether forming *p*-nitrophenylbenzylether.

(*b*) Proceeding as above, but allowing the mixture to stand for six weeks instead of two days, a mixture containing two layers was obtained—a dark-red lower layer and a blackish-red upper layer, which contained much crystalline matter.

The results got on examination of the mixture were very similar to those obtained from the product of the action of 4 molecular amounts of nitric acid on phenylbenzylether (q. v.)—the main product being 4-nitrophenylbenzyl-ether, together with a little matter which was soluble in alkali.

(*c*) When a mixture similar to (*a*) was allowed to stand four months and was re-saturated with nitrogen peroxide at intervals of three weeks, the solution became yellow with the separation of a white solid.

The carbon tetrachloride was distilled off under reduced pressure. On passing a current of steam through the yellow residue left in the flask, white plates, not unlike benzoic acid, distilled with the steam, whilst a red tarry substance, with much white solid matter, was left in the non-volatile portion. The latter solid was extracted with a little ether and the ether solution was washed with dilute potash. The ether layer gave colourless needles, melting at 186° C., which proved to be identical with 4·4′-dinitrophenylbenzylether prepared synthetically by the action of *p*-nitrobenzyl chloride on sodium *p*-nitrophenolate. The potash layer gave, on acidification and extraction with ether, a yellowish-white solid and tarry matter, neither of which, however, was obtained in sufficient quantity to establish its identity.

(*d*) *In the Absence of Solvents.*—A shallow glass vessel containing 4 g. of phenylbenzylether was placed beside a similar vessel containing liquid nitrogen peroxide, the two vessels being enclosed under a bell-jar. The phenylbenzylether immediately darkened in colour, and, after a few hours, appeared as a black, more or less oily solid. After three days the brownish-black oily nitration product was examined. It possessed a pleasant aromatic odour. The oily mass was mixed with ether, and the purple ethereal solution (A) was decanted from the brown tarry residue (B). After repeated crystallisation from alcohol the latter substance, which weighed about 2·4 g., melted at 105–106° C. A mixture of it with 4-nitrophenylbenzylether melted at the same temperature, the two substances being, therefore, identical. The ethereal solution (A) was washed with dilute potash, and in this way the solute was divided into two portions (α) and (β), the latter being soluble and the former insoluble in potash. α (about 1 g.) consisted of a brownish oily solid, which, after recrystallisation from alcohol, proved to be 4-nitro-

phenylbenzylether. β (about 0·6 g.) was also oily in consistency and red in colour. On extracting the crystalline matter with warm petroleum ether, yellow leaves, melting at 106–109° C., were obtained, which, after recrystallisation from dilute alcohol (25 per cent.), melted at 109–111° C. When further purified by distillation with steam and sublimation, the product melted at 119–120° C., and proved to be benzoic acid. The nitration of phenylbenzylether by this method gave therefore 75 per cent. of 4-nitrophenylbenzylether, 15 per cent. of benzoic acid, and 10 per cent. of oily or tarry matter.

(*e*) The experiment described above was repeated, allowing the phenylbenzylether to remain in the atmosphere of nitrogen peroxide for two months. The nitrated product had a somewhat lighter brown colour than that in the last experiment, and, as before, was separated by means of ether into two fractions, A and B. These fractions were then separately distilled in a current of steam, and the two portions which volatilized were united (C). The latter was a yellow-coloured solution which deposited 0·4 g. of colourless leaves on cooling. This substance, after recrystallisation, melted at 119–120° C., and proved to be benzoic acid. The mixture also contained a thin film of a brown-coloured oil, which had an aromatic odour, and was possibly benzaldehyde. The yellow aqueous portion of C was extracted with ether and the ether solution was washed with potash. The ethereal layer on evaporation left only a trace of a brown oily solid, whereas the potash layer, after acidification with diluted hydrochloric acid and extraction with ether, gave grey plates, melting between 79° and 91° C. This mixture may have contained a nitrophenol, as its alkaline solution had a yellow colour which disappeared on acidification. Fractional crystallisation from either water or alcohol failed to isolate the impurity in the body, the main constituent (0·6 g.) of which was benzoic acid.

The portion of A which remained (0·8 g.) in the distilling flask consisted of an orange-coloured solid, which, after recrystallisation from alcohol, melted at 105–106° C., and was identical with 4-nitrophenylbenzylether. The corresponding portion of B consisted of a dark-coloured tar. This was dissolved in a large volume of warm alcohol, and after some days 0·5 to 0·7 g. of a black tar and 1·1 to 1·3 g. of a light red oily solid were obtained. The latter was freed from oil by pressing it between absorption pads and then dissolved in alcohol, from which it separated in the form of light yellow prisms, melting at 103–105° C. As a mixture of it with 4-nitrophenylbenzylether melted at 104–105° C., it must have been slightly impure 4-nitrophenylbenzylether.

The prolonged action of nitrogen peroxide on phenylbenzylether forms therefore 50–55 per cent. of 4-nitrophenylbenzylether, 25 per cent. of benzoic acid, and 23–18 per cent. of oily or tarry products containing a small amount of a sweet-smelling oil.

2. *Action of Nitrous Fumes on Phenylbenzylether.*

A current of nitrous fumes, generated by the action of nitric acid on arsenious oxide, was passed for half an hour into a solution of 2 g. of phenylbenzylether in 50 g. of carbon tetrachloride. The solution, which was brown in colour, was allowed to stand for six weeks in a stoppered flask. At the time of its examination the mixture contained two layers, a clear green lower layer and a dark-reddish upper layer, which contained much crystalline matter.

The carbon tetrachloride was driven off, as before, under reduced pressure. A little alcohol was added to the reddish residue in the distilling flask, a clear yellow solution resulting, with a little insoluble oily matter. The yellow solution on evaporation gave a reddish product, which was dissolved in a little ether

and washed with dilute potash. The ethereal layer on evaporation gave a brownish solid, which, after a few recrystallisations from methylated spirits, yielded colourless crystals, melting at 104° C., identical with 4-nitrophenylbenzylether.

The potash layer gave on acidification yellowish crystals, which melted at 114° C.; on crystallisation from methylated spirits they lost their yellow colour, and were not unlike benzoic acid.

3. *Action of Nitric Acid on Phenylbenzylether.*

(*a*) *In the Absence of Solvents.*—2 g. of phenylbenzylether was slowly added to 3 g. of nitric acid (Sp. g. 1·5) in a small round flask immersed in a freezing mixture.

On allowing the mixture to stand overnight a reddish oily solid, possessing an odour like that of benzaldehyde, was obtained. The solid was pressed free from oil and shaken up with a little ether. The ether solution was then separated from the undissolved tar and washed with dilute aqueous potash. The ether layer on evaporation gave about 1 g. of 4-nitrophenylbenzylether, while the potash layer yielded on acidification a dark-red solid, the quantity of which was insufficient for further investigation.

When the acid (2 c.c.) was added gradually to the phenylbenzylether (2 g.), a rather violent reaction ensued with the formation of much charred matter.

(*b*) *In Ether Solution.*—1·92 c.c. (4 molecular amounts) of nitric acid (Sp. g. 1·42) was added to a solution of 2 g. of phenylbenzylether in 50 g. of ordinary ether. The mixture was allowed to remain in a stoppered flask for six weeks; it developed no coloration; and the phenylbenzylether was recovered unchanged.

Experiments similar to the foregoing were carried out, using 1, 2, and 3 molecular amounts of nitric acid, and, as might be expected, no interaction was found to have taken place.

(*c*) *In Carbon Tetrachloride Solution.*—Mixtures containing 1, 2, 3, and 4 molecular amounts of nitric acid (Sp. g. 1·5) and 2 g. of phenylbenzylether, dissolved in 50 g. of carbon tetrachloride, were allowed to remain in stoppered flasks for a period of six weeks.

The mixtures were at first dark-red in colour. After some time the colours became much clearer with the separation of crystalline matter in the cases of the solutions to which 2, 3, and 4 molecular amounts of nitric acid had been added.

On examining the contents of the flask to which 1 molecular amount of nitric acid had been added, phenylbenzylether was recovered unchanged together with a little yellow oily matter.

In the flasks to which 2 and 3 molecular amounts of nitric acid had been added, 4-nitrophenylbenzylether was identified, with an increasing amount of oily matter and traces of a reddish crystalline substance.

In the case of the solution to which 4 molecular amounts of nitric acid had been added, the red oil left after the distillation of the carbon tetrachloride was steam-distilled. The portion volatile with steam was soluble in ammonia, giving a yellow solution which became colourless on acidification with hydrochloric acid. The acid solution was extracted with ether; a while crystalline substance, melting at 101–105° C., resulted, which was not unlike benzoic acid in appearance, whilst a mixture of it with the latter melted at 113–115° C.; several reprecipitations and recrystallisations of this white substance, however, failed to improve its melting-point.

The red oily portion, which was not volatile with steam, was dissolved in chloroform and washed with dilute potash. The portion soluble in chloroform proved to be 4-nitrophenylbenzylether (about 1·2 g.), whilst the potash fraction yielded a small quantity of oily red plates.

(*d*) *In Acetic Acid Solution.*—1·8 c.c. (4 molecular amounts) of nitric acid (Sp. g. 1·5) were added to a solution of 2 g. of phenylbenzylether in 50 g. of glacial acetic acid. The mixture was allowed to remain in a stoppered flask for three weeks. The contents of the flask were at first deep purple in colour, but gradually changed to light-red. The colour changes indicated the formation of an oxonium salt, but when the mixture was poured into water phenylbenzylether was recovered unchanged.

Experiments with smaller molecular amounts of nitric acid were also performed and, as might be expected, yielded negative results.

On repeating the experiment, this time allowing the mixture to stand six weeks and then evaporating the solvent in a vacuum over soda-lime, yellowish-white prisms, melting at 94–96° C., were obtained. The green oily residue, left after removal of the crystals, had a distinct smell of benzaldehyde. On dissolving the crystalline matter in ether and washing with dilute potash, the ethereal layer on evaporation gave white crystals melting at 107–109° C., while the potash layer gave reddish oily crystals, melting at 101–109° C. Both fractions seemed to be mixtures of decomposition products of phenylbenzyl-ether and its nitro derivatives; the quantities produced, however, were too small to establish the identity of its constituents.

4. *Action of Nitric Acid on 4-Nitrophenylbenzylether.*

(*a*) *In Carbon Tetrachloride Solution.*—0·57 c.c. (6 molecular amounts) of nitric acid (Sp. g. 1·5) were added to 0·5 g. of 4-nitrophenylbenzylether and 50 g. of carbon tetrachloride. The mixture was shaken automatically for four weeks in a stoppered flask. Much white matter separated. The carbon tetrachloride was driven off under reduced pressure, and a little alcohol was added to the white residue in the distilling flask. The mixture was warmed slightly and filtered while hot. A white compound remained on the filter, whilst a yellowish-white substance separated from the filtrate on cooling—the latter proved to be unchanged 4-nitrophenylbenzylether.

The white compound left on the filter melted at 167–177° C., and after repeated crystallisation from chloroform, colourless needles, melting at 186° C., were obtained. A mixture of the latter with 4·4′-dinitrophenylbenzylether melted at the same temperature, the two substances being therefore identical. Under these conditions nitric acid reacts with 4-nitrophenylbenzylether forming 4·4′-dinitrophenylbenzylether.

(*b*) *In Acetic Acid Solution.*—1·14 c.c. (6 molecular amounts) of nitric acid (Sp. g. 1·5) were added to a solution of 1 g. of 4-nitrophenylbenzylether in 55 g. of glacial acetic acid. The mixture was allowed to remain in a stoppered flask for four weeks, during which time it developed no coloration. On pouring the mixture into water a white precipitate was formed, which was filtered, washed free from acid, and recrystallised from methylated spirits. Prismatic crystals were obtained, which melted at 105–106° C., and a mixture of them with 4-nitrophenylbenzylether melted at the same temperature. Apparently, therefore, 4-nitrophenylbenzylether is unaffected by nitric acid in acetic acid solution.

(*c*) *In Alcohol Solution.*—0·9 c.c. (4 molecular amounts) of nitric acid

(Sp. g. 1·5) was added to a mixture of 1 g. of 4-nitrophenylbenzylether and 50 g. of absolute alcohol—very little of the 4-nitrophenylbenzylether being soluble in the cold alcohol. The mixture was shaken automatically for a fortnight in a stoppered flask; it underwent no apparent change. The white matter was isolated from the contents of flask by filtration, and, after washing and drying, melted at 104–105° C., whilst a mixture of it with 4-nitrophenylbenzylether melted at 105–106° C. Under these circumstances 4-nitrophenylbenzylether did not appear to interact with nitric acid.

Proceeding as above, using, however, 6 molecular amounts of nitric acid and increasing the period of automatic shaking to 6 weeks, 4-nitrophenylbenzylether was again recovered unchanged.

Thus carbon tetrachloride again appears to exert a preferential nitrating influence as compared with such solvents as alcohol, ether, and glacial acetic acid.

(*d*) *In the Absence of Solvents.*—8 g. of 4-nitrophenylbenzylether was slowly added to 48 g. of nitric acid (Sp. g. 1·5) in a small round flask. The mixture was well shaken after each addition, and the temperature was not allowed to rise above 0° C. The 4-nitrophenylbenzylether became dark-brown on addition to the nitric acid, but, on shaking, a yellow solution was formed. About 3 g. of the 4-nitrophenylbenzylether went into solution, but in another experiment, in which the temperature was allowed to rise to 25° C., the whole 8 g. dissolved, giving a red-coloured solution; the products in each case, however, were much the same. After allowing this yellow solution to stand overnight the contents were poured into water, a white precipitate resulting. This white substance was washed free from acid and dried. It was then boiled with a little benzene, and filtered whilst hot. A white solid (A) was left on the filter, while the filtrate on cooling yielded another white body (B). (A) melted at 200–202° C., and after a few recrystallisations from glacial acetic acid, gave white platy prisms, melting from 207–208° C.

0·1289 g. of the substance gave on analysis 14·2 c.c. of nitrogen at 14° C. and 770 m.m.

corresponding to N 13·1
$C_{13}H_9O_7N_3$ requires N 13·2.

The substance is therefore a trinitro derivative of phenylbenzylether, and since it was prepared by the nitration of 4·4′-dinitrophenylbenzylether and also by the nitration of 2-nitrophenylbenzylether, it must be 2·4·4′-trinitrophenylbenzylether.

The other nitration product (B) was fractionated by means of alcohol. One fraction soluble in hot alcohol had a semi-crystalline appearance and melted at 157–160° C., but further recrystallisations from alcohol and glacial acetic acid failed to improve its crystalline appearance or its melting-point.

The other sub-fraction of B, insoluble in boiling alcohol, crystallised from chloroform in long silky needles melting at 188° C.

0·1487 g. of the substance gave 16·7 c.c. of nitrogen at 13° C. and 758 m.m.

corresponding to N 13·1
$C_{13}H_9O_7N_3$ requires N 13·2.

The substance was therefore another trinitro derivative of phenylbenzylether, and since it was also prepared by the nitration of 2-nitrophenylbenzylether (Par. 5) it must contain nitro groups in the positions 2 and 4. Since it is not identical with 2·4·4′-trinitrophenylbenzylether (m.p. 207–208° C.) nor with 2·4·6-trinitrophenylbenzylether (m.p. 147° C.) it must be 2·4·2′-trinitrophenylbenzylether.

5. *Action of Nitric Acid on 2-Nitrophenylbenzylether.*

2-Nitrophenylbenzylether was prepared synthetically by heating equivalent quantities of 0-nitrophenol, benzyl chloride, and sodium ethylate on a water-bath for 5 or 6 hours. The purified substance melted at 29° C.

4·2 g. of this 2-nitrophenylbenzylether was gradually added to 16·8 c.c. of nitric acid (Sp. g. 1·5) in a small round flask, which was kept cool by immersion in a freezing mixture.

The clear red solution so obtained was allowed to stand overnight, during which time much crystalline matter separated. The contents were then poured into a mixture of ice and water, and the yellowish-white precipitate isolated by filtration, washed free from acid and dried. The yellow solid thus obtained was boiled with benzene, and filtered while hot. The filtrate on cooling gave white crystals, which, after repeated recrystallisations from chloroform, melted at 187–188° C., and proved to be identical with 2·4·2′-trinitrophenylbenzylether [mentioned in par. 4 (*d*)].

The white matter insoluble in boiling benzene after recrystallisation from glacial acetic acid melted at 207–208° C., and was identical with 2·4·4′-trinitrophenylbenzylether.

6. *Action of Nitric Acid on 4·4-Dinitrophenylbenzylether.*

In the Absence of Solvents.—1 g. of 4·4′-dinitrophenylbenzylether was gradually added to 3 g. of nitric acid (Sp. g. 1·5) at 30–40° C. in a small round flask and allowed to stand overnight, during which time some colourless crystals separated. 50 c.c. of water was then added to the contents of the flask, and the yellowish-white precipitate, which was isolated by filtration, was washed free from acid and dried.

On recrystallisation from glacial acetic acid, colourless, platy prisms were obtained, which melted at 207–208° C., whilst a mixture of it with 2·4·4′-trinitrophenylbenzylether melted at the same temperature. Thus nitric acid acts on 4·4′-dinitrophenylbenzylether giving 2·4·4′-trinitrophenylbenzylether.

2·4·4′-trinitrophenylbenzylether is insoluble in water, ether, chloroform, alcohol, or benzene, either hot or cold. It is scarcely soluble in cold xylene or glacial acetic acid, but dissolves fairly easily on boiling.

7. *Action of Nitric Acid on 2·4·4′-Trinitrophenylbenzylether.*

(*a*) *In the Absence of Solvents.*—1·0 g. of 2·4·4′-trinitrophenylbenzylether was added slowly with shaking to 4 c.c. of fuming nitric acid (Sp. g. 1·52) in a small round flask. No heat was evolved, and no apparent change was observed. The yellow mixture was allowed to stand overnight and then poured into water. A yellowish-white precipitate was formed, which, after isolation, was washed, dried, and recrystallised from glacial acetic acid. Colourless prisms of unchanged 2·4·4′-trinitrophenylbenzylether were obtained.

Under these conditions nitric acid is without action on 2·4·4′-trinitrophenylbenzylether.

(*b*) The above experiment was repeated, but the mixture was this time heated on a water-bath for 6 or 7 hours and allowed to stand overnight. The mixture was then poured into water and the precipitate examined as before. 2·4·4′-trinitrophenylbenzylether was again recovered unchanged.

(*c*) The experiment was next attempted in the presence of 4 c.c. of fuming sulphuric acid (20 per cent. SO_3)—1 g. of the 2·4·4′-trinitrophenylbenzylether

being added to a cold mixture of 4 c.c. of fuming nitric acid (Sp. g. 1·52) and 4 c.c. of fuming sulphuric acid in a small round flask.

No heat was evolved; the light-yellow solution was allowed to stand overnight, during which time colourless crystals separated. The contents of the small flask were then poured into a mixture of ice and water, a voluminous yellowish-white precipitate resulting. The precipitate was filtered, washed free from acid, and dried. On crystallising the dried product from chloroform, colourless monoclinic plates were obtained, which melted with decomposition at 146° C.

They were fairly soluble in boiling chloroform, and somewhat more so in glacial acetic acid and hot benzene.

0·1239 g. of the substance gave on analysis 18·1 c.c. of nitrogen at 17° C. and 775 m.m.

corresponding to N 17·1
$C_{13}H_7O_{11}N_5$ requires N 17·1.

The compound is therefore a pentanitro derivative of phenylbenzylether.

On subjecting 2·4·2′-trinitrophenylbenzylether to the same treatment as 2·4·4′-trinitrophenylbenzylether, the same pentanitro compound, melting at 146° C., is formed; therefore this body must contain nitro groups in the positions 2·4·2′·4′ respectively.

7. *Action of Nitric Acid on the Pentanitro Compound.*

In the Absence of Solvents.—1 g. of the pentanitro compound was heated on a water-bath for 12 hours with 4 c.c. of fuming sulphuric acid and 4 c.c. of fuming acid (Sp. g. 1·52) in a small round flask.

After 6 hours fuming acid (4 c.c) was again added, and the heating was continued. The contents of the flask were finally poured into water, yielding a clear yellow solution, from which, by extraction with ether, a yellow oily compound was isolated. This separated from chloroform in almost colourless crystals, which melted with decomposition at 139–140° C. A mixture of this substance with the pentanitro compound melted at 122–125° C. It dissolved in dilute aqueous potash, giving a deep-red coloured solution. It was probably a decomposition product of nitrated phenylbenzylether, but it was not obtained in sufficient quantity for analysis.

SUMMARY.

1. Nitrogen peroxide converted phenylbenzylether into 4-nitrophenylbenzylether, and this in turn into its 4·4′-dinitro derivative together with some benzoic acid and a little oily matter.

2. A similar result was obtained by the action of nitric acid on a carbon tetrachloride solution of the ether, but in glacial acetic acid or ether solution on short standing no appreciable nitration occurred.

3. Nitric acid converted 4-nitro- and 2-nitrophenylbenzylether into 2·4·4′- and 2·4·2′-trinitrophenylbenzylether, and these were both converted by a mixture of concentrated sulphuric and nitric acids into the same pentanitrophenylbenzylether.

4. Attempts to prepare a hexanitro derivative of the ether led to the formation of decomposition products.

The above investigation was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for the materials employed in the research.

No. 37.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON ETHYL-β-NAPHTHYLETHER.

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(Read December 18, 1923. Printed February 27, 1924.)

Introduction.

In the preceding communication we showed that phenylbenzylether reacts with nitrogen peroxide vapour with formation of a mononitro derivative and oxidation products of the ether, while with a solution of the peroxide it gives a dinitro derivative. Similar results were obtained by the action of nitric acid on solutions of the ether in carbon tetrachloride. It was only by the action of concentrated nitric acid on the ether, or its nitro derivative, that a trinitro- or a pentanitrophenylbenzylether could be obtained.

In the present communication we describe the results of similar experiments which were carried out with ethyl-β-naphthylether with a view to determining whether ethers of the naphthols nitrated at the ordinary temperature more easily than ethers of the phenols.

Several nitro derivatives of ethyl-β-naphthylether have been already described. Thus Wittkampf [Ber. Dtsch. Chem. Ges., xvii (1884), p. 394] obtained 1-nitroethyl-β-naphthylether by the action of nitric acid on the ether, and established its constitution by converting it into 1-nitro-β-naphthylamine. In a similar experiment F. Gaess [Journ. f. pr. Chem. (2), xliii, p. 23] obtained 8-nitroethyl-β-naphthylether and crystals, melting at 114° C., which probably consisted of 6-nitroethyl-β-naphthylether. Gaess also obtained 1·6- and 1·8-dinitroethyl-β-naphthylether.

Staedel [Ber. Dtsch. Chem. Ges., xiv (1881), p. 900] prepared a trinitroethyl-β-naphthylether, melting at 186° C., but did not determine the orientation of the nitro groups in his compound.

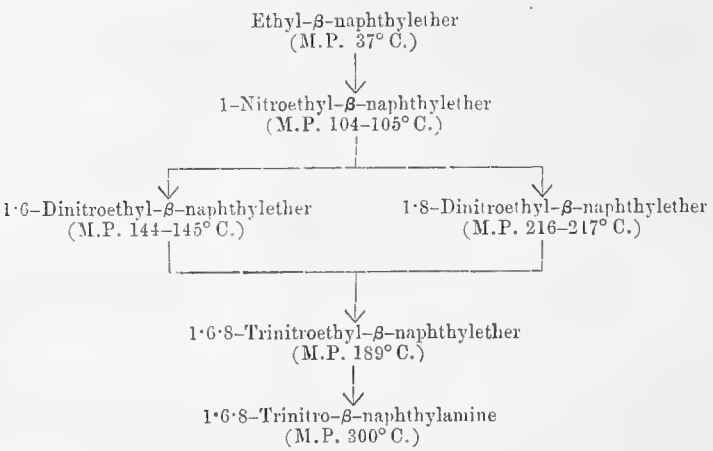
In our experiments we have found that ethyl-β-naphthylether reacted with nitrogen peroxide vapour more readily than phenylbenzylether, forming 1·6- and 1·8-dinitroethyl-β-naphthylether. In a similar experiment in carbon tetrachloride solution, the 1-nitro and 1·8-dinitro derivatives were the main products.

With one molecular amount of nitric acid in carbon tetrachloride solution and at the ordinary temperature the ether was converted into its 1-nitro derivative, while with four molecular amounts of the acid 1·6- and 1·8-dinitroethyl-β-naphthylether were formed in nearly equal amounts. The same two nitro compounds were also formed by the action of nitric acid on a dilute carbon tetrachloride solution of the mononitro ether.

Concentrated nitric acid acted readily on the ether forming the 1·6- and 1·8-dinitro derivatives; and when the temperature was allowed to rise to 50° C., a trinitro compound was obtained not only from the ether itself, but also from both its 1·6- and 1·8-dinitro derivatives. This body is, therefore, 1·6·8-trinitroethyl-β-naphthylether. It was converted by alcoholic ammonia into 1·6·8-trinitroethyl-β-naphthylamine, which melted with decomposition at 300° C.

An attempt to prepare higher nitrated derivatives of the trinitro body led to the formation of decomposition products.

The results obtained by us are shown in the following diagram :—



Experimental.

1. *Action of Nitrogen Peroxide on Ethyl-β-naphthylether.*

(*a*) *In the Absence of Solvents.*—A shallow glass vessel containing 1·5 g. of ethyl-β-naphthylether was allowed to remain in an enclosed space filled with nitrogen peroxide vapour. The ether first turned brown, then became liquid, and finally changed to an orange-red oily solid. After three days the solid was washed with ether. The ethereal washings contained a trace of red oily matter, soluble in alkali, and a solid, which crystallised from alcohol in colourless needles, melting at 144–145° C., and gave on analysis the following results :—

0·1946 g. substance gave 17·8 c.c. moist nitrogen at 13° C. and 760 m.m.

corresponding to N 10·8
$C_{12}H_{10}O_5N_2$ requires N 10·7.

The substance was, therefore, a dinitro derivative of ethyl-β-naphthylether. When heated in a sealed tube with alcoholic ammonia, it was converted into 1·6-dinitro-β-naphthylamine, which separated as golden yellow tabular prisms, melting with decomposition at 247° C. The substance, which melted at 144–145° C., was, therefore, 1·6-dinitroethyl-β-naphthylether. It is very soluble in warm organic solvents, moderately soluble in cold chloroform or acetic acid, and only slightly soluble in alcohol or benzene.

The residue left on washing the nitration product with ether was recrystallised from boiling benzene. Colourless rectangular prisms, melting at 216–217° C.,

were obtained. This substance, which was also a dinitroethyl-β-naphthylether, gave on analysis the following results:—

0·1512 g. substance gave 14 c.c. moist nitrogen at 13° C. and 760 m.m.

corresponding to N 11·0
$C_{12}H_{10}O_5N_2$ requires N 10·7.

Since alcoholic ammonia converted it into 1·8-dinitro-β-naphthylamine, melting at 223–224° C., it must have been 1·8-dinitroethyl-β-naphthylether. It is sparingly soluble in cold alcohol, benzene, or acetic acid, and moderately soluble in hot benzene or hot acetic acid.

Nitrogen peroxide vapour, therefore, reacts easily and smoothly with ethyl-β-naphthylether, converting it into its 1·6- and 1·8-dinitro derivatives.

(*b*) *In Glacial Acetic Acid Solution.*—Vapour of nitrogen peroxide, generated by heating lead nitrate, was passed for 20 minutes into a solution of 2 g. of ethyl-β-naphthylether in 50 g. of 95 per cent. acetic acid. Some crystals, which separated on allowing the solution to remain overnight, were filtered off. After recrystallisation from acetic acid they consisted of light yellow platy prisms, which melted at 103–104° C., and gave on analysis the following results:—

0·2087 g. substance gave 11·9 c.c. moist nitrogen at 14° C. and 769 m.m.

corresponding to N 6·78
$C_{12}H_{11}O_3N$ requires N 6·45.

The substance proved to be 1-nitroethyl-β-naphthylether. When heated in a sealed tube to 180° C. for eight hours with 20 parts of alcoholic ammonia, it was converted into 1-nitro-β-naphthylamine, melting at 126–127° C. 1-nitro β-naphthylether is sparingly soluble in cold alcohol or acetic acid, and is moderately soluble in carbon tetrachloride or ether.

The parent liquid, from which the mononitro compound had separated, was allowed to remain in a stoppered flask for several days. The solid matter deposited was filtered off and recrystallised from glacial acetic acid. It melted at 216–217° C., and proved to be 1·8-dinitroethyl-β-naphthylether.

The parent liquid was poured into water, and the precipitate was recrystallised several times from alcohol. It melted at 144–145° C., and proved to be 1·6-dinitroethyl-β-naphthylether.

In another experiment, in which anhydrous acetic acid was used as the solvent, a dark-brown solution resulted. From this no crystalline nitro derivative of ethyl-β-naphthylether could be isolated.

(*c*) *In Carbon Tetrachloride Solution.*—A solution of 2 g. of ethyl-β-naphthylether in 50 g. of carbon tetrachloride was saturated with nitrogen peroxide, and allowed to remain in a stoppered bottle for three weeks. The mixture then contained a dark oily upper layer and a clear light-red lower layer. The solvent was distilled under reduced pressure, and a red, oily solid remained. Portion of this solid dissolved in warm alcohol, giving a clear red solution, and the rest remained undissolved in the form of a brownish-white solid. The latter, on recrystallisation from glacial acetic acid, was obtained as colourless prisms, melting at 216–217° C., and these proved to be 1·8-dinitroethyl-β-naphthylether.

A red viscous mass with traces of a white solid was left by evaporation of the reddish alcoholic solution. The solid was extracted with petroleum ether,

from which it separated as creamy-white plates, melting at 102–103° C., and consisting of 1-nitroethyl-β-naphthylether. The red oily matter did not crystallise.

By the action of nitrogen peroxide on ethyl-β-naphthylether in carbon tetrachloride solution 1·8-dinitroethyl-β-naphthylether (60 per cent.) was the main product, whilst 1-nitroethyl-β-naphthylether (10 per cent.) and oils (30 per cent.) were also formed.

2. *Action of Nitrous Fumes on Ethyl-β-naphthylether.*

Nitrous fumes, generated by the action of nitric acid on arsenious oxide, passed for half an hour into a solution of 2 g. of ethyl-β-naphthylether in 50 g. of 95 per cent. acetic acid. A crystalline solid gradually separated, and this was, after ten days, filtered. The crystals proved to be 1-nitroethyl-β-naphthylether. When the red filtrate was poured into water, an orange precipitate was obtained. This also proved to be 1-nitroethyl-β-naphthylether.

In a similar experiment in which glacial acetic acid was the solvent, we obtained finally a dark oily residue which did not crystallise.

3. *Action of Nitric Acid on Ethyl-β-naphthylether.*

(*a*) *In the Absence of Solvents.*—3 g. of ethyl-β-naphthylether was added slowly to 12 c.c. of fuming nitric acid (Sp. g. 1·52) in a small round flask. The addition resulted in the production of a red solution with the evolution of much heat. The contents, which were not allowed to rise above 15° C., were let stand overnight and then poured into water. An orange-red precipitate was formed, which was filtered, washed, and dried. The dry solid, on fractional crystallisation from warm alcohol, gave two portions, one more soluble, melting at 125–130° C., and the other less soluble, melting at 180–197° C. After recrystallising both mixtures from glacial acetic acid, colourless needles, melting at 143–144° C., together with platy prisms, melting at 216° C., were obtained, which proved identical with 1·6- and 1·8-dinitroethyl-β-naphthylether respectively. By the action of nitric acid, then, on ethyl-β-naphthylether, the 1·6- and 1·8-dinitro derivatives were formed in nearly equal parts, together with a little (0·1–0·2 g.) red oily matter. In a similar experiment in which the temperature was allowed to rise to 50–55° C., the 1·6·8-trinitro derivative was the only crystalline product with 0·4–0·5 g. of oily or brown tarry matter.

(*b*) *In Acetic Acid Solution.*—0·45 c.c. (1 molecular amount) of nitric acid (Sp. g. 1·52) was added to a solution of 2 g. of ethyl-β-naphthylether in 50 g. of glacial acetic acid. The solution was at first clear yellow in colour, but on short standing in a stoppered flask it deepened to red. After 2 days 0·2 of yellow crystals separated. These melted at 103–4° C. after recrystallisation from alcohol, and proved to be 1-nitroethyl-β-naphthylether.

After separating the crystals by filtration, the parent liquid was returned to the stoppered flask. No further change occurred during the following week. The clear red solution was then poured into water, and the resulting precipitate was isolated, washed with water, and dried. The dried mass, after recrystallisation from alcohol, melted at 103–4° C., and was identical with 1-nitroethyl-β-naphthylether.

Similar experiments were undertaken with 2, and also with 4, molecular amounts of nitric acid (Sp. g. 1·52), but in each case 1-nitroethyl-β-naphthylether formed the only nitration product.

(*c*) *In Carbon Tetrachloride Solution.*—0·45 g. (1 molecular amount) of nitric

acid (Sp. g. 1·52) was added to a solution of 2 g. of ethyl-β-naphthylether in 50 g. of carbon tetrachloride, contained in a stoppered flask. The colour of the solution was at first yellow, but it quickly deepened to orange, and finally to red. Dark oily particles gradually appeared together with traces of crystalline matter. The contents were examined after remaining 6 days at the room temperature. The crystalline matter, which was isolated by filtration, melted at 205° C., and seemed to be impure 1·8-dinitroethyl-β-naphthylether. (Its formation was due no doubt to the unequal distribution of the nitric acid.) The carbon tetrachloride was distilled under reduced pressure, a red oily solid remaining in the distilling flask. The solid was dissolved in warm alcohol, and the solution on cooling yielded brownish-white prisms, melting at 103–4° C. A mixture of these with 1-nitroethyl-β-naphthylether melted at the same temperature, the two bodies being, therefore, identical.

1 molecular amount of nitric acid then reacts with ethyl-β-naphthylether in carbon tetrachloride solution, forming principally the 1-nitro derivative, with 5–10 per cent. of oily or tarry substances, and about 1 per cent. of a crystalline product, which is probably the 1·8-dinitro derivative of the ether.

In a similar experiment 1·9 c.c. (4 molecular amounts) of nitric acid (Sp. g. 1·52) was added to 2 g. of ethyl-β-naphthylether in 50 g. of carbon tetrachloride.

The solution was at first orange-yellow, and finally brownish-red. After 5 minutes a copious brown coloured precipitate was thrown down. The carbon tetrachloride was distilled off after 3 days, leaving a nearly white solid residue. On fractionating from warm alcohol, two kinds of crystals were obtained—slender acicular prisms, melting at 143° C., and long slender rectangular prisms, melting at 217° C. The first mentioned prisms proved to be 1·6-dinitro-, and the last 1·8-dinitroethyl-β-naphthylether.

By the action of 4 molecular amounts of nitric acid, then, on a 4 per cent. carbon tetrachloride solution of ethyl-β-naphthylether, the 1·6-dinitro- and the 1·8-dinitro derivatives of the ether are formed in nearly equal quantities.

4. *Action of Nitric Acid on 1-Nitroethyl-β-naphthylether.*

(*a*) *In the Absence of Solvents.*—About 5 g. of 1-nitroethyl-β-naphthylether were added slowly to 25 c.c. of fuming nitric acid in a small round flask cooled by immersion in water. The clear red solution was allowed to remain at the room temperature overnight, and was then poured into water. The orange red precipitate which formed was filtered, washed with water, and dried. A portion of this solid separated from hot alcohol in the form of colourless, apparently rectangular prisms, melting at 189–190° C. Another portion crystallised from benzene as monoclinic plates or leaves, which also melted at 189–190° C. A mixture of the two products melted at 189–190° C. The apparent difference in the crystalline structure of the two substances was only one of habit, since both gave oblique extinction, and therefore belonged to the monoclinic system. The two bodies were therefore identical, and gave on analysis the following results:—

0·0962 g. substance gave 11·6 c.c. nitrogen at 12° C. and 728 m.m.

corresponding to N 13·82.
$C_{12}H_9O_7N_3$ requires N 13·7.

The substance was therefore a trinitro derivative of ethyl-β-naphthylether, and, since it was obtained by the nitration of both the 1·6- and 1·8-dinitroethyl-β-naphthylether (see 5 and 6 below), it must have been 1·6·8-trinitro-

ethyl-β-naphthylether. It is only sparingly soluble in cold alcohol or benzene, but dissolves fairly easily in the hot solvents.

(*b*) *In Carbon Tetrachloride Solution.*—A solution of 2 g. of the mononitro ether in 100 g. of carbon tetrachloride was placed in a flask, and to it was added 2 c.c. (4 molecular amounts) of nitric acid (Sp. g. 1·52). During the addition of the acid the colour changed to yellow, and finally to orange. Much solid matter separated in the course of a few minutes. After 3 days the carbon tetrachloride was distilled under reduced pressure. Hot alcohol and chloroform extracted from the solid residue some 1·6-dinitroethyl-β-naphthylether, melting at 144–145° C. The white solid which had not dissolved in the chloroform was recrystallised from acetic acid. It melted at 216–217° C., and proved to be 1·8-dinitroethyl-β-naphthylether. The two dinitro compounds were formed in nearly equal amounts.

5. *Action of Nitric Acid on* 1·6-*Dinitroethyl-β-naphthylether.*

About 0·2 g. of 1·6-dinitroethyl-β-naphthylether was added slowly to 0·8 c.c. of fuming nitric acid in a small round flask. During the reaction little or no heat was evolved, but the colour of the solution became orange, and finally red. On standing overnight the contents of the flask became semi-solid. The mixture was poured into water, and the solid was filtered, washed, and dried. It was dissolved in boiling benzene, from which it separated as glistening white plates, which melted at 189–190° C., and proved to be identical with the 1·6·8-trinitroethyl-β-naphthylether already described.

When this trinitro derivative was heated to 180° C. for 8 hours in a sealed tube with 30 parts of alcoholic ammonia a good yield of trinitro-β-naphthylamine was obtained. The latter compound separated from hot acetone as golden yellow prisms, which darkened at 287° C., and melted with decomposition at 300–301° C. It gave on analysis the following results:—

0·1788 g. substance gave 31 c.c. moist nitrogen at 12° C. and 766 m.m.

corresponding to N 20·54
$C_{10}H_6O_6N_4$ requires N 20·15.

This ether is probably identical with the trinitroethyl-β-naphthylether already prepared by Staedel [Liebig's Ann. ccxvii (1883), p. 174].

6. *Action of Nitric Acid on* 1·8-*Dinitroethyl-β-naphthylether.*

An experiment similar to the last was carried out with 1·8-dinitroethyl-β-naphthylether, and again a similar reddish solution of the nitrated compound was formed without the evolution of much heat. After remaining overnight the contents of the flask were mixed with water and the solid was filtered, washed, and dried. It separated from hot benzene as colourless leaves or plates, melting at 189–190° C., and identical with the 1·6·8-trinitroethyl-β-naphthylether described above.

An attempt was made to nitrate this substance still further by heating 0·5 g. of it with 2 c.c. of fuming nitric acid for 8 hours. The contents of the flask were then allowed to remain overnight, and finally were poured into water. A clear yellow solution was obtained. On evaporating the aqueous solution a small amount of a brownish solid remained. This dissolved in potash, forming a deep-red solution. It was also very soluble in water, alcohol, or acetic acid, but was nearly insoluble in petroleum ether, chloroform, or benzene. It could not be obtained in a pure form, and was probably a decomposition product of the nitrated ether.

SUMMARY.

1. By the action of the oxides and the oxyacids of nitrogen on ethyl-β-naphthylether in dilute solution the main product was 1-nitroethyl-β-naphthylether.

2. Nitrogen peroxide in the vapour phase converted ethyl-β-naphthylether into 1·6- and 1·8-dinitroethyl-β-naphthylether. In carbon tetrachloride solution it formed the 1·8-dinitro- and 1-nitroethyl-β-naphthylether, while in acetic acid solution the 1-nitro compound was the main product.

3. Nitric acid in carbon tetrachloride solution converted the ether into 1·6- and 1·8-dinitroethyl-β-naphthylether.

4. Nitric acid converted 1-nitro-, 1·6-dinitro, and 1·8-dinitroethyl-β-naphthylether into the same trinitro derivative which was, therefore, 1·6·8-trinitroethyl-β-naphthylether.

The above investigation was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for the materials employed in the research.

No. 38.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON DIPHENYLETHYLENEETHER

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(Read December 18, 1923. Printed February 27, 1924.)

Introduction.

It has been shown by one of us in conjunction with J. Keane (No. 37) that ethyl-β-naphthylether interacts with the oxides and the oxyacids of nitrogen with greater ease than phenylbenzylether. Each of these ethers contains two nitratable rings—in the same radicle in the case of the naphthylether, and in different radicles in that of phenylbenzylether.

Diphenylethyleneether, another ether which contains two nitratable rings, resembles phenylbenzylether in so far as these rings are in different radicles. On the other hand, as this substance approaches the aryl-alphyl ethers in constitution, and is moreover a di-ether, it seemed of interest to examine its behaviour towards nitrating agents, more especially under the conditions of low temperatures and low concentrations employed in the previous investigations.

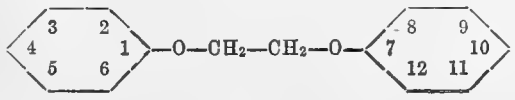
The action of the oxides or the oxyacids of nitrogen on the ether, which can be conveniently prepared from potassium phenolate and ethylene dibromide, or ethylene dichloride, by the method of W. H. Perkin [Journ. Chem. Soc., lxix (1888), p. 165–166], has not been previously examined.

Some of its nitro derivatives have, however, been already obtained by the combination of nitrophenols with ethylene dihalides. Thus A. Weddige [Journ. f. pr. Ch. (2), xxi (1880), p. 127] prepared 2·8-dinitrodiphenylethyleneether[1] and 4·10-dinitrodiphenylethyleneether from ethylene dibromide and the potassium derivatives of the corresponding nitrophenols. He also obtained a substance [Journ. f. pr. Ch. (2), xxiv (1881), pp. 241–256], melting at 86° C., which he termed 2-nitrodiphenylethyleneether, from phenylbromethylether and a body which he assumed to be 2-nitrophenol. E. Wagner [Journ. f. pr. Ch. (2), xxvii (1883), p. 201] prepared 3·9-dinitrodiphenylethyleneether by a similar reaction.

Before attempting to isolate the products of the interaction of the oxides and the oxyacids of nitrogen with the ether, we deemed it advisable to prepare the simpler nitro derivatives of the latter from ethylene dibromide, or dichloride, and the potassium derivatives of the corresponding nitrophenols, so as to determine the properties of the pure substances, with a view to facilitating their isolation from the nitration mixtures.

By the action of potassium *p*-nitrophenolate on phenylbromethylether we got a compound (4-nitrodiphenylethyleneether) which corresponded in melting-

[1] The nomenclature adopted in this communication is based on the following structural formula for diphenylethyleneether:—



point (86° C.) and other properties with the 2-nitrodiphenylethyleneether of Weddige (*loc. cit.*). On the other hand, from 2-nitrophenol we obtained 2-nitrodiphenylethylenether, which melted at 97° C. Owing to the discrepancy between our results and those of Weddige, the preparation of the ortho compound was repeated, using carefully purified materials, but the product again melted at 97° C.

Weddige's results with regard to the 2·8- and the 4·10-dinitro compounds we were able to confirm.

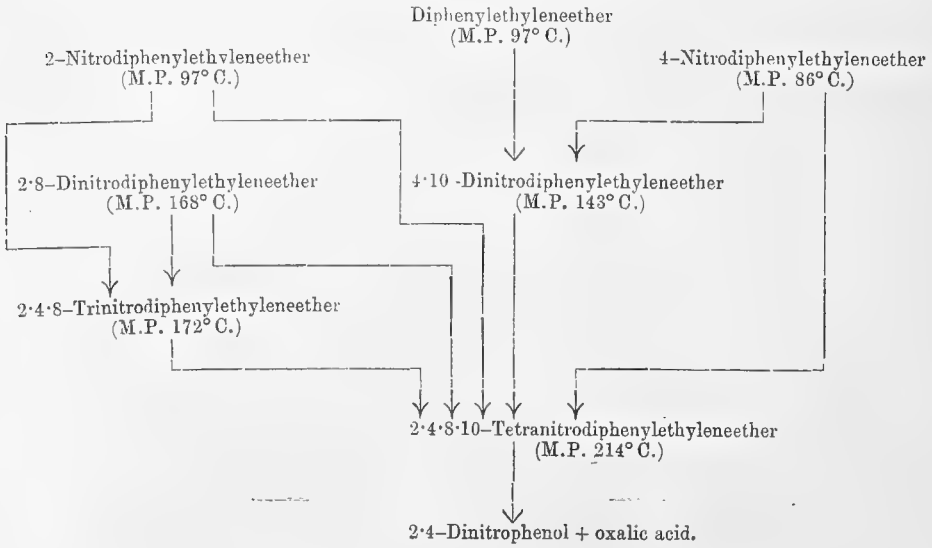
The asymmetrical 2·10-dinitro derivative, which has not been previously described, we prepared in an analogous manner from 2-nitrophenylbromethylether and potassium *p*-nitrophenol.

By the action of nitric acid on the 2·8-dinitro derivative we obtained, in addition to a tetranitro derivative, a trinitro compound, melting at 172° C., and which was therefore very probably 2·4·8-trinitrodiphenylethyleneether. As the same tetranitro derivative was obtained by the nitration of 4·10-dinitrodiphenylethyleneether, its nitro radicles must be in the positions 2·4·8·10, and the substance which melted at 214° C. is therefore 2·4·8·10-tetranitrodiphenylethyleneether. Attempts to convert the tetranitro compound into a higher nitro derivative by further nitration were unsuccessful, portion of the substance being decomposed with formation of 2·4-dinitrophenol, but the greater part of it being recovered unchanged.

In acetic acid solution, at low concentrations of the solutes and at the ordinary temperature, nitric acid, even on prolonged standing (10 weeks), did not nitrate the ether. Under similar conditions we obtained from carbon tetrachloride solutions of the interacting substances 4·10-dinitrodiphenylethyleneether, 2·4·8·10-tetranitrodiphenylethyleneether, oxalic acid, and 2·4-dinitrophenol.

Nitrogen peroxide in the vapour phase or in solution converted the ether into 4·10-dinitrodiphenylethyleneether. In the former case a small quantity of a substance which exploded violently at 203° C. was obtained, and in the latter case, in addition to the dinitro compound, we also isolated some 2·4-dinitrophenol.

The course of the nitration of the original ether and its nitro derivatives may be conveniently illustrated by the following diagram :—



EXPERIMENTAL.

1. *Action of Nitrogen Peroxide on Diphenylethyleneether.*

(*a*) *In the Absence of Solvents.*—Diphenylethyleneether (10 g.) and dry liquid nitrogen peroxide were placed in shallow dishes, side by side, under a bell-jar. The ether absorbed the nitrogen peroxide, and quickly changed into a dark-brown, tarry mass. The nitrogen peroxide was renewed at intervals during the first 15 days, after which time the absorption of the fumes became much slower. At the end of 5 weeks the tarry mass had become again, to a large extent, crystalline. On treating the mixture with chloroform a portion (3·1 g.) dissolved, forming a red solution from which, on evaporation, red crystals separated. These crystals became yellowish-white on standing in the air and melted at 143–144° C. The portion which was not so readily soluble in chloroform dissolved in hot alcohol, giving a green solution, from which 2·2 g. of green crystals separated on cooling. These crystals also melted at 143–144° C., and their melting-point was not affected by the addition to them either of the red crystals mentioned above or of 4·10-dinitrodiphenylethyleneether, which was prepared by the method of Weddige [Journ. f. pr. Ch. (2), xxi (1880), p. 127] from *p*-nitrophenol and ethylene dibromide. When the alcoholic filtrate was concentrated about 7 g. of an oil remained, from which light petroleum separated a very small amount of a crystalline body melting, and exploding violently, at 203° C.

(*b*) *In Carbon Tetrachloride Solution.*—A solution of 5 g. of diphenylethyleneether in 200 g. of carbon tetrachloride was saturated in the cold with nitrogen peroxide vapour and was allowed to remain several weeks in a stoppered flask. A substance which rapidly separated gradually became crystalline, the colour of the solution at the same time changing from brownish-red to light orange-red. The crystalline solid was filtered and washed with ether and alcohol. It weighed 4 g. After recrystallisation from xylene, it melted at 143–144° C., and proved to be 4·10-dinitrodiphenylethyleneether. A further quantity of this substance mixed with some 2·4-dinitrophenol was obtained from alcoholic filtrate.

The carbon tetrachloride solution was washed with aqueous potash and then, on evaporation, gave a small amount of unchanged diphenylethyleneether. From the reddish aqueous potash solution, by acidification and extraction with ether, about 0·4 g. of 2·4-dinitrophenol was obtained.

2. *Action of Nitric Acid on Diphenylethyleneether.*

(*a*) *In the Absence of Solvents.*—Diphenylethyleneether (10 g.) was added slowly to 12 g. of cold concentrated nitric acid (Sp. g. 1·4). From the mixture, which was allowed to remain at the temperature of the room for two days, most of the original ether was recovered, together with a very small amount of oily matter, which did not crystallise. When, however, the mixture was heated on the water-bath for several hours, some 4·10-dinitrodiphenylethyleneether was obtained.

(*b*) When cold fuming acid (Sp. g. 1·52) was employed, in an experiment similar to the last, about 5 g. of 4·10-dinitrodiphenylethyleneether and 1 g. of a substance which was less soluble in acetic acid were obtained. The less soluble substance separated from boiling acetic acid and xylene in the form of yellowish-white flat prisms, melting at 214° C. It gave on analysis the following results:—

0·1830 g. substance gave 22·4 c.c. moist nitrogen at 16° C. and 764 m.m.

corresponding to N 14·28

$C_{14}H_{10}O_{10}N_4$ requires N 14·21.

The substance melting at 214° C., which is sparingly soluble in most solvents, is therefore tetranitrodiphenylethyleneether. When heated on the water-bath with aqueous, or still better with alcoholic potash, it slowly decomposed with formation of 2·4 dinitrophenol.

(*c*) *In Glacial Acetic Acid Solution.*—1, 2, 3, 4, and 6 molecular amounts of nitric acid (Sp. g. 1·52) were added respectively to 5 flasks, each of which contained 5 g. of diphenylethyleneether dissolved in 400 g. of glacial acetic acid. The mixtures were allowed to remain in the stoppered flasks for 10 weeks. From the solutions, which were colourless or faintly orange, the diphenylethyleneether was in each case recovered unchanged.

In another experiment 24 molecular amounts of nitric acid were added to a flask containing 2·5 g. of the ether dissolved in 200 g. of glacial acetic acid. The mixture became warm and the colour of the solution changed through orange, green, purple, and violet, to brown in the course of a few days. The deep-brown solution was examined after 9 weeks, and from it, in addition to the parent ether, only a small quantity of a green oil was obtained.

(*d*) *In Carbon Tetrachloride Solution.*—Solutions of 5 g. of ethylenediphenylether in 200 g. of carbon tetrachloride to which 1, 2, 3, 4, and 6 molecular amounts of nitric acid (Sp. g. 1·52) had been added rapidly turned dark-brown. The colour then gradually became lighter, and finally, after about 3 weeks, it was in all these cases light-orange.

(1) The solution to which 1 molecular amount of nitric acid had been added was filtered, after remaining at the temperature of the room for 18 weeks, from a small amount (0·15 g.) of a suspended solid, which proved to be a mixture of tetranitrodiphenylethyleneether with oxalic acid and a minute quantity of a lower melting (140–150° C.) fraction, which probably contained 4·10-dinitrodiphenylethyleneether.

From the carbon tetrachloride filtrate 2·4-dinitrophenol was extracted with alkali, and by evaporating the carbon tetrachloride we got about 4·3 g. of unchanged diphenylethyleneether.

(2) From the solution to which 2 molecular amounts of nitric acid had been added, 3·8 g. of the original ether were recovered, together with small amounts of oxalic acid.

The tetranitro compound, which was probably also formed in the reaction, was not isolated.

(3) The solid suspended in the solution to which 3 molecular amounts of nitric acid had been added, and which, like the last, had been allowed to remain at the temperature of the room for 23 weeks, weighed 1·2 g., and consisted of tetranitrodiphenylethyleneether, with some oxalic acid. From the carbon tetrachloride filtrate 3 g. of the original ether and 0·8 of dinitrophenol were isolated. A small amount of a reddish oily substance obtained probably contained 4·10-dinitrodiphenylethyleneether.

(4) The solution to which 4 molecular amounts of nitric acid had been added was allowed to remain at the temperature of the room for 11 weeks. From it 2·4-dinitrophenol and oxalic acid in larger amounts than those got in the last experiment were isolated. Rather more than 1 g. of tetranitrodiphenylethyleneether and 1·3 g. of the original ether were also obtained, together with a reddish oily substance similar to that mentioned in the last experiment.

(5) The solution to which 6 molecular amounts of nitric acid had been added on short standing (3 weeks) gave a tarry mass, from which the original ether, 2·4·8·10-tetranitrodiphenylethyleneether and 2·4-dinitrophenol, were isolated. When the action was allowed to progress for 25 weeks the solution

contained 4·5 g. of suspended solids, amongst which were some large colourless prisms of oxalic acid (about 1 g.). The filtered solids were washed with water, alcohol, and ether, and then recrystallised from xylene, from which about 1 g. of pure tetranitrodiphenylethyleneether, melting at 214° C., separated. Aqueous potash extracted 0·3 g. of pure 2·4-dinitrophenol from the carbon tetrachloride filtrate. A small amount of a reddish oily semi-crystalline substance was also obtained, but was not further examined.

3. *Action of Nitric Acid on Nitrodiphenylethyleneether.*

2-Nitrodiphenylethyleneether (2 g.) was added slowly to 5 parts nitric acid (Sp. g. 1·51) with which it reacted energetically with formation of a red solution, and, after a short time, with a separation of a crystalline solid. The mixture was let remain at the temperature of the room for 2 days; it was then poured into water, and the solid which separated was filtered and dried. Alcohol separated from this a very small quantity of an oily body, which was not further examined. By recrystallising the residue from xylene it was separated into tetranitrodiphenylethyleneether, melting at 214° C., and a more soluble crystalline solid, melting at 172° C. The latter substance gave on analysis the following results:—

0·137 g. substance gave 13·8 c.c. moist nitrogen at 16° C. and 764 m.m.

corresponding to N 11·89
$C_{14}H_{11}O_8N_3$ requires N 12·03.

The substance was therefore a trinitrodiphenylethyleneether.

4. *Action of Nitric Acid on 4-Nitrodiphenylethyleneether.*

3 g. of *p*-nitrodiphenylethyleneether were added slowly to 15 g. of nitric (Sp. g. 1·51), and the mixture was allowed to remain overnight at the temperature of the room. It was then poured into water, and the solid which separated was filtered and washed with water, alcohol, and ether. It weighed 2·9 g., and consisted of nearly pure tetranitrodiphenylethyleneether. A dark-coloured impure substance, melting between 140 and 160° C., was also obtained, but the amount was insufficient for further examination.

5. *Action of Nitric Acid on 2·8-Dinitrodiphenylethyleneether.*

Nitration of the dinitro derivative, by a method analogous to that employed above in the case of the 2-nitro derivative, gave a product from which we isolated trinitrodiphenylethyleneether, melting at 172° C., and tetranitrodiphenylethyleneether, melting at 214° C.

6. *Action of Nitric Acid on 4·10-Dinitrodiphenylethyleneether.*

4·10-Dinitrodiphenyleneether (1 g.) was added to 5 parts of nitric acid (Sp. g. 1·51), and the crystals which separated after about 3 hours were removed, washed, dried, and recrystallised from xylene. About 0·95 g. of tetranitrodiphenylethyleneether, melting at 214° C., were thus obtained. When the nitric acid solution, which had been separated from the crystals, was poured into water about 0·2 g. of the solid separated, and this, after crystallisation from alcohol and xylene, melted between 185 and 210° C., probably consisting of a mixture of the tetranitro compound with the unchanged parent substance.

7. *Action of Nitric Acid on 2·4·8·10-Tetranitrodiphenylethyleneether.*

Tetranitrodiphenylethyleneether was heated for an hour and a half on the water-bath with 5 parts of mixed acids, and the mixture was then poured into water.

The product consisted mainly of unchanged tetranitrodiphenylethyleneether together with a small amount of 2·4-dinitrophenol, melting at 114° C.

8. *Synthetical Preparations of the Nitro Derivatives.*

(*a*) *2-Nitrodiphenylethyleneether.*—Phenylbromethylether (10 g.), which was prepared from ethylene dibromide and potassium phenolate by the method of W. H. Perkin [Journ. Chem. Soc., lxix (1888), p. 165–6], was added to a solution of potash (3 g.) and 2-nitrophenol (8 g.) in alcohol (50 g.), and the mixture was heated to gentle boiling under a reflux condenser for 16 to 18 hours. The residue left after distillation of the alcohol was washed with water and recrystallised several times from alcohol. It melted at 97° C. and gave on analysis the following results:—

0·181 g. substance gave 8·6 c.c. moist nitrogen at 16° C. and 753 m.m.

corresponding to N 5·6
$C_{14}H_{13}O_4N$ requires N 5·4.

2-Nitrodiphenylethyleneether crystallises from alcohol in the form of rectangular plates which are sparingly soluble in cold alcohol, soluble in ether, and readily soluble in hot alcohol or chloroform. It is insoluble in aqueous potash.

(*b*) *4-Nitrodiphenylethyleneether.*—A solution of 2 g. potash and 6 g. 4-nitrophenol and 10·5 g. of phenylbromethylether in 50 g. of alcohol was heated under a reflux condenser for 24 hours. The alcohol was removed by distillation, and the product was freed from potassium bromide and excess of 4-nitrophenol by washing with dilute alkali. After several recrystallisations from alcohol it melted at 86° C., and gave on analysis the following results:—

0·209 g. substance gave 10·1 c.c. moist nitrogen at 16° C. and 757 m.m.

corresponding to N 5·69
$C_{14}H_{13}O_4N$ requires N 5·4.

4-Nitrodiphenylethyleneether crystallises from ether and alcohol in the form of prisms which are insoluble in potash, soluble in alcohol, chloroform, and acetone, sparingly soluble in ether.

(*c*) *2·10-Dinitrodiphenylethyleneether.*—2-Nitrophenylbromethylether, which was prepared from 2-nitrophenol and ethylene dibromide by the method already described by Weddige [Journ. f. pr. Ch. (2), xxiv (1881), p. 246], was heated for 26 hours under a reflux condenser with an alcoholic solution of the potassium derivative of 4-nitrophenol. The alcohol was then distilled, and the residue was washed with dilute potash until it was free from potassium bromide and 4-nitrophenol. After recrystallisation from alcohol the substance melted at 117·5° C. It gave on analysis the following results:—

0·184 g. substance gave 15·2 c.c. moist nitrogen at 15° C. and 760 m.m.

corresponding to N 9·6
$C_{14}H_{12}O_6N_2$ requires N 9·2.

2·10-Dinitrodiphenylethyleneether crystallises from alcohol in the form of rectangular prisms. It is sparingly soluble in cold alcohol, but readily soluble in warm alcohol or acetone.

SUMMARY.

1. By the action of phenylbromethylether on 2-nitrophenol and 4-nitrophenol, 2- and 4-nitrodiphenylethyleneether were obtained. These melted respectively at 97° C. and 86° C. In a similar manner 2·10-dinitrodiphenylethyleneether, melting at 117·5° C., was prepared.

2. Concentrated nitric acid converted 2-nitro- as well as 2·8-dinitrodiphenylethyleneether into 2·4·8-trinitrodiphenylethyleneether, melting at 172° C. The latter substance, and also 4·10-dinitrodiphenylethyleneether, were in turn converted into 2·4·8·10-tetranitrodiphenylethyleneether, which melted at 214° C.
Attempts to nitrate the tetranitro ether still further led to its decomposition into 2·4-dinitrophenol and oxalic acid.

3. Nitrogen peroxide in the vapour phase acted slowly on the ether, forming 4·10-dinitrodiphenylethyleneether and an oil containing a very small amount of an explosive solid, melting and exploding at 203° C.
In carbon tetrachloride solution nitrogen peroxide converted the ether into its 4·10-dinitro derivative and 2·4-dinitrophenol.

4. Nitric acid in dilute acetic acid solution had no appreciable action on the ether, but in carbon tetrachloride solution 4·10-dinitro- and 2·4·8·10-tetranitrodiphenylethyleneether, with oxalic acid and 2·4-dinitrophenol, were formed.

In conclusion we wish to state that the above research was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for a grant in aid of the investigation.

No. 39.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON DIPHENYLETHER.

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Introduction.

In previous communications from this laboratory the behaviour of some secondary amines, urethanes, ureas, and ethers towards the oxides and the oxyacids of nitrogen has been described.

Of the different substances examined, that which nitrated most smoothly was diphenylnitrosamine. Its parent amine, diphenylamine, underwent complex side-reactions during the course of its nitration [Proc. R.I.A., xxxiv, B, pp. 194, 212]. It seemed likely, therefore, that the difference in the behaviour of the two bodies was due to the replacement of the oxidisable hydrogen atom of the imino radicle by the nitroso group. It was shown later, however, that when this hydrogen atom was replaced by a carbethoxy radicle the urethane thus formed nitrated much less easily [Proc. R.D.S., xvii, N.S., No. 14] than diphenylnitrosamine.

Diphenylether corresponds closely in constitution with diphenylamine, and as it does not contain an easily oxidisable radicle, it might be expected to behave on nitration like diphenylnitrosamine.

The action of nitric acid on diphenylether has been previously studied by Mailhe and Muret [Comptes Rendus, cliv (1912), p. 715; Bull. Soc. Chim., x (1913), p. 1011]. According to these chemists fuming nitric acid converted the ether into 4·10-dinitrodiphenylether, a trinitro derivative, a tetranitro derivative, melting at 95° C., and a pentanitro derivative, melting at 86–88° C. In their later communication they stated that this pentanitro derivative did not exist. By the action of nitric acid on a solution of the ether in glacial acetic acid they obtained 4-nitrodiphenylether.

A. N. Cook [Journ. Amer. Chem. Soc., xxii (1910), p. 1285] converted 2·4-dinitrodiphenylether into a trinitro derivative, which we found to be 2·4·10-trinitrodiphenylether, and which we nitrated further to 2·4·8·10-tetranitrodiphenylether, melting at 195° C.

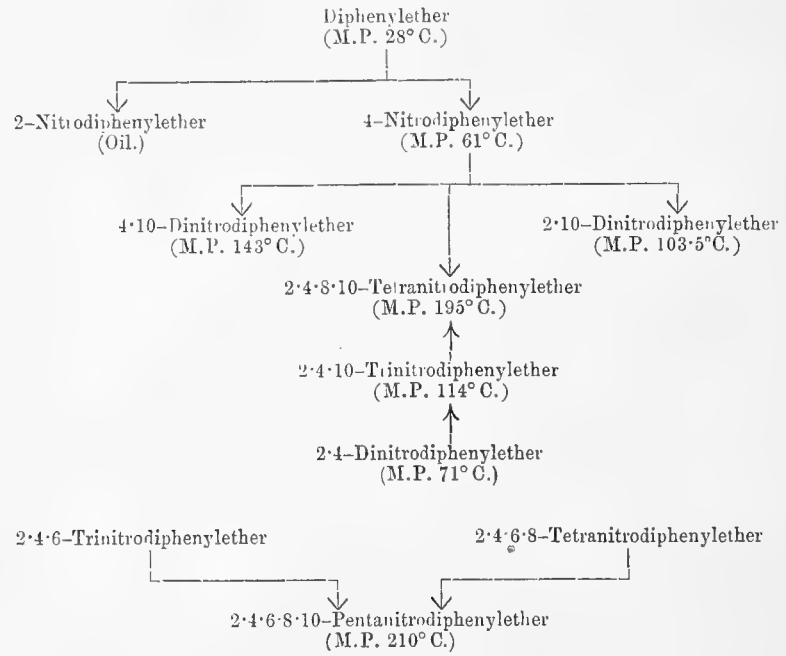
In our experiments nitric acid, in the absence of solvents, converted diphenylether into 4-nitro-, 2·10-, and 4·10-dinitro-, and 2·4·8·10-tetranitrodiphenylether. Similarly from 2·4·6-trinitro- and 2·4·6·8-tetranitrodiphenylether we prepared a pentanitro compound, melting at 210° C., and which was probably 2·4·6 8·10-pentanitrodiphenylether.

At low concentrations of the interacting substances and at the ordinary temperature, nitric acid had scarcely any action on diphenylether in acetic acid solution. When, however, the solution contained 24 molecular amounts of nitric acid 4-nitrodiphenylether, 2·4-dinitrophenol, and, probably, 2-nitrodiphenylether were formed. In carbon tetrachloride solution, even at low concentrations of the acid and the ether, the latter was converted into 4-nitro-, 2·10-, and 4·10-dinitrodiphenylether, and 2·4-dinitrophenol.

Nitrogen peroxide acted rapidly on diphenylether producing 4-nitro-, 2·10-, and 4·10-dinitrodiphenylether, with 2·4-dinitrophenol. Nitrogen sesquioxide behaved like the peroxide.

Although diphenylether nitrates more easily than diphenylurethane, and more smoothly than diphenylamine, it is not in either of these respects so readily acted upon as diphenylnitrosamine. Moreover, its tetranitro derivative decomposes rather easily into dinitrophenol.

The course of the nitration of diphenylether and its nitro derivatives may be conveniently represented by the following diagram :—



Experimental.

1. *Preparation of Diphenylether and its Nitro Derivatives.*

Before examining the behaviour of diphenylether towards the oxides and the oxyacids of nitrogen it was necessary to select a convenient method for its preparation, and also to determine the properties of such derivatives of it as could be obtained from the nitrophenols at our disposal.

(*a*) *Diphenylether.*—We found the methods of Gladstone and Tribe [Journ. Chem. Soc., xli (1882), p. 8]; Merz and Weith [Ber. Dtsch. Chem. Ges., xiv (1881), p. 189]; and Hoffmeister [Liebig's Annalen, clix (1871), p. 191], inconvenient for the preparation of this substance, which can, on the other hand, be readily made by the method of Ullmann and Sponagel [Ber. Dtsch. Chem. Ges., xxxviii (1905), p. 22117],

A mixture of 47·2 g. of phenol, 24·8 g. of potash, 62·8 g. of bromobenzene, and 0·4 g. of copper bronze was heated in an oil-bath under a reflux condenser for 3 hours to a temperature which was gradually raised from 190° C. to 230° C. When the reaction-product was distilled in a current of steam the unchanged bromobenzene passed over first and was followed by the diphenylether, which quickly solidified. The colourless crystalline diphenylether melted at 28° C., and dissolved easily in the ordinary organic solvents.

(*b*) *4-Nitrodiphenylether,* which melted at 61° C. and dissolved easily in ether or benzene, and moderately in cold alcohol or ligroin, was readily obtained by the method of Haeussermann and Teichmann [Ber. Dtsch. Chem. Ges., xxix (1896), p. 1446], in which 4-nitrochlorobenzene and potassium phenolate are heated in phenol solution to 150° C. for five hours. The product was freed from phenol by washing it with aqueous alkali, and from excess of 4-nitrochlorobenzene by distilling the latter in a current of steam. The purified substance was then recrystallised from hot alcohol.

(*c*) *2·4-Dinitrodiphenylether* was obtained from 2·4-dinitrochlorobenzene and potassium phenolate by the method previously employed for its preparation by Willgerodt [Ber. Dtsch. Chem. Ges., xxii (1879), p. 767; compare Maikopar, ibid., vi (1873), p. 564]. It separated from alcohol in the form of long acicular crystals, which melted at 71° C.

(*d*) *2·10-Dinitrodiphenylether,* which was previously obtained by Häussermann and Bauer [Ber. Dtsch. Chem. Ges., xxix (1896), p. 2083], was prepared by their method from 4-nitrochlorobenzene and the potassium derivatives of 2-nitrophenol. It consisted of colourless, acicular crystals, which melted at 103·5° C., and were sparingly soluble in cold, but easily in hot, alcohol.

(*e*) *2·4·6-Trinitrodiphenylether.*—Willgerodt [Ber. Dtsch. Chem. Ges., xii (1879), p. 1278] obtained this body from picryl chloride and potassium phenolate. We obtained it by a similar method. It melted, as Willgerodt states, at 153° C.

(*f*) *2·4·10-Trinitrodiphenylether.*—Following the method by which Willgerodt and Huetlin first obtained this compound [Ber. Dtsch. Chem. Ges., xvii (1884), p. 1765], we prepared it by heating the potassium derivative of 4-nitrophenol with an alcoholic solution of 2·4-dinitrochlorobenzene in a sealed tube to 150° C. for 6 hours. The pure compound consisted of thin six-sided, tabular crystals, which melted at 114° C., were soluble in hot alcohol, difficultly soluble in ether, and nearly insoluble in ligroin.

2. *Action of Nitrogen Peroxide on Diphenylether.*

(*a*) *In the Absence of Solvents.*—Diphenylether (4 g.) and liquid nitrogen peroxide were placed in shallow glass dishes, side by side, under a bell-jar, and allowed to remain at the temperature of the room for a month. Initially the absorption of the nitrogen peroxide was rapid, and this substance was, therefore, renewed from time to time as required. The solid at first became dark in colour and oily in consistency; it finally changed into a mixture of a crystalline body with a dark-red oil. The nitrogen peroxide was removed at the end of the month by spontaneous evaporation, and the oily matter was separated from the crystals by extraction with ether. The solid left undissolved by the ether separated from hot alcohol in the form of colourless needles, which melted at 103° C. As its melting-point was not affected by addition to the substance of 2·10-dinitrodiphenylether (prepared as described above), the compound must have been identical with the latter body.

When the ethereal solution of the oil was washed with potash, the alkaline layer became red in colour, and from it, by acidification, extraction with

ether, and recrystallisation from dilute alcohol, 2·4-dinitrophenol was obtained. When the ether solution, which had been washed with dilute alkali, was allowed to evaporate, a dark-red oily substance remained, and this, when shaken with acetone, to a large extent dissolved, leaving a small amount of a crystalline solid, which melted at 142° C., and which gave on analysis the following results :—

0·1000 g. substance gave 9·4 c.c. moist nitrogen at 16° C. and 764 m.m.

corresponding to N 11·1
$C_{12}H_8O_5N_2$ requires N 10·8.

The latter compound was, therefore, a dinitrodiphenylether, and must have been identical with the 4·10-dinitrodiphenylether, melting at 143° C., which Häussermann and Teichmann [Ber. Dtsch. Chem. Ges., xxix (1896), p. 1448] obtained from 4-nitrochlorobenzene and the potassium derivative of 4-nitrophenol.

The light-red oil, left on evaporation of the acetone, did not crystallise.

In another experiment, under somewhat different conditions of temperature, the main product was 4·10-dinitrodiphenylether, and with it were mixed 2·4-dinitrophenol and some unchanged diphenylether.

(*b*) *In Carbon Tetrachloride Solution.*—Nitrogen peroxide vapour was passed for some time through a solution of 4 g. of diphenylether in 80 g. of carbon tetrachloride. After remaining for 6 weeks at the temperature of the room, the reddish-coloured solution was filtered from the red tarry solid, which had separated, and distilled. The tarry-red solid and the residue left on the distillation of the carbon tetrachloride were together dissolved in hot alcohol. When the solution was allowed to stand for some time colourless crystals of 2·10-dinitrodiphenylether were deposited. By evaporating the parent liquid from these crystals a red oil was again obtained. 2·4-Dinitrophenol was separated from the oil by dissolving the latter in ether and extracting it with dilute aqueous potash. The light-reddish oil, which remained when the ether had evaporated, was subjected to a steam-distillation. Some unchanged diphenylether passed over with the steam, but the light-yellow oil left in the distillation-flask did not crystallise. It dissolved easily in all solvents except benzene, and probably contained mononitrodiphenylethers.

3. *Action of Nitrous Fumes on Diphenylether.*

(*a*) *In Carbon Tetrachloride Solution.*—Nitrous fumes, prepared from nitric acid and arsenious oxide, were passed for a considerable time through a 5 per cent. solution of diphenylether in carbon tetrachloride.

After remaining 4 weeks in a stoppered flask, the solution had a light-red colour, and contained some colourless prismatic crystals floating on it. From it, by a method analogous to that just described, we obtained 2·10-dinitrodiphenylether, 2·4-dinitrophenol, and a yellow oil, which did not, however, crystallise.

(*b*) *In Acetic Acid Solution.*—In another experiment, in which glacial acetic acid was employed as the solvent, no solid separated from the deep-red solution. The latter, after remaining 4 weeks at the temperature of the room, was poured into a large volume of water, and the oil which separated was extracted with ether. The ethereal solution was freed from 2·4-dinitrophenol by washing it with dilute alkali, and then on evaporation left a reddish oil, which separated from hot alcohol as a light-yellow solid. The latter, when recrystallised from a small amount of petroleum ether, was obtained in the form of colour-

less plates, which melted at 56° C. As a mixture of it with 4-nitrodiphenylether (melting at 61° C.) melted about 58° C., and as the two bodies crystallised in truncated rhombohedral plates, having an obtuse angle of 112° and an acute angle of 68°, the substance melting at 56° C. was probably slightly impure 4-nitrodiphenylether. This compound, which was the chief product of the reaction, was very soluble in ether or benzene, and moderately soluble in cold alcohol.

4. *Action of Nitric Acid on Diphenylether.*

(*a*) *In the Absence of Solvents.*—3 g. of diphenylether was added slowly to 15 g. of nitric acid (Sp. g. 1·4), contained in a flask, and the contents were shaken. As the ether remained undissolved in the cold, the contents of the flask were heated on a water-bath for an hour, when a copious evolution of oxides of nitrogen took place. After heating for 5 hours a deep-red solution with a small amount of a dark-red oil floating on top of the acid layer was obtained. When the mixture was poured into a large excess of water a dark-red oil was precipitated. This was extracted with ether, and the ether layer was washed with alkali. The ether on evaporation gave a yellow oil, which was warmed with petroleum ether in which a portion of it dissolved. The petroleum ether on evaporation left a somewhat oily solid. This was dissolved in alcohol, which on concentration gave colourless plates, melting about 56° C., and probably consisting of slightly impure 4-nitrodiphenylether.

The oil which remained undissolved by the petroleum ether was light-yellow in colour, and was treated with various organic solvents from which, however, it did not crystallise. On further nitrating this oil with nitric acid (Sp. g. 1·52) a yellow crystalline solid was obtained which gave the following results on analysis :—

0·1000 g. substance gave 13·7 c.c. of moist nitrogen at 14° C. and 756 m.m.

corresponding to N 16·02
$C_{12}H_6O_9N_4$ requires N 16·0.

As it agreed in melting-point (195° C.) with the 2·4·8·10-tetranitro compound obtained from 2·4-dinitrochlorobenzene and 2·4-dinitrophenol, it must have been 2·4·8·10-tetranitrodiphenylether.

(*b*) *Action of Fuming Nitric Acid.*—Nitric Acid (Sp. g. 1·52) was cooled in a freezing mixture and diphenylether (1 g. of the ether to 5 g. of the acid) was slowly added. A small amount of charring occurred, and there was a copious evolution of oxides of nitrogen. The ether quickly dissolved in the acid giving a red solution which, after remaining overnight at the temperature of the room, was poured into water. The white solid which was precipitated was washed with alkali. After recrystallisation from acetone a solid separated, which, when again crystallised from acetic acid, melted at 195° C., and was identical with the tetranitro compound obtained in the last experiment. The acetone filtrate on concentration gave a solid, which, after recrystallisation from benzene, melted at 143° C., and consisted therefore of 4·10-dinitrodiphenylether.

(*c*) *Action of Mixed Acids.*—The sole product of the action at the ordinary temperature of a mixture of 5 parts of nitric acid (Sp. g. 1·52) and 5 parts of concentrated sulphuric acid on diphenylether was 2·4·8·10-tetranitrodiphenylether, melting at 195° C.

(*d*) *In Acetic Acid Solution at low concentrations.*—To 5 solutions, containing

in each case 2 g. of diphenylether in 40 g. of acetic acid, 1, 2, 3, 4, and 5 molecular amounts of nitric acid (Sp. g. 1·52) were added. A light-yellow coloration was developed, which deepened, in the case of the solutions containing 4 and 5 molecular amounts of the acid, to a light red. When the contents of each bottle were poured into water a colourless oil separated. The oil rapidly changed into a crystalline solid, which melted at 28° C., and proved to be unchanged diphenylether. About 90 per cent. of the ether was recovered in each case.

(*e*) *In Acetic Acid Solution at ordinary concentrations.*—Using two similar solutions of the ether in acetic acid containing, however, 12 and 24 molecular amounts of nitric acid respectively, deep-red solutions were obtained with a considerable evolution of heat. On pouring either of the solutions into water a light-red oil was precipitated. The oil was extracted with ether, and the ether layer was freed from 2·4-dinitrophenol by washing it with dilute alkali. The ether on evaporation left a solid which separated from alcohol in the form of prisms, melting at 56° C., and probably consisting of slightly impure 4-nitrodiphenylether.

(*f*) *In Carbon Tetrachloride Solution.*—To each of three solutions containing 2 g. of diphenylether in 40 c.c. of carbon tetrachloride were added 1, 3, and 5 molecular amounts of nitric acid (Sp. g. 1·52) respectively. These solutions were allowed to remain at the temperature of the room for 6 weeks. The bottle to which 1 molecular amount of acid had been added was at first yellow in colour, and contained a few transparent prisms mixed with a deep-red oil floating on top of the carbon tetrachloride. As the reaction progressed the oil was gradually converted into a brown-tarry substance. The carbon tetrachloride was distilled, and the oil which remained was extracted with ether. The ether layer was freed from 2·4-dinitrophenol by washing it with dilute alkali. The ether on evaporation left an oil which on distillation with steam was found to contain unchanged diphenylether. The residual oil gave a few crystals which were washed with alcohol, and were then found to be 4-nitrodiphenylether.

The remaining solutions were examined in a different manner. The carbon tetrachloride was orange-yellow in colour and had a dark-red oily layer above it, containing large thick prisms. An equal volume of water was added to the mixture, and the carbon tetrachloride was separated from the aqueous layer, which was extracted with ether. When the ether solution was freed from 2·4-dinitrophenol, by washing it with dilute potash, it left on evaporation a somewhat oily solid. On crystallising this solid from benzene it separated in the form of blunt prisms, which melted at 143° C., and consisted of 4·10-dinitrodiphenylether. When the carbon tetrachloride layer was washed with dilute alkali and allowed to remain for some time a solid separated, and this was filtered. The filtrate on complete evaporation left an oil containing some unchanged diphenylether. The solid which had separated from the carbon tetrachloride was warmed with alcohol. A portion which had not dissolved proved to be 4·10-dinitrodiphenylether. By evaporating the alcoholic solution a solid was obtained. This was dissolved in warm petroleum ether from which it separated as colourless crystals, melting about 95° C., and obviously consisting of 2·10-dinitrodiphenylether, which melts, when pure, at 103·5° C.

Similar results were obtained from the solutions to which 3 and 5 molecular amounts of nitric acid had been added, different amounts of the nitro compounds being, however, obtained from the different solutions.

5. *Action of Nitric Acid on 4-Nitrodiphenylether.*

About 1 g. of 4-nitrodiphenylether was added to 5 g. of nitric acid (Sp. g. 1·4), and the mixture was heated for an hour on the water-bath. The colour of the solution became dark red, and oxides of nitrogen were evolved. The solid which separated when the mixture was poured into water was filtered, dried, and washed with ether. The undissolved portion, which was purified by recrystallisation from benzene, melted at 143°C., and consisted of 4·10-dinitrodiphenylether. The dissolved portion was recovered by evaporating the ether. It melted about 95° C., and probably consisted of 2·10-dinitrodiphenylether in a somewhat impure condition.

In a similar experiment with fuming nitric acid, the mononitroether dissolved with evolution of much heat. The solution was allowed to remain overnight at the temperature of the room, and was then poured into water. The yellow solid which separated was dried and recrystallised from benzene. It melted at 195° C., and proved to be 2·4·8·10-tetranitrodiphenylether.

6. *Action of Nitric Acid on 2·4-Dinitrodiphenylether.*

By nitrating 2·4-dinitrodiphenylether by a method analogous to that of the last experiment, a deep-red solution was obtained, and from this, by addition of water, a light-yellow solid was precipitated. The solid was dissolved in boiling alcohol, and the solution was allowed to crystallise. The first fraction which separated consisted of rectangular prisms, which melted about 110° C., and were found to be slightly impure 2·4·10-trinitrodiphenylether. The second fraction was separated by means of alcohol into two substances. One of these was 2·4·8·10-tetranitrodiphenylether, melting at 195° C. The other melted at 153° C., but, as its amount was not sufficient for an analysis, we were unable to determine whether it was 2·4·6-trinitro- or 2·4·6·10-tetranitrodiphenylether, each of which, according to Willgerodt [Ber. Dtsch. Chem. Ges., xii (1879), p. 1278; xvi (1884), p. 1766], melts at 153° C.

7. *Action of Nitric Acid on 2·4·6-Trinitrodiphenylether and 2·4·10-Trinitrodiphenylether.*

(*a*) About 1 g. of 2·4·6-trinitrodiphenylether was added to 5 g. of fuming nitric acid. On remaining at the room temperature a white solid separated. The mixture was poured into water, the solid was filtered, and recrystallised from acetic acid. It consisted of colourless needles, which melted at 210° C., and were sparingly soluble in alcohol, but easily in acetone. It gave on analysis the following results:—

0·1200 g. substance gave 18·65 c.c. moist nitrogen at 15° C. and 754 m.m.

corresponding to N 18·05
$C_{12}H_5O_{11}N_5$ requires N 17·8.

The substance was therefore a pentanitrodiphenylether.

(*b*) The nitration of 2·4·6-trinitro-, or 2·4·6·8-tetranitrodiphenylether, with a mixture of 5 parts of fuming nitric acid and 5 parts of concentrated sulphuric acid, gave in each case the same pentanitrodiphenylether, melting at 210° C. The latter compound was probably 2·4·6·8·10-pentanitrodiphenylether.

(*c*) Fuming nitric acid converted 2·4·10-trinitrodiphenylether into 2·4·8·10-tetranitrodiphenylether, melting at 195° C.

SUMMARY.

1. Nitrogen peroxide vapour acted rapidly on diphenylether forming 2·10- and 4·10-dinitrodiphenylether together with some 2·4-dinitrophenol. In carbon tetrachloride solution the main products were 2·10-dinitrodiphenylether and 2·4-dinitrophenol, but in acetic acid solution the chief product was 4-nitrodiphenylether.

2. Nitric acid at low concentrations in acetic acid solution had no appreciable action on diphenylether. At more or less high concentrations 4-nitrodiphenylether was formed.

In carbon tetrachloride solution, even at low concentrations of the nitric acid, 4-nitro-, 2·10-, and 4·10-dinitrodiphenylether, and 2·4-dinitrophenol were formed.

3. Concentrated nitric acid converted the ether into 4-nitro-, 4·10-dinitro- and 2·4·8·10-tetranitrodiphenylether. The last substance, which was also obtained from 2·4-dinitrochlorobenzene and 2·4-dinitrophenol, melted at 195° C.

4. By the action of nitric acid on 4-nitrodiphenylether, 4·10- and 2·10-dinitro-, with 2·4·8·10-tetranitrodiphenylether, were formed. This tetranitro compound was also prepared by the nitration of 2·4-dinitro-, or 2·4·10-trinitrodiphenylether.

5. A pentanitrodiphenylether, which was probably the 2·4·6·8·10-pentanitro derivative, was obtained by nitrating 2·4·6-trinitro, or 2·4·6·8-tetranitrodiphenylether. It melted at 210° C.

The above research was undertaken at the request of the Research Section of Nobel's Explosives Company, to whom we are indebted for a grant in aid of the investigation.

No. 40.

THE ACTION OF THE OXIDES AND THE OXYACIDS OF NITROGEN ON DIPHENYLENE OXIDE.

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Introduction.

A comparison of nitration under similar conditions has shown [H. Ryan and P. Ryan, Proc. R.I.A., xxxiv, B, pp. 194, 212] that diphenylnitrosamine is more smoothly nitrated than diphenylamine. Interesting results have also been obtained by comparing the ease of nitration of other substituted diphenylamines, *e.g.*, urethanes, with diphenylamine and diphenylnitrosamine. Thus H. Ryan and A. Donnellan [Proc. R.D.S., xvii, N.S., p. 113] found that diphenylurethane was less easily nitrated than diphenylamine, while in the paper preceding the present one H. Ryan and P. J. Drumm describe an investigation on diphenylether, which differs from diphenylamine only by the substitution of an oxygen atom for an imino radicle.

In the present investigation a similar ether, diphenylene oxide, was examined, and its behaviour towards nitrating agents was compared with that of diphenylether. In the case of diphenylene oxide, unlike that of diphenylether, there was a complete absence of decomposition products, derivatives of the former being evidently less easily decomposed than those of the latter substance. Also diphenylene oxide was less easily nitrated than diphenylether, for concentrated nitric acid in the cold was almost without action upon it. Furthermore, the highest nitro derivative of diphenylene oxide we were able to obtain was the tetranitro compound, while in the case of diphenylether a pentanitro compound was prepared. We also found that, as in the case of diphenylether, nitrations in carbon tetrachloride solutions gave higher nitro derivatives than those under similar conditions where the solvent was glacial acetic acid. Hence, it is evident that the solvent exercises an influence upon the reaction.

Diphenylene oxide has been obtained, mostly in small yield, by various methods, *e.g.*, by the distillation of calcium phenolate [Niederhäusern, Ber. Dtsch. Chem. Ges., xv (1882), p. 1120]; by heating 2·2′-dihydroxydiphenyl with zinc chloride [Kraemer and Weissgerber, Ber. Dtsch. Chem. Ges., xxiv (1891), p. 1663]; by passing diphenylether through a red-hot tube [Täuber and Halberstadt, Ber. Dtsch. Chem. Ges., xxix (1896), p. 1876]; and by the oxidation of diphenylene [Dobbie, Fox, and Gauge, Journ. Chem. Soc., ciii (1913), p. 41]. A good yield of diphenylene oxide was obtained by Sabatier and Mailhe

[Comptes Rendus, cli (1909), p. 492] by passing the vapour of phenol over heated thoria. For the purpose of the present investigation the oxide was obtained in fair yield (15 per cent.) by heating a mixture of litharge and phenol for eight hours, and then subjecting the product to destructive distillation [Compare Galewsky, Liebig's Annalen, cclxiv (1891), p. 189].

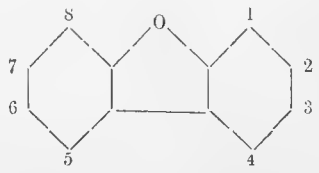
The nitration of diphenylene oxide has been already attempted, but with somewhat inconsistent results. Borsche and Bothe [Ber. Dtsch. Chem. Soc., xli (1908), p. 1940], by the action of fuming nitric acid on a solution of the oxide in glacial acetic acid, obtained a mononitro derivative, melting at 181–182° C. Mailhe [Comptes Rendus, cliv (1912), p. 1515] states that this compound melts at 175° C.; but our product was found to have the same melting-point as that of the former investigators. Borsche and Bothe were of opinion (*loc. cit.*) that this body was 3-nitrodiphenylene oxide[1], and this contention is borne out by considerations based on the theory of induced alternate polarities recently put forward [Robinson and Kermack, Journ. Chem. Soc., cxxi (1922), p. 427; Lapworth, ibid., p. 416].

Galewsky (*loc. cit.*, p. 129) attempted to prepare a mononitro derivative by direct nitration of diphenylene oxide, but was not successful, a dinitro compound only being obtained. Hoffmeister [Liebig's Annalen, clix (1871), p. 214] described a dinitrodiphenylene oxide, melting about 200° C. This substance was probably an impure form of the dinitrodiphenylene oxide, melting at 245° C., prepared by Mailhe [*loc. cit.*; see also Bull. Soc. Chim. (1912), i, p. 1011] in a similar manner, and which he considered to be 3·6-dinitrodiphenylene oxide. This assumption is also in accordance with the theory of alternate polarities. Mailhe also claimed (*loc. cit.*) to have obtained by nitration a tetranitro compound, melting at 168° C., a pentanitro compound, melting at 122° C., and a hexanitro derivative, melting at 135° C., but he afterwards stated that the pentanitro and the hexanitro derivatives did not exist. He described also a trinitrodiphenylene oxide, melting at 142–143° C., and a tetranitrodiphenylene oxide, melting at 172° C.

The state of our knowledge concerning the nitro derivatives of diphenylene oxide before the present investigation was undertaken may be summarised as follows:—

Derivative.	Melting-point.	
Mononitro	182° C. (Borsche).	175° C. (Mailhe).
Dinitro	ca. 200° C. (Hoffmeister).	245° C. (Mailhe).
Trinitro	—	143° C. (Mailhe).
Tetranitro	252° C. (Borsche & Scholten).	172° C. (Mailhe).

[1] The nomenclature adopted in this communication is based on the following formula:—



In our experiments, which were for the most part carried out at the ordinary temperature and at low concentrations, the action upon diphenylene oxide of the oxides and oxyacids of nitrogen was investigated.

Nitrogen peroxide vapour converted diphenylene oxide into a dinitro derivative, melting at 245° C. In glacial acetic acid solution the main product was, however, a mononitrodiphenylene oxide, melting at 182° C. Under the same conditions in carbon tetrachloride solution the mononitro and the dinitro derivatives were formed.

Nitrous fumes, when passed into a solution of diphenylene oxide in glacial acetic acid, gave the mononitro compound, and, in addition, a small quantity of the oxide was recovered.

The treatment of diphenylene oxide directly with fuming nitric acid caused the formation of a mixture of the mononitro and the dinitro derivatives.

When dilute solutions of diphenylene oxide in glacial acetic acid were treated with 1 and 4 molecular amounts of fuming nitric acid, respectively, and the solutions were allowed to remain at the room temperature for 5 months, there was no appreciable action, the oxide being recovered unchanged in each case. In similar experiments carried out with carbon tetrachloride as the solvent mononitrodiphenylene oxide was formed.

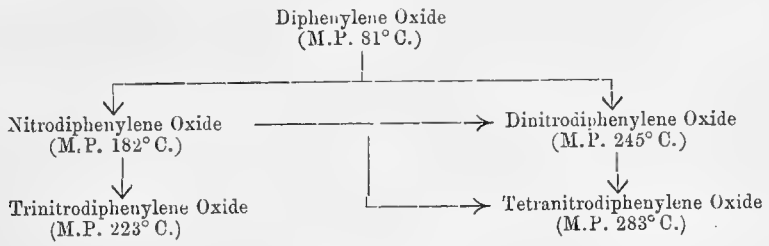
By heating solutions of diphenylene oxide in glacial acetic acid, or carbon tetrachloride, with small amounts of fuming nitric acid, very good yields of mononitrodiphenylene oxide were obtained.

Dinitrodiphenylene oxide, melting at 245° C., was prepared most conveniently by the action of fuming nitric acid on the mononitro compound in glacial acetic acid solution.

Trinitrodiphenylene oxide was not formed in large yield by nitration. By treating the mononitro compound directly with fuming nitric acid a small quantity of trinitrodiphenylene oxide, melting at 223° C., was isolated in addition to the dinitro derivative. It is probable that this trinitro compound is 1·3·6-trinitrodiphenylene oxide. In this reaction a small quantity of a tetranitrodiphenylene oxide was also formed, but the best method of obtaining the latter body is by the direct action of a mixture of fuming nitric and concentrated sulphuric acids on the dinitro compound. The tetranitrodiphenylene oxide melted at 283° C., and is probably 1·3·6·8-tetranitrodiphenylene oxide. Recently Borsche and Scholten [Ber. Dtsch. Chem. Ges., 1 (1917), p. 607] claim to have obtained 1·3·6·8-tetranitrodiphenylene oxide, melting at 252·5° C.

Attempts to convert the tetranitro compound into a more highly nitrated derivative were unsuccessful.

Our results may be summarised as follows:—



EXPERIMENTAL.

1. *Action of Nitrogen Peroxide on Diphenylene Oxide.*

(*a*) *In the Absence of Solvents.*—2 g. of diphenylene oxide and some dry liquid nitrogen peroxide were placed in shallow dishes under a bell-jar. After

remaining at the room temperature for a day the oxide assumed a yellow colour, which deepened slightly as the reaction progressed. The experiment was continued for 5 weeks, the supply of nitrogen peroxide being only once replenished, as its absorption was slow. The yellow product was washed with ligroin, and, after recrystallisation from glacial acetic acid, fine white needles, melting at 245° C., separated in a very good yield. This compound was therefore evidently identical with the dinitrodiphenylene oxide obtained by Mailhe by the action of nitric acid on the oxide.

(*b*) *In Glacial Acetic Acid Solution.*—1 g. of diphenylene oxide was dissolved in 20 c.c. of glacial acetic acid, and dry nitrogen peroxide vapour was passed into the solution until the latter was saturated. The reddish-brown solution was allowed to remain in a stoppered bottle at the room temperature, a relatively large quantity of a yellow solid being deposited overnight. After 3 months the product was poured into water; the yellow solid was washed free from acid, and after recrystallisation from glacial acetic acid it consisted of yellow silky needles, melting at 182° C. It was evidently identical with the mononitrodiphenylene oxide obtained by Borsche and Bothe (*loc. cit.*), by the action of fuming nitric acid on a solution of diphenylene oxide in acetic acid.

(*c*) *In Carbon Tetrachloride Solution.*—A solution of 2 g. of diphenylene oxide in 40 g. of carbon tetrachloride was saturated with nitrogen peroxide vapour. Some heat was evolved, and on cooling a yellow solid was deposited. The mixture was allowed to remain at the room temperature for 3 months. The solvent was then evaporated, and the solid product was extracted several times with boiling alcohol until only a very small amount of residue was left. From the filtrate a good yield of the above-mentioned mononitro derivative was obtained, while the residue on recrystallisation from glacial acetic acid gave a small quantity of dinitrodiphenylene oxide, melting at 245° C.

2. *Action of Nitrous Fumes on Diphenylene Oxide.*

Nitrous fumes, generated by the action of arsenious oxide on nitric acid, were passed into a solution of 2 g. of diphenylene oxide in 40 g. of glacial acetic acid. After remaining at the room temperature a small quantity of a yellow solid was deposited from the dark-green solution, and this increased gradually in amount. After 4 weeks the solution was filtered from the solid residue, which on crystallisation was found to be the mononitro derivative already described. The filtrate on evaporation gave a further yield of this substance in addition to some unchanged oxide.

3. *Action of Fuming Nitric Acid on Diphenylene Oxide.*

(*a*) *In the Absence of Solvents.*—2 g. of diphenylene oxide was added gradually to fuming nitric acid (10 c.c.). A vigorous reaction ensued, and a yellow solid was obtained on cooling the mixture. This solid was washed with water, dried, and extracted with boiling ligroin, and finally with boiling alcohol. The undissolved residue consisted of dinitrodiphenylene oxide, melting at 245° C., and from the alcoholic filtrate the mononitro compound, melting at 182° C., was isolated.

(*b*) *In Glacial Acetic Acid Solution.*—To two solutions, each of which contained 1 g. of diphenylene oxide in 100 g. of glacial acid, 1 and 4 molecular amounts of fuming nitric acid were added respectively. The reactions were evidently very slight, even on allowing the solutions to remain at the room temperature for 5 months, since the only substance isolated was, in both cases, the original oxide.

(*c*) *In Carbon Tetrachloride Solution.*—A solution containing 1 g. of diphenylene oxide and 1 molecular amount of fuming nitric acid in 100 g. of carbon tetrachloride was prepared. This solution was also allowed to remain at the room temperature for 5 months, when, on evaporation of the solvent, a red oil remained. After extracting this with ligroin, the parent substance was obtained from the ligroin extract, and a small quantity of the mononitro derivative previously mentioned was isolated from the undissolved portion

A similar experiment was also performed with the employment, however, of 4 molecular amounts of fuming nitric acid. After 5 months the yellow solution was evaporated, leaving a yellowish-white solid. This was washed with ligroin, a small quantity of the original oxide being isolated from the extract. The undissolved residue consisted of mononitrodiphenylene oxide, melting at 182° C.

(*d*) An almost quantitative yield of mononitrodiphenylene oxide was obtained by the following method:—5 g. of the oxide were dissolved in 20 c.c. of glacial acetic acid, and 5 c.c. of fuming nitric acid were added slowly with shaking. When the initial violent reaction had subsided, the mixture was heated for 5 minutes on the water-bath, and on cooling a yellow solid cake was formed. It was washed with water and recrystallised from glacial acetic acid, and was then found to be pure mononitrodiphenylene oxide.

(*e*) An equally good yield of the mononitro compound was obtained by substituting carbon tetrachloride for acetic acid as the solvent. In this case the solid cake was deposited during the addition of the acid. On recrystallising this from glacial acetic acid it gave the pure mononitro compound.

4. *Preparation of the Nitro Derivatives.*

(*a*) *Dinitrodiphenylene Oxide.*—This compound was readily obtained in the following manner:—To a concentrated solution of 5 g. of the mononitro derivative in glacial acetic acid 25 c.c. of fuming nitric acid was added slowly. A somewhat vigorous reaction occurred, and, when it had subsided, the mixture was heated for about 20 minutes on the water-bath. The white crystalline deposit formed on cooling was washed with water, and recrystallised from acetic acid. The product consisted of fine white needles of dinitrodiphenylene oxide, melting at 245° C. The yield was 3 g.

Dinitrodiphenylene oxide is almost insoluble in ligroin, slightly soluble in alcohol, moderately soluble in glacial acetic acid or xylene, and readily soluble in acetone.

(*b*) *Trinitrodiphenylene Oxide.*—Fuming nitric acid (10 c.c.) was added slowly with shaking to 2 g. of mononitrodiphenylene oxide. A vigorous reaction ensued with evolution of heat. The reddish-yellow solution was allowed to remain at the room temperature for a few minutes, and was then poured into water. The solid product was washed several times with boiling alcohol. The residue was extracted with boiling benzene, from which a small quantity of colourless blunt prisms, melting at 223° C., was isolated. The alcoholic extract contained dinitrodiphenylene oxide. The portion left undissolved by the benzene crystallised from xylene, yielding a small amount of colourless thin plates, melting at 283° C. The compound melting at 223° C. was a trinitrodiphenylene oxide, and it gave on analysis the following results:—

0·1027 g. substance gave 12·3 c.c. moist nitrogen at 18° C. and 766 m.m.

corresponding to N 14·1
$C_{12}H_5O_7N_3$ requires N 13·9.

Trinitrodiphenylene oxide is almost insoluble in ligroin, slightly soluble in alcohol, somewhat soluble in benzene or glacial acetic acid, and readily soluble in acetone.

(*c*) *Tetranitrodiphenylene Oxide.*—2 g. of dinitrodiphenylene oxide was dissolved in a mixture of 10 c.c. of fuming nitric acid and 10 c.c. of concentrated sulphuric acid. The mixture was heated for several hours on the water-bath, a small quantity of colourless crystals being deposited during the course of the experiment. The product was poured into water, and the white solid deposited was washed with water, and dried. The residue left after washing with hot benzene crystallised from xylene as colourless plates, melting at 283° C., which gave on analysis the following results:—

0·1075 g. substance gave 14·7 c.c. moist nitrogen at 16° C. and 770 m.m.

corresponding to N 16·3
$C_{12}H_4O_9N_4$ requires N 16·1.

Tetranitrodiphenylene oxide is very slightly soluble in alcohol, somewhat more soluble in benzene, and readily soluble in hot acetic acid, xylene, or acetone.

Summary.

1. Diphenylene oxide interacts with nitrogen peroxide or nitric acid much less readily than diphenylnitrosamine.

2. Nitrogen peroxide vapour converted diphenylene oxide into its dinitro derivative. Nitrogen peroxide in solution in glacial acetic acid, gave the mononitro compound; in carbon tetrachloride it gave the mononitro and the dinitro derivatives.

3. Fuming nitric acid converted the oxide into its mononitro and its dinitro derivatives. In cold dilute solution in glacial acetic acid the acid had scarcely any action on the oxide, but in carbon tetrachloride the mononitro compound was formed. This body was also produced by the action of fuming nitric acid on a hot solution of the oxide in glacial acetic acid.

4. Dinitrodiphenylene oxide was readily produced by the action of fuming nitric acid on a solution of mononitrodiphenylene oxide in glacial acetic acid.

5. Trinitro- and tetranitrodiphenylene oxides were both formed by the action of fuming nitric acid on the mononitro compound. The tetranitro derivative was also readily obtained by the action of a mixture of fuming nitric acid and concentrated sulphuric acid on dinitrodiphenylene oxide.

In conclusion we wish to state that the above research was undertaken at the request of the Research Section of Nobel's Explosives Company, and that we are also indebted to the Department of Scientific and Industrial Research for a grant in aid of the investigation.

No. 41.

THE HABITATS OF *LIMNAEA TRUNCATULA* AND *L. PEREGER* IN RELATION TO HYROGEN ION CONCENTRATION.

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(Read January 29. Printed February 22, 1924.)

L. truncatula has long been the accepted intermediate host of the liver fluke *Fasciola hepatica,* and it has recently been pointed out by Taylor (1922) that sheep are infected with liver fluke in parts of Scotland from which *L. truncatula* is absent. *L. pereger* is, however, found there, and has been proved to be infected with the cercariae of *F. hepatica.* The authors (1923) have already shown that the distribution of many snails is limited by acidity, whereas others are found in acid or almost neutral, but never in alkaline habitats. Attention was drawn by Wallace (1922) to the opinion of Welsh farmers that liver fluke was more prevalent on land which had been limed than on untreated pastures. Since the sheep pastures of Wales are notoriously poor in calcium carbonate the liming must produce a considerable change in the soil reaction, but the reaction is unlikely to be at all as alkaline as in limestone or chalk districts. There appeared, therefore, to be indications that an almost neutral reaction favoured *L. truncatula.* In view of the fact that in certain wet years the ravages of liver fluke among sheep become disastrous, it appeared to be of interest to ascertain what characteristics might be used to define localities in which *L. truncatula* or *L. pereger* could develop or could abound. This appears to be the more desirable, inasmuch as it is often the custom to bring sheep down from hill pastures free from these snails to winter in lowlands, which are normally less acid than the hills. The work of Walton (1923), on the destruction of *L. truncatula* by means of copper sulphate, has, however, demonstrated that habitats naturally very favourable may be almost or entirely cleared of this snail.

L. truncatula probably attains its greatest abundance in water-logged pastures. Boycott (1919) does not consider it as a water-snail, though it occurs in three "drying" ponds out of a total of one hundred and forty-one "closed" ponds in the parish of Aldenham. It is nowhere recorded by him among the sixty-nine land mollusca of Aldenham and its district (1921). This district, therefore, is apparently not a favourable habitat for *L. truncatula*; *L. pereger,* on the other hand, is truly a water-snail. Boycott (1919) records its occurrence in fifty-one out of one hundred and eight ponds examined by him, as well as in lakes and streams, though not in any of the seventeen "drying" ponds. Stelfox (1911) records the general occurrence of *L. pereger* in the Clare Island survey, and remarks upon the swarms of a small form

of *L. truncatula* found upon the bare rock-faces of the sandstone cliff of Croaghmore, which faces the Atlantic on the west of the island. The snail is found up to nearly 1,000 ft. Taylor (1920) does not include *L. truncatula* among the mollusca found in the low-lying district of Audruicq, in Picardy, and comments upon the absence of limestone. Kendall (1921) records the finding of *L. truncatula* in the Oundle district in river marsh—rush-grown shallows and moist river margins, also in small streams, but not in "natural marsh"—an extensive tract of boggy ground with common rush, cotton grass, bog bean, marestail, and peppermint.

Through the kindness of Sister Monica Taylor, Mr. C. L. Walton, Miss K. Carpenter, Mr. T. H. Taylor, and Mr. W. M. Temple the authors have been provided with certain samples and notes concerning the distribution of these snails in Scotland, Wales, and Yorkshire, as well as near Plymouth. The values for $pH = \log \frac{1}{H}$ are recorded for these soils or waters, as are also the electrical conductivities of the waters measured at 0° C., at which temperature N/100 potassium chloride has the value 0·00078, and was used to standardise the cell.

The data presented in the table opposite are obviously not as numerous as could be desired, but are put on record and discussed now, as the subject is foreign to the authors' general line of work, and it is not their intention to pursue it further. The crosses given in the table denote a rough attempt at giving an idea of the relative abundance of the two species.

It must be explained that *pH* 6·9–7·6, for example, does not mean that the value lay between those limits, but that when examined it was *pH* 6·9, and when the water was thoroughly well aerated and the free carbon dioxide was reduced thereby so as to be approximately in equilibrium with the air, the *pH* value then rose to *pH* 7·6. The condition of soft water at *pH* 6·9 is therefore very different from that of hard water at the same *pH* value, for though the reaction is the same the hard water is charged with a great excess of carbon dioxide above the equilibrium value, and is correspondingly poor in oxygen, whereas a soft water at this *pH* value may be thoroughly well aerated. It is obvious that the oxygen content of water is of great biological importance. The change in *pH* value produced by aeration may, therefore, be taken as giving a rough idea of the degree to which the water is saturated with oxygen.

The electrical conductivity of the water gives a measure of its salt content, which, when dealing with normal natural waters, may be taken as roughly proportional to its hardness. Hard water has a conductivity of $270–290 \times 10^{-6}$, whereas a very soft water like the Plymouth town supply from Burrator reservoir, on Dartmoor, has a conductivity $C = 26–28 \times 10^{-6}$, with a *pH* value of 6·4 to 6·8 according to season; these values are unaltered by aeration, but a spring water at *pH* 6·8 and having $C = 270 \times 10^{-6}$ rises to over *pH* 8 on aeration.

On inspecting the table it may be seen that, though the records are not numerous, they may be taken as typical of districts of some size. As regards the conductivity a wide range may be noted; this indicates a yet wider range in "hardness," for even the soft waters of the R. Ystwyth and its neighbours have $C = 39–72 \times 10^{-6}$, the Aberystwyth* town supply being as low as $C = 19 \times 10^{-6}$. *L. truncatula* has been found in water having conductivity

* The authors are indebted to Miss K. Carpenter for these samples.

Date.	Locality.	Nature of habitat.	*L. truncatula.*	*L. pereger.*	pH.	C × 10^6.	Notes.
12/2/'23.	Dunbarton, basaltic district.	Soil by stream.	+	0	6·6	—	Always infected with *F. hepatica.*
20/2/'23.	Do.	Stream.	+	0	6·4 – 6·7*	59	Do.
20/3/'23.	Dunbarton, calciferous sandstone district.	Ditch.	+ +	0	6·9 – 7·6	186	Never infected, no sheep.
20/3/'23.	Mugdock reservoir grounds.	Offset from stream.	0	+ + +	6·8 – 7·2	72	*L. truncatula* never found.
22/3/'23.	Dunbarton, old red sandstone quarry, drainage from fields.	Pond.	+	+ + +	6·6 – 7·3	220	*L. pereger* highly infected with *F. hepatica.*
14/2/'23.	Bodorgan, Anglesey.	Stream in shallow ditch.	+	+ + +	6·8 – 7·4	192	A few *L. truncatula* along sides.
14/2/'23.	Do.	Mud from ditch.	+	+ + +	7·2	—	—
5/6/'23.	Near Aberystwyth.	Soil from site.	+	+	5·8	—	—
5/6/'23.	Do.	Do.	0	+	5·8	—	—
5/6/'23.	Do.	Do.	+	0	7·5	—	—
22/3/'23.	Near Leeds.	Stream in flood.	0	+ +	7·1 – 7·4	227	Infected with two species of cercariae.†
8/10/'23.	Allerton Park, Knaresboro'.	Lake with sedges.	+ +	0	7·2	—	Deer infected with fluke.
1/4/'23.	Plymouth.	Ditch in lane, flowing.	+ +	0	7·6 – 8·2	294	—
2/4/'23.	Do.	Stagnant ditch in meadow with duckweed.	+	+ +	6·9 – 7·8	213	—
2/4/'23.	Do.	Do., but spirogyra abundant.	+ +	+	7·7 – 8·1	213	—
8/3/'23.	Near Malldraeth Marsh, Llangefni, Wales.	Pools 2 in. deep in water-logged pasture.	+ + + +	0	7·8 – 8·0	290	—

* For explanation of second figure see text.

† Neither was *F. hepatica.*

ranging from 59–294 × 10^{-6}, and *L. pereger* from 72–227 × 10^{-6}. When taken in conjunction with the failure to find the snails in the R. Yealm, where the water was at $C = 32 \times 10^{-6}$, it seems justifiable to conclude that the lower limits recorded have an approximate value as setting a genuine bound to the habitats of these species. The greatest abundance of *L. truncatula* was, however, in a highly calcareous water near Malldraeth Marsh.

On considering the hydrogen ion concentration it is seen that *L. truncatula* has been found in water between *pH* 6·4 and 7·8, and *L. pereger* from *pH* 6·6–7·7. No differential significance can be attached to these figures, but it seems that *L. pereger* can endure deeper and less well-aerated water than can *L. truncatula*, which is amphibious. The records show that snails may be infected even in the most acid of the waters studied.

It is, however, noticeable that none of the records of soil are more acid than *pH* 5·8. Streams draining Dartmoor may be even more acid than the R. Yealm, *pH* 6·4, which receives tributaries that lessen its acidity from the Culm Measures. Values such as *pH* 5·4 above the Culm, on granite, and *pH* 5·0, for a bog pool, have been observed; and among the China-clay pits *pH* 6·2 to 6·4, with the low value 31 × 10^{-6} for conductivity. It appears, therefore, that there must be great stretches of upland pastures in which the water is too acid and too poor in salts for either of these species to exist. The acidity of the soil in these districts is even greater than is that of the water. Wet peat bogs, where cotton grass abounds, may be as acid as *pH* 4·1; heather bogs, *pH* 4·6; and the siliceous soil of uplands, good permanent pastures with wild white clover, cocksfoot, sheep's fescue, crested dogstail, and bent, may be as acid as *pH* 5·4. It is, therefore, not unpleasing to find that such habitats appear to be too acid for *L. truncatula* to live. It is evident, therefore, that the habitats given in Roebuck's census (1921) "*L. pereger*: fresh and stagnant water generally" and "*L. truncatula*: ponds, ditches, and wet places generally," require modification in the direction of the exclusion of fresh water of very low conductivity, and also of high acidity in water or soil. It may also be pointed out that *L. truncatula* has been recorded (1900) by one of us as occurring in quantity on the mud between the tidal limits of the upper part of the R. Tweed, where the water was slightly brackish. The nature of the habitats makes it clear that though both species can harbour *F. hepatica*, yet *L. truncatula* is the more likely to cause infection.

Summary.

1. The habitats of *L. pereger* and *L. truncatula* appear to differ in the fact that whereas the former is truly a water-snail, and can endure even somewhat stagnant water; the latter is amphibious, and can live either in shallow, well-aerated water or on moist land, or even on cliffs in a region of high humidity.

2. The observed ranges for the two species are almost identical as regards acidity and salt content of the water, those for *L. pereger* being *pH* 6·6 to 7·7 and $C = 72–227 \times 10^{-6}$ at 0° C., and for *L. truncatula*, *pH* 6·4–7·8 and $C = 59–294 \times 10^{-6}$. It is noticeable that the records do not include upland waters of very low salt content, with conductivity 20–30 × 10^{-6}, nor regions of high acidity, more acid than *pH* 6·4 for water or *pH* 5·8 for land records.

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[*Continued on p. 3 of cover.*

No. 42.

EXPERIMENTS ON THE POSSIBLE EFFECT OF VITAMINS ON QUANTITY OF MILK AND BUTTER FAT.

By E. J. SHEEHY, F.R.C.Sc.I., B.Sc., M.R.I.A.
[Bio-Chemical Laboratory, D.A.T.I.]

(Read February 26. Printed April 24, 1924.)

That vitamins, or accessory food factors, are necessary for growth and continued metabolic activity of the body, is no longer a matter of controversy. At least three different accessory factors, now known as A, B, and C, function for different purposes: A supports growth and strengthens resistance to disease, B also supports growth and helps to maintain nervous efficiency, and C prevents skin diseases, vascular, and other disorders. The vegetable kingdom is the ultimate source of the vitamins, which cannot be synthesised de novo by animals, but the suckling mother can, to a certain extent, utilise the reserves of her own body to supply the vitamins in her milk,[1] even where her diet is deficient in these materials. When these reserves are exhausted, however, the vitamin content of milk is proportional to that of the food from which the milk is derived.[2]

While a definite relationship between the vitamin content of the food of a lactating animal and its milk is established, there is no information as to the part played by these factors in the processes of milk production. There is evidence to show that the activity of the mammary gland cells is dependent on internal secretions from other parts of the body. Developments in the ovary and in the uterus are succeeded by evolution of the mammary cells,[3] and there is an immediate response in milk production after the injection of a small dose of pituitrin into the blood of a lactating animal.[4] Probably there are other internal secretions, as well as some chemical substances of food, which act as hormones for the mammary cells, and the possible effect of some or all of the accessory food factors suggested itself.

To investigate this problem three goats were selected and fed with a view to exhausting the stores of vitamins A, B, and C in animals I, II, and III, respectively, for some time previous to parturition and subsequent to it. This was done by feeding each goat on a ration complete in all respects except the vitamin whose effect on the milk yield was afterwards to be investigated. When the animals had milked for several weeks, each was given, without otherwise changing the diet, the particular factor in which the previous food had been lacking, and the effect of the addition noted. The determinations made were total milk, butter fat in milk, and the weight of the animal. The

[1] The Newer Knowledge of Nutrition. M'Collum, 1919. Chapter vi.
[2] Jr. Biol. Chem., xxvii, 33. *Ibid.*, xlv, 119. *Ibid.*, l, 339. Biochem. Jr., xv, 540. Jr. Agric. Sc., xiii, 144.
[3] Physiology of Reproduction. Marshall, 1910, page 580. Proc. Roy. Soc., lxxxviiB, 422.
[4] Proc. Roy. Soc., lxxxivB, 16. Jour. Dairy Sc., i, 475. *Ibid.*, iv, 474. Quarterly Jr. Physiol., vi, p. 315.

animals were weighed once per week always at the same hour, and the milk fat was tested by the Gerber method in tubes previously checked by the Adams paper coil method. The goats were housed at the Albert Agricultural College, and experimental work was performed partly there and partly at the College of Science.

The details of the original scheme are outlined in Table I.

TABLE I.

	Goat I.	Goat II.	Goat III.
Date of Parturition.	11 May, '23.	30 March, '23.	20 April, '23.
Deficient Diet.	Roots, ... 7 lb. per day Dried Yeast, ... 1 lb. ,, White Wheaten Flour, ... 1½ lb. ,, Coco Fat, ... ½ lb. ,, Linseed Oil, ... ½ pint per week Above lacking in Vitamin A.	Fish Meal, ... ¾ lb. per day White Wheaten Flour, ... 2¾ lb. ,, Linseed Oil, ... 1 pint per week Above lacking in Vitamin B.	Crushed Oats, 1¾ lb. per day Fish Meal, ... ½ lb. ,, Dried Yeast, ... ¾ lb. ,, Bran, ... ½ lb. ,, Linseed Oil, ... 1 pint per week Above lacking in Vitamin C.
Complete Diet.	Similar to above with the Linseed Oil replaced by Cod Liver Oil, i.e. with Vitamin A added.	Similar to above plus 6 ounces of marmite per week, i.e. with Vitamin B added.	Similar to above plus 30 c.cs. of orange juice per day, i.e. with Vitamin C added.

Simultaneously it was shown, by tests on rats, that the deficient diet fed to goat I contained but a trace, if any, of vitamin A, and that fed to goat II similarly lacked vitamin B, and, by tests on guinea pigs, that the diet of goat III was likewise deficient in vitamin C.

Unfortunately goat III did not give sufficient milk to warrant proceeding with her, and consequently the test with vitamin C was necessarily abandoned. Goats I and II gave a good flow of milk and remained quite normal for a time; but after some weeks of lactation both became constipated, and their weight dropped. This produced a temporary disturbance in the yields of milk and butter fat, and in order to restore normal health the diet was necessarily so altered temporarily that some vitamins were incidentally given to the animals during the convalescent stage. The disturbance in general health was not due, however, to lack of vitamins in the previous diet, because normal health continued on that same diet supplemented by an extra half-pint of linseed oil per week—a material known to contain none of these substances. It became necessary to feed the two animals for another considerable period on diet free from vitamin A and B respectively in order to exhaust any vitamin reserve in the body before applying a test. In this connexion it

TABLE II.

	Goat I.				Goat II.			
	Date	Daily Milk Yield (lb.)	% Fat in Daily Milk	Mean % Fat over period	Date	Daily Milk Yield (lb.)	% Fat in Daily Milk	Mean % Fat over period
Vitamin A lacking in diet from July 23 to September 30.	September 1	2¾	3·8		Vitamin B lacking in diet from June 6 to July 6. June 15	4	5·0	
	2	2¾	4·0		16	4	4·7	
	3	2¾	4·2		17	4	4·2	
	4	2¾	3·5		18	4	4·9	
	5	2¾	4·3		19	4	4·0	
	6	2¾	3·7		20	4	5·0	
	7	2¾	4·5		21	4	4·3	
	8	2¾	4·3		22	4	4·8	
	9	2¾	4·5		23	4	4·1	
	10	2¾	3·7		24	4	4·5	
	11	2¾	4·2		25	4	5·0	
	12	2¾	4·1		26	4	4·5	4·59
	13	2¾	4·4		27	4	4·2	
	14	2¾	4·3		28	4	4·7	
	15	2¾	4·7	4·17	29	4	4·5	
	16	2¾	4·5		30	4	4·3	
	17	2¾	3·8		July 1	4	5·1	
	18	2¾	4·4		2	4	4·4	
	19	2¾	4·4		3	4	4·6	
	20	2¾	4·5		4	4	4·2	
	21	2¾	3·8		5	4	5·5	
	22	2¾	4·5		6	4	4·5	
	23	2¾	4·2		Vitamin B provided from July 7 to July 26. 7	4	4·2	
	24	2¾	4·0		8	4	4·0	
	25	2¾	4·2		9	4	5·0	
	26	2¾	3·5		10	4	4·8	
	27	2¾	4·3		11	4	4·1	
	28	2¾	3·9		12	4	4·5	
	29	2¾	4·2		13	4	4·6	
	30	2¾	4·8		14	4	4·5	
Vitamin A provided from October 1 to October 14.	October 1	2¾	4·5		15	3¾	4·9	
	2	2¾	4·3		16	3¾	4·5	4·56
	3	2¾	3·6		17	3¾	4·7	
	4	2¾	4·2		18	3¾	4·7	
	5	2¾	4·1		19	3¾	4·0	
	6	2¾	4·1		20	3¾	5·0	
	7	2¾	3·5		21	4	5·4	
	8	2¾	4·7	4·15	22	4	4·9	
	9	2¾	4·0		23	4	3·9	
	10	2¾	4·5		24	4	4·5	
	11	2¾	3·6		25	4	4·9	
	12	2¾	4·4		26	4	4·2	
	13	2¾	4·3		Vitamin B lacking in diet from July 27 to August 9. 27	4	5·1	
	14	2¾	4·3		28	4	4·7	
					29	4	5·2	
					30	4	4·3	
					31	4	4·5	
					August 1	4	4·7	
					2	4	4·5	4·66
					3	4	5·1	
					4	4	4·1	
					5	4¼	4·6	
					6	4¼	4·9	
					7	4¼	4·6	
					8	4¼	4·7	
					9	4¼	4·2	
Vitamin A lacking in diet from October 15 onwards.	15	2¾	3·6		Vitamin B provided from Aug. 10 onwards. 10	4¼	5·1	
	16	2¾	4·3		11	4¼	4·7	
	17	2¾	4·0		12	4¼	4·8	
	18	2¾	3·5		13	4	4·7	4·75
	19	2¾	4·6		14	4	4·8	
	20	2¾	3·6	4·09	15	4	4·3	
	21	2¾	4·2		16	4	4·9	
	22	2¾	3·9					
	23	2¾	4·8					
	24	2¾	4·4					

has been shown that the body stores up greater quantities of vitamin A[1] than of either B or C. The lactation period was, in consequence of this irregularity, well advanced before the effect of adding the vitamins could be determined. When, eventually, the tests were proceeded with, the yields of both animals were fortunately regular. Table II gives particulars of the results. In the case of goat I the addition of vitamin A was effected by substituting one pint of cod liver oil for one pint of linseed oil; and in the case of goat II the addition of vitamin B was effected as in the original scheme, i.e., by adding six ounces of marmite per week.

The results indicate that the addition of vitamin A to a diet, so composed of ordinary unpurified food as to contain the minimum of that factor, does not affect either the quantity or the fat content of the milk. The same conclusion holds for vitamin B. Apparently neither A nor B is concerned with the activity of the mammary gland cells, or else their presence in extremely small quantity suffices. At any rate it would not appear as if the quantity of either milk or butter fat can be influenced by the feeding of the accessory food factors. It is, however, realised that the tests reported in this paper were so limited in extent that a conclusive statement from these determinations, on the possible effects of the accessory food substances in this connexion, cannot be made.

Summary.

Neither the quantity of milk nor its richness in fat appears to be influenced by the quantity of either vitamin A or B in the diet of the lactating goat.

[1] Report on the Present Knowledge Concerning Accessory Food Factors (Vitamins), 1919. Medical Research Committee, chapter ii.

No. 43.

A MECHANICAL DEVICE FOR SEALING OFF RADIUM EMANATION TUBES.

By H. H. POOLE, Sc.D.

(Read April 29. Printed July 3, 1924.)

In the extraction and purification of radium emanation for therapeutic purposes it is essential that the operator should be guarded against the dangerous effects of excessive exposure to the radiations emitted. The problem which has hitherto presented the greatest difficulty in this respect in the Irish Radium Institute is that of the protection of the operator's fingers during the division, by sealing off, of the long capillary tube, containing the emanation, into pieces, each about 1·5 cm. long, suitable for insertion in serum needles. It is essential that each seal should be absolutely reliable, and that there should be no enlargement of the tube beyond the diameter of 0·85 mm., which is the maximum allowed by the standard needle. As the process involves the simultaneous rotation and drawing out of the tube, it is difficult to ensure satisfactory results if the parts to be separated are held in forceps. Even the use of rubber gloves, or finger stalls, renders the process so much more difficult that the time required for the sealing off is considerably lengthened. As the division of the tube into short lengths is carried out immediately after the emanation has been drawn into it, so that the activity of the tubes is increasing every instant, this attempt at protection is not of much use, so that the usual practice has hitherto been to hold the tube in the bare fingers, and trust to speedy work. This method has proved satisfactory, in so far as no serious damage has occurred to any operator's fingers, but the small β and γ ray activity that soon appears in the tubes is sufficient to cause an appreciable amount of temporary injury, so that the apparatus, photographs of which are shown in figs. 1 and 2, has been constructed to obviate all handling of the tubes during the sub-division. The apparatus has been in continuous use for some weeks, and works extremely well.

It is constructed chiefly of meccano parts, and the scale may be judged from the fact that the holes in the metal strips which form the framework are $\frac{1}{2}$ inch apart, between centres. As may be seen from fig. 2, the device may be described as a crude form of miniature lathe, with two collinear chucks, in which the capillary tube may be held, rotated independently at the same speed. Each chuck is allowed about 3 mm. end play in its bearings, so that the space between their near ends may be anything between 7·5 mm. and 13·5 mm. They are normally pressed apart by the two pivoted fingers and the wire spring seen in the photographs, but may be locked in the "near" position by the cam seen near the bottom of fig. 2. A swinging frame is suspended from the end of the

fixed cantilever, and carries a small gas jet, for which the nipple of a Primus stove proved very suitable. The position of this jet can be readily adjusted both horizontally and vertically, so that when the arm on the left side of the apparatus is raised the small flame is brought to a position midway between the chucks. The swinging framework is sufficiently flexible to enable the flame to be moved towards either chuck by applying a slight side pressure to the operating arm.

The construction of the chucks is best shown in fig. 3, which is drawn to scale, actual size; one of them is shown in section. Each consists of a piece of steel rod, 14 mm. long by 6·3 mm. ($\frac{1}{4}''$) diameter, with a central hole, which for the greater part of its length is 3·2 mm. ($\frac{1}{8}''$) in diameter, but at one end is contracted to 1·7 mm., thus forming an internal shoulder. A piece of rubber

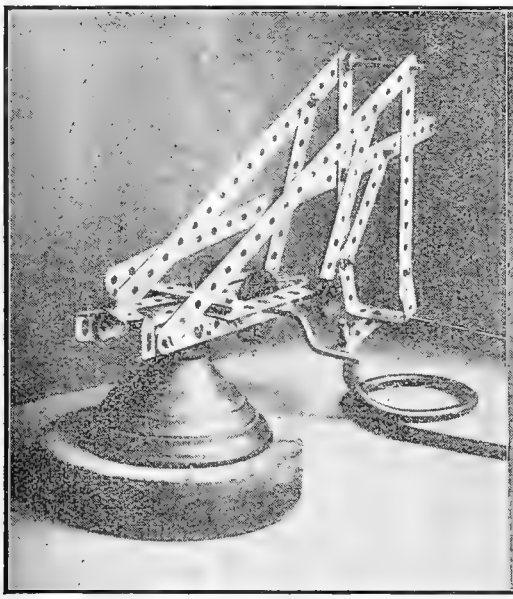

Fig 1.

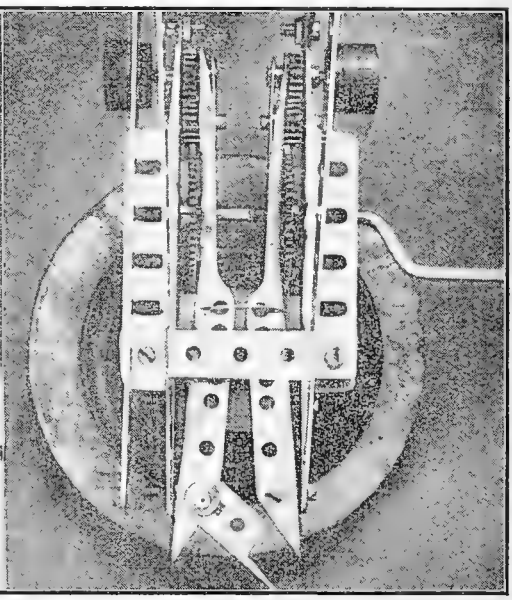

Fig. 2.

cycle-valve tubing, 6·5 mm. long, fits inside the larger part of the hole, and may be pressed against the shoulder by a screw plunger ($\frac{5}{32}''$ Whitworth thread), which is also perforated axially by a hole 1·7 mm. diameter. This plunger is fitted with a milled head, about 2 cm. diameter, which, for lightness' sake, is made of vulcanite. Thus an emanation capillary may very easily be slipped right through the chuck when the head is unscrewed, but, on screwing up the latter, the rubber tube is compressed longitudinally, so that the central hole is contracted, and the capillary firmly gripped by the rubber. In fig. 3 the left-hand chuck is shown in its "near" position, with the milled head screwed up, while the right-hand chuck, seen in section, is in the "far" position, with the head unscrewed to release the capillary, which is also shown. Other details, such as the supports for the jet holder, and some of the gearing are omitted.

The manipulation of the instrument is very simple. The fingers are locked in the "near" position, the milled heads unscrewed, and the chucks pushed as close together as possible. The emanation tube is then slipped in, a forceps being

used for holding it, and gripped in such a position that the ink-spot, marking the point where it is desired to effect a division, is midway between the chucks. A tube is seen in position in fig. 1, and the division marks on it are just visible. The fingers are then unlocked, and the chucks, which are geared up in the ratio 3 : 1, are rotated at some 400 to 500 r.p.m. The gas flame is then applied to the desired point on the capillary, which is very quickly drawn out and sealed off. The flame is then moved slightly along the axis of the capillary, so as to apply it to each separate sealed end in turn, in order to strengthen the seals. A short finished tube is most readily removed from an unscrewed chuck by tilting

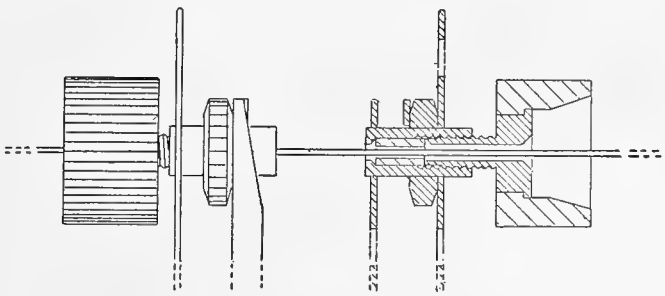

Fig. 3.

the entire appliance over a sheet of white blotting paper, when a slight shake usually causes the tube to slip out. Longer tubes may be handled with forceps. Every tube is then tested by slipping it into a standard needle. If, as occasionally happens, a seal is too unsymmetrical or too large to enter the needle, the tube is again inserted in the sealing apparatus for rectification. Want of symmetry can generally be corrected by careful heating of a stationary tube. An enlarged seal must be treated by fusing on another piece of capillary tube, and redrawing out just at the seal. This operation can quite easily be carried out in the apparatus.

No. 44.

NOTES ON THE FILTRATION AND OTHER ERRORS IN THE DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF SOILS.

By W. R. G. ATKINS, Sc.D.

(Read May 27. Printed July 5, 1924.)

Introduction.

In many investigations in which a soil extract is examined it is necessary to enquire into the most suitable proportions of soil and water to be taken for the determinations, and such an enquiry is necessary both for the electrometric and colorimetric methods of measuring hydrogen ion concentration. Since exact work by the latter method necessitates clear solutions, it is important to study the means available for obtaining these. Attention must also be paid to the choice of the most suitable indicators for the range required.

This paper embodies the experience obtained by the writer on these matters during the last four years, and is brought forward now because it seems possible that some of the errors consequent upon variations in technique have not been fully appreciated.

Proportion of soil to water.

There is a general impression that for purposes of hydrogen ion determination the precise proportions of soil and water taken is a matter of little importance. Observations to this effect have been made by various workers, notably by Sharp and Hoagland (1916) and by Arrhenius (1922, 2). This arises in part from the low solubility of the constituents of the soil which liberate hydrogen or hydroxyl ions, so that a relatively minute amount of soil may suffice to produce a saturated solution. There is, furthermore, the apparent anomaly that dilution may have but little effect upon the hydrogen ion concentration. This is fully considered by Clark (1922), but it may be said that from the writer's experience markedly acid soils of a sandy nature are more likely to give dilution errors than are clay or humic soils which are more highly buffered. Joseph (1923) has shown that for the alkaline soils of the Sudan the proportions of the mixture cannot be neglected. He adopts the one to five ratio advocated for general soil work by Hoagland, Martin, and Stewart (1921).

With these reservations the precise weighing out of the proportions is not as a general rule necessary for work to an accuracy of pH 0·1, as may be gathered from the figures given in the following tables.

Table I shows a comparison of the pH values obtained by extracting soils (*a*) for three to four hours, with two parts of water to one of soil by weight, and (*b*) by pouring off the extract and adding distilled water to the residue so as to make the mud-water mixture up to the original volume; this was shaken up at intervals during a further fifteen hours, making eighteen hours in all. All the samples were centrifuged till clear and examined colorimetrically.

TABLE I.

EFFECT OF TIME OF EXTRACTION AND RE-EXTRACTION OF SOIL UPON THE *pH* VALUE OF THE EXTRACT. SOIL, ONE PART; WATER, TWO PARTS.

Sample.	3–4 hrs. extraction.	Further 15 hrs. re-extraction.
	pH.	*pH.*
1	5·0	5·2
2	6·0	6·0
26	5·4	5·6
27	6·0	6·0
28	6·0	6·0
16	7·6	7·6

Here Nos. 1 and 26 show a decrease in acidity, when the soil is re-extracted, amounting to *pH* 0·2, quite a serious discrepancy; but in the other samples identical results were obtained.

These results are confirmed by those shown in Table II as regards time of extraction and the first re-extraction. In this series half the sample was retained and examined again after a total extraction period of eighteen hours; the other half was drained after three to four hours, and fresh water was then added as described previously. Then an examination was made after another fifteen hours, viz. eighteen in all, and the draining and re-filling were repeated a second and a third time after further periods of twenty-six and sixteen hours, namely, total times of forty-four and sixty hours respectively.

TABLE II.

EFFECT OF EXTRACTION AND RE-EXTRACTION OF SOIL UPON THE *pH* VALUE OF THE EXTRACT. SOIL, ONE PART; WATER, FIVE PARTS BY WEIGHT.

Sample.	Extraction.		Re-extraction, total time.		
	3–4 hrs.	18 hrs.	18 hrs.	44 hrs.	60 hrs.
	pH.	*pH.*	*pH.*	*pH.*	*pH.*
5	6·0	6·0	6·05	6·35	6·45
6	5·8	5·8	5·8	6·0	6·25
24	8·1	—	8·0	7·8	7·6
25	7·8	—	7·8	7·65	—
30	7·65	—	7·65	7·55	7·5

The second and third re-extractions have noticeable effects, tending to bring the *pH* values nearer to neutrality. The strong buffer action of the soils is well brought out by these figures. In this connexion the work of Arrhenius (1922, 1) on clay as an ampholyte should be remembered. This action of clay must greatly reduce the effect of the carbon dioxide dissolved in the soil solution, so that the *pH* value is not regulated mainly by the carbonate, bicarbonate, and carbon dioxide equilibrium as in alkaline fresh or salt water. In the writer's paper on plant distribution (1922) too great importance was attributed to bicarbonates and too little to silicates, aluminates, and such complexes.

The preparation of a clear soil extract.

Certain sandy soils and silts settle so rapidly that no trouble is experienced owing to turbidity. With most soils, however, three or four hours is not sufficient to give a really clear solution, though, if they stand overnight, many

of them will settle out sufficiently to give a portion which is satisfactory for use. This, however, may involve a new source of error, the biological production of carbon dioxide, especially in the more fertile soils. This is quite insufficient to affect appreciably the turbid extract, which is heavily buffered by the soil particles, but may lead to the lowering of the *pH* value of the supernatant liquid, especially if alkaline. Errors of from *pH* 0·1 to *pH* 0·5 may be encountered from this source; the agitation of the liquid, with several changes of air, serves to extract this carbonic acid, and a result approximating to the true initial value may be obtained. Thus the method of subsidence, though convenient where practicable, has its limitations.

As a routine practice in all his work, the writer has cleared soil solutions by means of an electric centrifuge capable of running at 9,000 r.p.m., but usually run at a lower speed owing to much trouble due to breakage of tubes. This has proved quite satisfactory with almost all soils examined, though a few—while clear enough to permit of print being read through the suspensions—were yet slightly turbid. A number of soils give clear extracts with a slight yellowish tint; with these a comparator should always be used, otherwise the *pH* values are too low by 0·1, 0·2, or more, with indicators such as phenol red and brom thymol blue, or too high with indicators, such as methyl red, in which the red to yellow colour change is in the reverse direction as compared with phenol red. Should a tube break in the centrifuge the liquid must be rejected, even if clear, as it is markedly too alkaline owing to contact with the freshly broken glass surface. The use of colloidal iron was tentatively suggested by Gillespie (1920) as of possible value for clearing solutions. According to the writer's experience its use is liable to give an error in the direction of an increase in the acidity. Gimingham (1923) has recently introduced percolation through a column of soil as a method of obtaining a clear solution.

The use of a centrifuge would not be possible everywhere, and filtration, if permissible, would be far more convenient. Olsen (1923) states that with Swedish "Berzelius" acid-extracted filter-papers his colorimetric were within *pH* 0·2 of his unfiltered electrometric determinations made upon wood and meadow soils, using one volume of soil to one of water. These soils were mostly rich in humus.

With arable soils, however, he records errors of as much as *pH* 0·5. It may be remarked that Olsen uses distilled water for his extractions, as is also the writer's practice, but Wherry uses any natural water close to neutrality, which appears to introduce an error owing to the buffer action of a natural water at *pH* 7·2, due largely to silicates and bicarbonates. Wherry's (1922) methods are admittedly approximate, being designed for rapid field work. Salisbury (1922), too, follows Olsen in filtering his solutions made from 10 grams of undried soil with 50 c.c. of water neutral to brom thymol blue. The first filtrate is discarded, and the results obtained by this technique agreed, in the samples tested by him, with those given by clearing with the centrifuge. Kelley (1923) and others have tried diluting turbid extracts to two or three volumes, and using a comparator. The error given is said to be small, and with highly buffered clay soils this is probably true. The writer considers indiscriminate dilution to be a risky procedure, though at times it proves useful.

The tables which follow show that filtration may introduce serious errors. Thus alkaline soils appear nearer neutrality when filtered through an acid-extracted filter-paper, and acid soils may have their acidity shown as erroneously low even when filtered through acid-extracted paper, and to a more marked extent when ordinary filter-paper is used, such as Whatman, No. 1. Munktell's No. 0 is evidently the "Berzelius" paper used by Olsen, and is acid-extracted.

Table III.

Comparison of centrifuged and filtered soil extracts. Soil, one part; water, five parts. Total volume about 25 c.c.; time of extraction, eighteen hours.

Sample.	Centrifuged.	Filtered.		Paper.
		1st 5 c.c.	remainder.	
	pH.	*pH.*	*pH.*	
3	5·6	5·65	5·7	Whatman No. 1.
4	5·4	6·0	6·0	„ „
24	8·0	7·8	7·8*	„ „
25	7·8	7·7	7·7†	„ „
17‡	4·55	6·2 (10 c.c.)	5·5	„ „
17	4·55	—	4·8	Munktell's No. 0.
17A§	5·1	—	5·5	„ „
63	8·0	—	7·4	„ „

* Still turbid, but fit to examine.
† Clear, slightly yellow.
‡ Peat 1, water 6; peat on paper.
§ No. 17 diluted with equal volume of distilled water.

In the above table both methods agree well with some soils, whereas in others, No. 4 and No. 17 for example, wide discrepancies appear. Thus No. 17 is changed *pH* 0·95 namely, almost a tenfold reduction in acidity, by filtering through unextracted and *pH* 0·25 through extracted paper. On the other hand, No. 63 has its alkalinity much reduced by using acid-extracted paper.

Table IV.

Effect of filtration on *pH* of peat extract.

Sample.	Composition.		*pH.*	Notes.
17	10 grms. peat, 60 c.c. water		4·55	Centrifuged.
17B	Liquid drained off from No. 17, 180 c.c. distilled water added to residue		4·95	Do.
Do.	Do.	do.	5·25	1st 80 c.c. of filtrate, Munktell's No. 0.
Do.	Do.	do.	5·02	2nd 80 c.c. of filtrate.

In Table IV are shown the results of an attempt to satisfy the acid-absorbing power of the acid-extracted paper by making the first filtrate very large. The latter here shows an error of *pH* 0·3, whereas in the second it is only *pH* 0·07. The error may thus be much lessened by the rejection of a large first filtrate.

The differences between various filter-papers is shown in Table V, which records *pH* values ascertained approximately by spot tests using the usual indicators, all of which were tried on each paper with concordant results. The values about *pH* 4·4–4·8 are probably less accurate than the others, since methyl red is in many respects not so reliable as are indicators of the sulphone phthalein series.

TABLE V.

pH VALUES OF FILTER-PAPER, SPOT TESTS.

Paper.	*pH*.
Whatman, No. 1, 11 cm., old batch, ...	7·6
Do. do. new batch, ...	6·8–7·0
Do. No. 1, 12·5 cm., old batch, ...	7·4–7·6
Do. No. 4, „ „	6·8–7·0
Do. No. 40, 11 cm., HCl and HF, ...	4·4–4·8
Munktell's No. 0, 7 cm., HCl, ...	4·4–4·8
Do. „ 9 cm., HCl, ...	4·4–4·8
Schleicher and Shull, No. 589, 7 cm., HCl and HF, ...	4·4–4·8
Chardin, for agar agar, ...	4·4–4·8
J. Green, extraction thimble, ...	5·2

It was furthermore found that washing was not effective in lessening the action of acid-extracted paper in absorbing acid. Thus when 100–150 c.c. of distilled water was filtered through Munktell's No. 0 paper it came out at *pH* 5·65, namely a little acid had been taken up by the paper. Peat extract, found to be at *pH* 4·55 when centrifuged, was then filtered through both washed and unwashed paper, and the filtrates (10 c.c.) were both at *pH* 4·8. With unextracted paper there was a noticeable improvement through washing, but the error still remained very great, viz. *pH* 1·45. This paper (Whatman No. 1) when washed as before with 100–150 c.c. of water at *pH* 5·6 reduced the acidity of the latter to *pH* 6·2, having evidently parted with alkali. When peat extract at *pH* 4·55 was filtered through washed and unwashed paper the 10 c.c. filtrate was at *pH* 6·2 for the former and *pH* 6·0 for the latter.

Indicators.

Those of the Clark and Lub's series have been found by the writer to be very satisfactory and stable in solution, with the exception of methyl red. Cohen (1922) has introduced brom cresol green as a substitute for methyl red, and its use is to be preferred. It is like the other sulphone phthaleins in being unabsorbed by peat and plant tissues, whereas methyl red is rapidly taken out of the solution, quite apart from being decolorised by bacterial action.

The indicators should be used in their half transformed condition, phenol red for example being neither a deep red nor yellow, but a faint pink. This is of importance when dealing with lightly buffered soil extracts and natural waters. The use of alcohol as a solvent should be avoided where possible, though for methyl red it is certainly convenient. Alcohol is apt to undergo oxidation, producing traces of acetic acid, and so it is usually faintly acid; acetic acid and sodium acetate act as buffers, so their addition in the indicator solution should be avoided. For this and other reasons when there is a difference between the *pH* values given by a soil extract with methyl red and brom cresol purple the value shown by the latter should be accepted or the newly introduced brom cresol green used.

In the course of a discussion on certain of the points raised in this paper, Dr. A. F. Joseph suggested that the alkaline soils of the Sudan, in which the clay was deflocculated by the alkali, might not be satisfactorily cleared by the centrifuge. He accordingly sent a sample for trial, which contained 29·6 per cent. of clay, and was extremely alkaline, *pH* 10·0, as determined electrometrically.

On making up a 1 : 5 mixture with distilled water, and centrifuging for ten minutes, after standing with repeated shaking for three hours, it was found that a solution resulted which was clear enough to permit of print being read through it, though it still had an opalescence. The liquid had a very faint straw tint. Another portion was passed through a sieve, with 100 meshes to the inch, and a 1 : 5 mixture was made up. This portion was, therefore, richer in the clay fraction. When centrifuged beside the unsieved sample, and for the same time, the supernatant liquid was somewhat less clear and had a marked straw tint. The *pH* value, determined colorimetrically, was close to 9·4, using thymol phthalein as an indicator, but with thymol blue the untreated sample appeared to be about *pH* 9·8 and the sieved *pH* 9·55. It seems that small amounts of carbon dioxide readily affect the soil extract when no longer buffered by soil particles. It was also suggested by Dr. Joseph that the results of Table V, showing the *pH* values of filter-papers, might be tested further by adding to a number of papers just enough water to soak them thoroughly and leave a small residue of liquid when they were lightly pressed. This was accordingly done, and it was found that 2 c.c. thus obtained from Whatman No. 1, 11 cm. diameter paper, gave the value *pH* 7·1 with carefully adjusted brom thymol blue, thus agreeing well with the results of the spot test. Munktell's No. 0, 9 cm. diameter, gave a press liquid at *pH* 5·5, that of Whatman No. 40, 7 cm. diameter, being at *pH* 5·55 with methyl red. These results are considerably less acid than the values of Table V. When spot tests were made on these papers, using brom cresol green as indicator, Munktell's No. 0 gave a yellow spot with a green edge and Whatman No. 40 a yellow spot with a blue edge, thus denoting a lesser acidity.

The writer wishes to acknowledge his indebtedness to the Department of Scientific and Industrial Research, London, for a grant covering the cost of the hydrogen ion determination outfit; to the Marine Biological Association, Plymouth, for general laboratory facilities; and to Dr. A. F. Joseph, Khartoum, for a sample of Sudan soil and his helpful criticism.

Summary.

1. As a general rule the effect of increasing or decreasing the soil to water proportion within limits does not alter the *pH* value appreciably—by as much as *pH* 0·1—for soils between *pH* 6 and 8. With more acid or more alkaline soils an alteration in *pH* value may be introduced by altering the proportions. For lightly buffered acid soils one part of soil to two of water seems a safe proportion to adopt; for other soils a one to five proportion seems to be convenient and reliable.

2. The *pH* value of some soil extracts is markedly modified by filtration, even when a first filtrate is rejected. Both untreated and acid-extracted filter-papers may reduce the acidity. The use of large volumes, about 160 c.c., of filtrate and a filter appropriate to the soil reduces these errors. Where possible clearing by the centrifuge is desirable, and has been the writer's regular practice.

3. The fibres of acid-extracted filter-papers act towards indicators as if as acid as about *pH* 4·8, but washing was not found to render them less acid. Unextracted papers are at about *pH* 7–7·6 and give up traces of alkali to distilled water.

4. The indicator brom cresol green is to be preferred to methyl red for the same *pH* range.

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No. 45.

VARIATIONS IN THE PERMEABILITY OF LEAF-CELLS.

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(Read June 24. Printed July 15, 1924.)

From many points of view changes in the permeability of cells and the factors controlling them are of interest. Variations in the permeability of the protoplasmic membranes lining the leaf-cells have special significance with regard to the problems of the export of dissolved substances from leaves to the rest of the plant and of their import from the stem to the leaf (6 and 7).

Certain conditions, however, render the study of the permeability of leaf-cells difficult. The plasmolytic method is unsatisfactory in their case, owing to the difficulty of obtaining good contact between the surfaces of these cells and the watery solutions used. The injury to the cells of leaf-tissues necessary to render them visible also introduces disturbances unknown in nature and magnitude.

Osterhout's important and pioneer researches (9, 10, 11, 12) have introduced the electrical method for the determination of changes in permeability in homogeneous tissues like those of the great kelps; he and others have shown that the electrical resistance of a tissue is a measure of the permeability of the protoplasmic membranes of its component cells. The methods of making electrical contact with the tissue under experiment, which he found suitable with Laminaria, are, however, not available for leaf-tissues, which are more or less spongy, and are coated with an unwettable cuticle. Consequently it was necessary, if the electrical resistance method was to be used, to find some other means of effecting this end.

After a considerable amount of experiment the following method was found satisfactory, and adopted:—A square centimetre is cut with a template from the leaf to be investigated. It is laid across two electrodes (*E*, fig. 1) formed of two square pieces of platinum foil 0·5 cm. on the side. Each electrode is given a rectangular fold so as to appear L-shaped in profile. The two horizontal parts face each other and support the leaf-square. To the vertical part of each electrode is soldered a piece of platinum wire (*D*) which passes through the sealed end of a glass tube. The end of the latter supports the electrode rigidly. The glass tubes are filled with mercury (*H*) and are fixed in a small frame made of three discs of cork (*A*, *B*, *C*) connected by a glass rod (*L*). The ends of flexible leads dipping into the mercury connect the electrodes with one of the arms of a Kohlrausch bridge. In order to secure good contact between the electrodes and the cut edge of the leaf-square a little sap, pressed from leaves of the experimental plant, is placed upon each electrode. The electrodes themselves are coated with platinum-black to obviate polarization effects. Before cutting out the leaf-square from the leaf, it is advisable to smear the upper surface of the latter with a thin film of vaseline. This prevents the sap on the electrodes from creeping across and short-circuiting the tissue.

The upper surface of the square is turned downwards as it lies on the electrodes. In the other arm of the bridge a high adjustable resistance (10,000–400,000 ohms) is introduced. In practice two pairs of electrodes were fixed in the frame so that two leaf-squares might be experimented upon simultaneously.

It was soon found that temperature had a large effect upon the resistance, and it was consequently necessary to have the temperature of the tissue under control. To effect this the frame was fitted into a large test-tube (*F*, fig. 1) and the whole immersed in a glass vessel containing about two litres of water. The water in this vessel could be cooled by the addition of ice, or heated by the passage of an electric current through a submerged wire of German silver supported on a bent glass rod (*R*). Adjustment of an external resistance made it possible to maintain the water round the test-tube at any desired temperature between 0° and 50° C. Before putting the frame into the test-tube a few drops of water were introduced to keep the space surrounding the leaf-square moist, and thus prevent the drying up of the leaf-square during the observations.

The resistance of the leaf-squares examined was found to be considerable, amounting to from 200,000 ohms to 600,000 ohms at 0° C.

The relation of resistance to temperature in the leaves of *Hedera helix* may be seen in figs. 4, 5, 6, and in the leaves of *Syringa vulgaris* in figs. 2 and 3. Here the ordinates are resistances measured in ten thousands of ohms (thus 12 on the vertical scale indicates 120,000 ohms), and the abscissae are temperatures in degrees centigrade. Fig. 4 contains the records of the behaviour of three leaf-squares of *Hedera helix*. The curve *A* shows the change of electrical resistance of a square cut on February 5th from an old last season's leaf of ivy, fixed on the electrodes as described and raised slowly through the range of 0°–50°. Each reading of resistance was carried out after the water round the test-tube was at the temperature recorded for 15–20 mins.

Next day the same leaf-square was heated through the same range, and its resistances are recorded in the curve *A'*.

B and *B'* are similar curves traced from the behaviour of a similar square cut from another old leaf. The curve *Bd* gives the resistances of the same square through the same range after it had been killed by exposure to chloroform vapour for somewhat over an hour.

C, *C'*, and *Cd* are similar records for a square cut from a young leaf also of the previous season.

The idiosyncrasies of the three squares are noticeable. There are considerable absolute differences in resistance of the three, and differences in the form of their curves.

They all show a marked fall of resistance with rise in temperature. In *A*, however, the rise between 30°–40° is accompanied by a rise in resistance. So far as my experience goes this is a rare occurrence.

The reduction after heating is another regular feature. Thus *A'*, *B'*, and *C'* are each lower than *A*, *B*, and *C* respectively. Heating appears to initiate a progressive change which continues to reduce the resistance. Thus in the case of the series of experiments recorded in *C* and *C'* it was found that the resistance of the leaf-square on being cooled to 0°, immediately after being raised to 50°, rose to $43{\cdot}3 \times 10^4$ ohms from $11{\cdot}5 \times 10^4$ ohms. Next day its resistance at 0° was only $26{\cdot}0 \times 10^4$ ohms. It had been kept during the night at a temperature of about 10°–11°.

This progressive and lasting reduction of resistance caused by exposure

to high temperatures explains the fact that, generally, the resistances observed in summer are less than those of the same plant found in winter and spring.

The amount of the reduction in resistance is by no means regular, as inspection of fig. 4 will show. Fig. 5 also gives a striking example. The squares *A* and *B* were cut from the same mature leaf of *Hedera helix*, and included symmetrically-situated pieces on opposite sides of the mid-rib. Their resistances throughout the first heating were almost identical. On the second heating a marked difference was noted. It is recorded in the curves *A′* and *B′*.

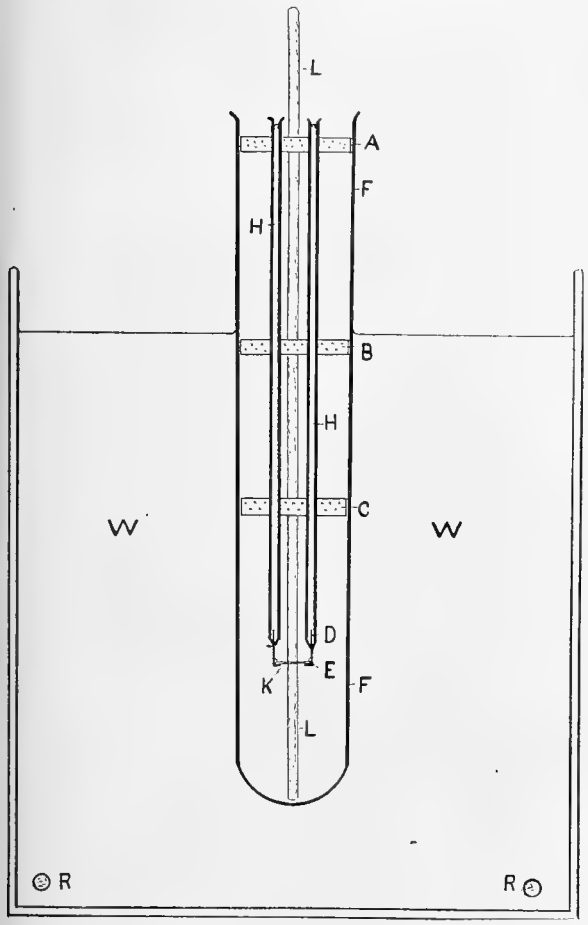

FIG. 1.

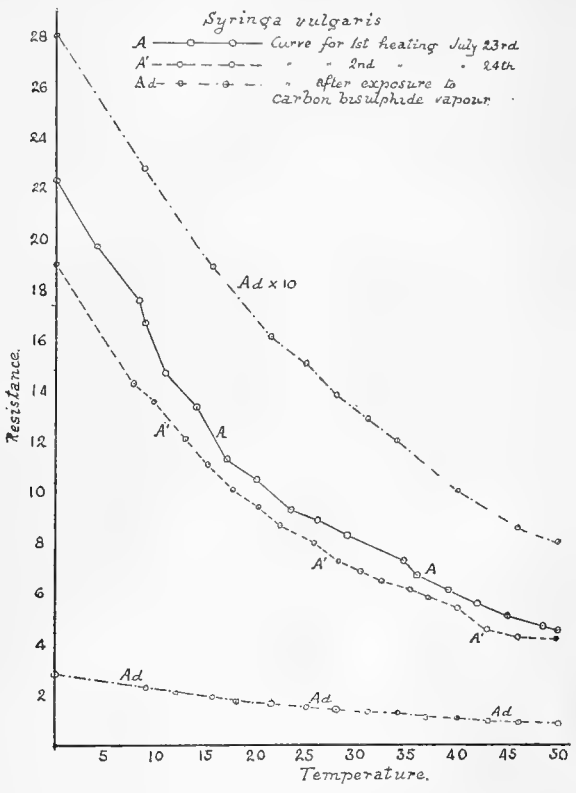

FIG. 2.

The changes induced by the heating do not always appear to be lethal. This is evidenced by the fact that sometimes the recovery of resistance after the heating may be almost complete, e.g. *B′*, fig. 5. Also a similar change is observed when the heating is carried only as high at 35°. Thus a leaf-square of *Hedera helix* heated from 0° to 35° on February 28th had a resistance of $41{\cdot}7 \times 10^4$ ohms at 0°, and $10{\cdot}8 \times 10^4$ ohms at 35°. On the next day its resistance at 0° was $30{\cdot}8 \times 10^4$ ohms and $11{\cdot}8 \times 10^4$ ohms at 35°.

The curves show a rapid fall in resistance between 0° and 15°. They are less steep at the higher temperatures, but often show a steeper bend between

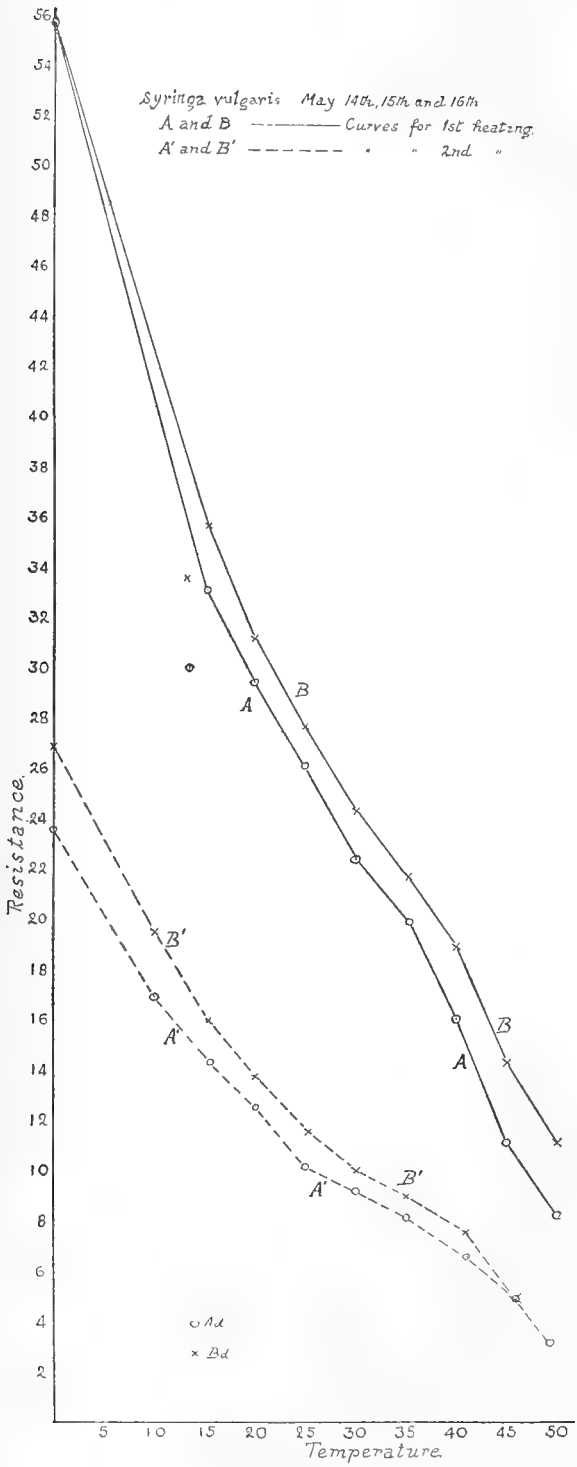
Syringa vulgaris May 14th, 15th and 16th
A and B ———— Curves for 1st heating.
A' and B' – – – – – – " " 2nd "
Resistance.
Temperature.
A
B
A'
B'
Ad
Bd

FIG. 3.

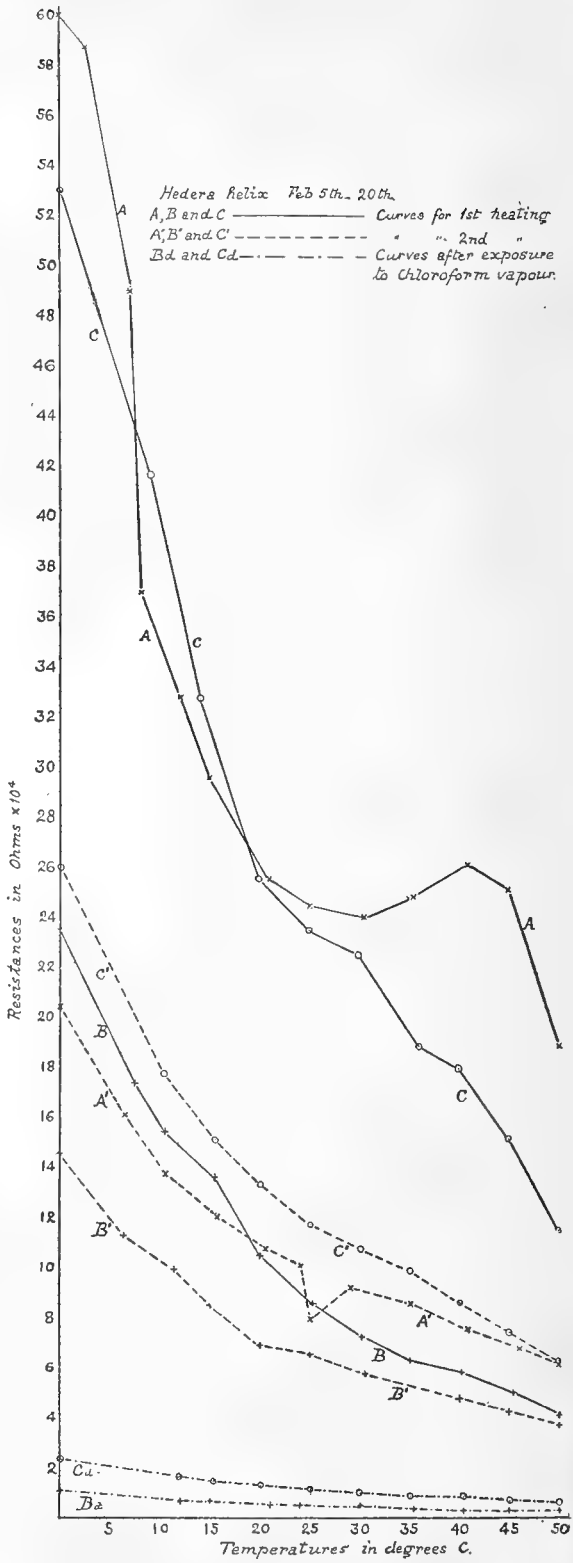
Hedera Helix Feb 5th. 20th.
A, B and C ———— Curves for 1st heating
A', B' and C' – – – – – – " " 2nd "
Bd and Cd —·— ·— — Curves after exposure to Chloroform vapour
Resistances in Ohms x 10⁴
Temperatures in degrees C.
A
B
C
A'
B'
C'
Cd
Bd

FIG. 4.

40° and 50°. The initial steepness is most marked in those with the highest resistance, viz. 40–60 × 10^4 ohms. The final steepening, which, in some cases at least, seems associated with lethal changes, is, so far as I have observed, most marked in young immature leaves which rapidly change colour about and over 40° C. (see fig. 6).

Over the interval of temperature used the conductivity temperature-coefficient for 10° (i.e. the ratio of the conductivity at T° + 10° to the conductivity at T° C.) varied considerably, viz. from 1·70 to 0·92. The average of the thirty observations plotted in fig. 4 gives 1·334 for the coefficient—a figure almost identical with that obtained by Osterhout for Laminaria. This figure is a measure of the variation of permeability with temperature.

The resistance of the leaves of *Hedera helix* appears to grow with age, and this in spite of the fact that during the interval growth in thickness takes place.

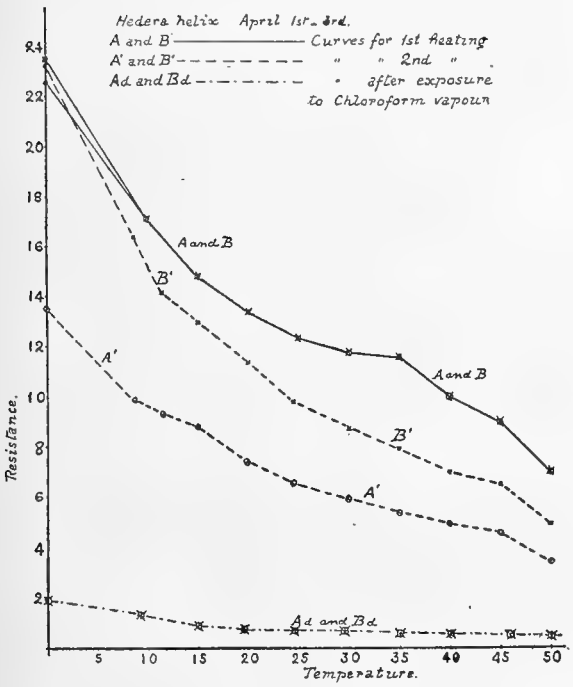

FIG. 5.

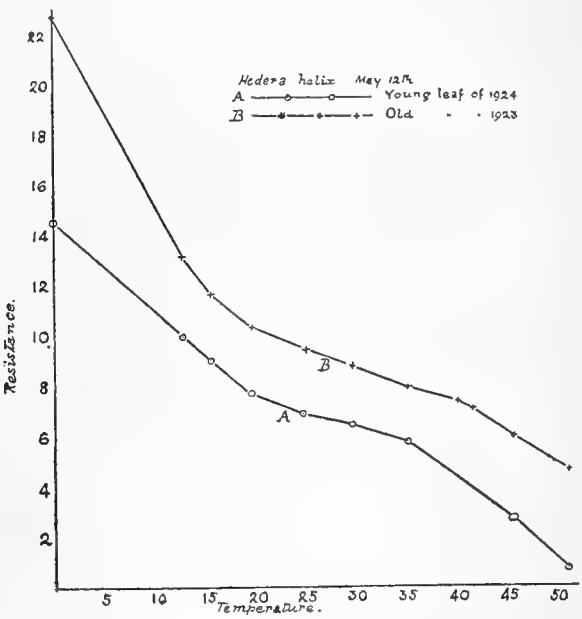

FIG. 6.

TABLE.

Age of leaf.		Date.	Temperature.	Thickness.	Resistance.
Young mature	...	9/5/'24	13°	0·250 mm.	22·8 × 10^4 ohms.
Old "	...	"	13°	0·375 mm.	28·9 × 10^4 "
Young "	...	14/4/'24	10·2°	0·238 mm.	17·9 × 10^4 "
Old "	...	"	10·2°	0·277 mm.	24·4 × 10^4 "
Young immature	...	12/5/'24	0°	0·200 mm.	14·49 × 10^4 "
Old mature	...	"	0°	0·400 mm.	22·75 × 10^4 "

The probable explanation of this rather surprising observation seems to be that the increase of thickness with age is due to the increase in size of the intercellular system, and does not involve an increase in the cross-section of the conducting cells, or an increase in the surfaces of contact of these cells. The resistance due to the protoplasm of the constituent cells evidently depends directly on this total surface. Expansion of the individual cells and of the intercellular spaces would tend to reduce this surface, and hence explain the growth of resistance with age.

The contrast between the resistances of the living and of the dead leaf is in every case very marked. That of the living leaf has been found to vary between twenty-two times and six times that of the dead leaf. The form of the curve for the dead tissue generally resembles that for the living tissue. This is made plain by fig. 2, in which $Ad \times 10$ is obtained by plotting the temperature-resistance curve for the dead tissue to ten times the vertical scale. The large difference between the dead and the living tissue is in agreement with the view that the electrical resistance of a tissue is controlled by the semi-permeable property of the cells composing it.

Figs. 2 and 3 summarize experiments on the leaves of *Syringa vulgaris* similar to those on *Hedera helix* already described. The same features, already noticed in the case of *Hedera helix,* are apparent in the curves for the leaves of *Syringa vulgaris.*

Preliminary experiments on the effect of light in altering the permeability have not given conclusive results, inasmuch as the effects observed might be attributed to the heating produced by the illumination.

The salient fact brought out by the foregoing experiments is the reduction of resistance or the increase of protoplasmic permeability produced by a rise of temperature. Thus we may expect the permeability of leaf-cells to become about doubled by a rise of temperature from 10° to 30°, and at 20° the permeability of the cells will be 50 per cent. greater than at 10°.

A rise in the temperature of the surroundings when the atmosphere round the leaves is saturated will of course produce a rise in the general temperature of the plant including its leaves. Under these conditions all the cells of the plant, if they behave like the leaf-cells, will become more permeable, and probably important effects result from this change.

In 1905 Brown and Escombe (2 and 3) came to the conclusion on theoretical grounds that the temperature of a leaf when insolated or exposed to diffuse light did not, in the specific cases considered, differ from that of its surroundings by more than + 1·64° or − 1·84°.

In the same year, however, F. F. Blackman and G. L. C. Matthaei (1) measured by thermo-electric means the temperatures of shaded and insolated leaves. It was found that a difference of 16° might be established, while a leaf exposed to diffuse light is often 1°–3° above its shaded surroundings. The leaves used were of *Prunus laurocerasus,* and the thermo-junction was embedded in the mid-rib of the leaf.

Recently I have carried out some similar experiments with leaves of *Hedera helix* and of *Syringa vulgaris.* The thermo-couple I used was made of constantan and copper. The elements of the couple were in the form of fine silk-covered wires, each end of the copper wire being soldered to a piece of constantan wire. In order to reduce the thermo-electric effect so as to give a convenient deflection with a sensitive galvanometer seven metres of No. 42 s.w.g. (diam. = 0·1 mm.) constantan wire were used. The copper element was formed of 50 cm. of No. 36 s.w.g. (diam. = 0·18 mm.) copper wire.

The extreme ends of the elements, stripped of their silk covering, were twisted together and soldered. The soldered junction so formed was reduced to about 0·25 mm. in length by an oblique cut of a sharp scissors. By this means the actual junction was very close to the point formed, so that when the double wire was pushed through even a thin leaf, the junction was still embedded in the tissue, as the point was just emerging. The other ends of the constantan wires were soldered to thicker copper wires, and these latter junctions, together with the support carrying the copper terminals, were immersed in a small vessel of petroleum. By this means, and by means of a connexion already described, thermo-electric errors were avoided (4, 5, 8).

The thermo-couple thus arranged gave a deflection of 26 mm. per 1° difference of temperature of the junctions.

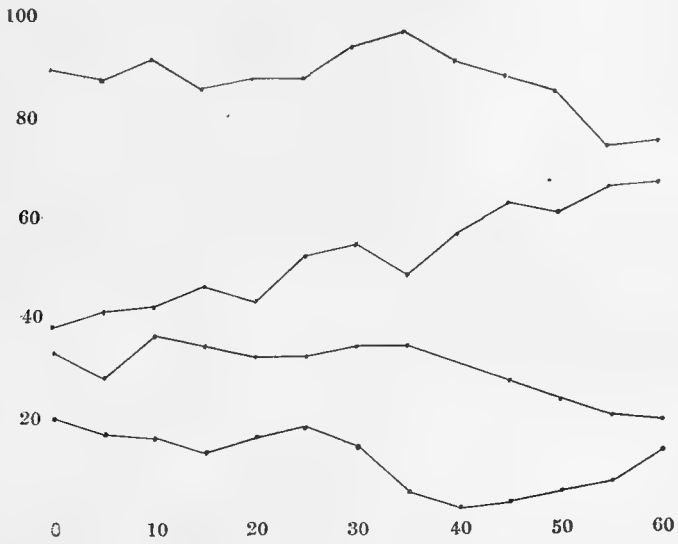

FIG 7.

In cool weather with intermittent sunshine early in May an insolated patch on a leaf of *Hedera helix* was found to be 4·7°–6·2 above an adjacent patch which was shaded by an overhanging leaf. Under similar circumstances a temperature difference of 6·3°–6·6° was found in leaves of *Syringa vulgaris*. In each case the periods of insolation were less than 5 mins., and the leaves were not normal to the direction of illumination. The leaves were exposed to a brisk breeze and were well supplied with water. Rapid temperature changes were observed, fluctuations of 1°–6° within a minute being not uncommon. Records of four sixty-second observations are shown in fig. 7. Bright diffuse light caused a rise of 1° or 2° above a shaded portion. In more favourable weather, differences as large as those found by Blackman and Matthaei would probably have been obtained.

From these experiments it is evident that during sunshine there are temperature-differences between shaded and insolated leaf-areas of 10° and more.

The results connecting resistance and temperature show that these temperature-differences will lead to large fluctuations in permeability, the cells in the heated areas becoming more permeable. It has already been pointed out that such differences in permeability acting in concert with the hydrostatic tension throughout the plant furnish a mechanism for the distribution of dissolved substances through the plant body.

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No. 46.

NOTES ON ACARINE OR ISLE OF WIGHT BEE DISEASE.

By LIEUT.-COLONEL C. SAMMAN, R.A.M.C.,

AND

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(Plates XVIII and XIX.)

(Read April 29. Printed August 21, 1924.)

Introduction.

In the year 1904 a serious, apparently new, disease of honey-bees broke out in the Isle of Wight, and, spreading rapidly, swept over the British Isles like a plague, completely wiping out the bees in many districts. At least two commissions of investigation failed to find the cause of this disease, and it was only so recently as 1919 that a band of workers in Aberdeen University were able to give the first correct explanation of the cause of Isle of Wight disease in bees. The organism which is the causative agent belongs to the order Acarina. It was first noted by Miss E. J. Harvey, who attached no special importance to its presence in the bee; but it was afterwards seen, and its importance immediately recognized by Bruce White, who has given a description of its effect on the host. The credit of the discovery of the cause of Isle of Wight bee disease therefore belongs to Bruce White, whose paper on its pathology is the only one up to this date in existence. White's paper was published simultaneously with other communications from Miss Harvey and Dr. John Rennie. Since Bruce White's discovery, John Rennie has carried out investigations on the bionomics of this disease, and it is principally to him that we owe what knowledge we have of the habits, pathogenicity, and treatment of this remarkable disease, which is characterized by many peculiarities not hitherto known in parasitology.

Our study of this disease in bees has extended about one year. During most of this time one of us has carried out routine diagnostic work for Irish bee-keepers, and it is only more recently, during winter, that a serious attempt has been made to extend our knowledge of the disease: the junior writer had long been interested in the Acarina, and welcomed the opportunity to undertake an investigation of the embryology and anatomy of the causative organism of Isle of Wight disease. Owing to technical difficulties, it is only recently that satisfactory sections of the parasites have been obtained.

For nearly a year this work has been carried out with facilities provided solely by the Zoological Department of Trinity College, Dublin. Since the Free State Government kindly granted us fifty pounds for expenses relative to this investigation, we have recently made a little progress. Our work has been

especially hampered by the difficulty in obtaining diseased bees. We must take this opportunity of thanking the Minister of Agriculture, Mr. Hogan, and some of the officials of the Department of Agriculture for the interest they have taken in our work and for the loan of some pamphlets.

For the benefit of those not especially acquainted with the subject, the following simple account is given:—

In the higher organism, like man, the function of bringing oxygen for respiration to the tissues and cells, as is well known, is carried out by the blood, but among certain of the animal groups, one of which contains the bees, there is found instead a separate system of air-tubes or tracheae which ramify or branch over the tissues carrying oxygen to the cells. Any blockage of these air-tubes brings partial or total asphyxia to the tissues served by the blocked tube, just as stoppage of a blood-vessel going to a part of the body would produce a like change.

Biologists have never ceased to wonder at the powers which exist in the thoracic-wing muscles of insects; the rapidity with which these muscles are able to operate, and their endurance are alike remarkable. The air-tubes or tracheae which serve these thoracic muscles with oxygen are the seat of the disease under discussion. The mite or acarid crawls in through the external opening of the tube or stigma, and breeds rapidly within the lumen of the trachea. In later stages the bees are unable to fly, and crawl about the hive or on objects near by: now bees only defecate when in flight, and consequently the "crawlers" retain their foecal matter. This naturally led the earlier investigators to consider that they were dealing with some complaint of the alimentary canal, and no one seems to have thought of a connexion between the state of the gut and a cause of the inability to use the wings. Thus the discovery of the acarids within the air-tubes has proved a remarkable one.

Reference to the microphotographs will give a good idea of the disease. In Pl. XVIII, fig. 1, is a photo of the adult male mite; it has four pairs of legs, with the piercing organs, palps, and chelicerae between the first two pairs. These mites are very slow walkers, and are unable to run like many of the free living acarids. In Pl. XIX, fig. 7, is a photo of the upper part of one of the thoracic tracheae; there is a main stem below, which branches to form a Y, from the upper part of which smaller air-tubes branch off, and finally form exceedingly fine twigs that carry air to the tissues. In Pl. XVIII, fig. 4, is another tube showing a branching lower down. The end on the left below *d* connects to the exterior by the air-pore or stigma. In Pl. XVIII, fig. 3, the lowermost part of the thoracic trachea is shown.

The mother mite crawls into the tracheal tube through the stigma, and generally comes to rest just inside. In Pl. XVIII, fig. 2, is a clean tube flattened under the cover-slip and photographed; in fig. 3 is a tube which shows the first stage of infection. The mother mite at ♀ has already laid two eggs, l^1 and l^2; in Pl. XIX, fig. 5, the family has increased, and the mites are working their way up the tube; while in fig. 6 the infection is heavy, and the female mites at ♀ have been breeding rapidly. Some time after the tube or trachea becomes infected another fact may be noted: in Pl. XVIII, fig. 4, is shown very clearly, at *dd*, a black smudge on the wall of the tube. This smudge is caused by the mites, and eventually spreads in large patches and areas, and finally the tube becomes blackened, as shown in Pl. XIX, fig. 8. Such bees are doomed, and are unable to fly, or at all events to fly far.

The mite is believed to feed by sucking the haemocoel or body cavity (or blood) fluid of the bee which surrounds these tubes, so that the mites, by pricking through the wall with their chelicerae are able to draw fluid for

their nutriment. According to John Rennie it is the feeding of the mites that weakens the bee.

Rennie's (1921) paper (jointly with White), in the Transactions of the Royal Society of Edinburgh, has been superseded by his late memoir (No. 6) published by the North of Scotland College of Agriculture, and entitled "Acarine Disease Explained." In the Transactions of the Royal Society of Edinburgh (as well as in Rennie's joint papers) is one by Bruce White on the "Pathology of Isle of Wight Disease in Hive Bees." White mentions that female mites may advance as far as the secondary tracheae before depositing eggs; in the later stages of attack the mites may attain the small tracheae, the thoracic air-sacs, and the vessels of the head. Regarding the tracheal system, White states that the change in colour of the tube is accompanied by an increasing hardness and brittleness of the parts, which become rigid. In the early stages of attack there may show, here and there, a few fragments of brownish matter, the faeces of the invading adults. Such granules increase in number, finally forming bands upon the tracheal wall. They are brownish or yellowish in colour, and when densely aggregated appear black.

White also states that such foecal matter may become inhaled into the smaller passages, forming emboli in the tracheoles.

The muscular system may be visibly affected, the fibres showing atrophic changes; but the number of such showing these signs is small. This author performed the ingenious experiment of blocking up the stigmata, or openings leading to the tubes, in healthy bees, and found that, in some cases, states resembling the symptoms of Isle of Wight disease were produced. White undoubtedly, in this way, has added very strong evidence to the view that the acarid is the causative agent of the disease.

Experiments on infection with Acarapis woodi have been made by Miss Elsie J. Harvey, one of Rennie's assistants. Miss Harvey came to the conclusion that bees were not usually, if at all, infected before emergence from cells. Clean bees placed in a queen cage could be infected if kept in a diseased hive. Miss Harvey showed that experimental infection is not easy to effect, but that the disease is spread by bodily contact between infective and non-infected bees.

Rennie, after Bruce White's and Miss Harvey's discovery of the causative agent of the disease, attempted to classify the mite. He placed it in Canestrini's genus "Tarsonemus" and gave it the specific name of "woodi" after A. H. E. Wood, a gentleman much interested in apiculture. Rennie's excursion into the treacherous grounds of systematic acarology was not altogether successful, for Hirst has removed this form from the genus Tarsonemus and placed it in the genus Acarapis, which is closely allied.

Hirst states that there is no nymphal stage either in Acarapis or Tarsonemus, this being entirely suppressed. This disease is spread by adult mites.

The Cure or Prevention of Isle of Wight or Acarine Disease.

We know that the causative organism of this disease is an acarid—the female of which is tracheate, the male non-tracheate. The healthy bees are infected by peripatetic females, which leave the thoracic tracheae of infected bees, and wander into the tracheae of non-infected bees. The acarids live on the haemocoel fluid of the bees.

The problem is to kill the acarids either (*a*) in the tracheae, by feeding the bees on some substance which might so affect the haemocoel fluid of the host, as to make it toxic to the parasites; or (*b*) in the tracheae by some gas or fumigant which might poison the mites without killing the bees themselves;

or (*c*) on the surface of the bees, by sprinkling the hive with some substance which would come into contact with the peripatetic gravid females, and thus prevent newly emerged bees from becoming infected; or finally, (*d*) to find some way of preventing clean stocks of bees from becoming infected.

John Rennie and various bee-keepers have tried many methods. The former mentions in one of his papers that many fumigants will kill the mites in experimental chambers: unfortunately Rennie does not appear to have given a list of these substances in any of his published work. In 1923, Wood announced that Rennie had found what might be assumed to be a cure for the disease, and, in his letter to an English bee journal, mentioned the name of an Aberdeen chemist from whom, for the sum of 2s. 6d., bee-keepers might obtain samples of this secret substance. In various bee journals from time to time "cures" for Isle of Wight bee disease have been announced. We ourselves have received visits from various bee-keepers who claimed to have cures for the disease.

We have thought it right to publish all our findings, in order that future workers might have the assistance of knowing our methods and our failures. Some, or all, of these methods may have been tried by Rennie—we do not know. We have not had Rennie's secret treatment analysed, but we have examined Irish bees which were said to have been treated with this cure, and were found still heavily infected. We do not wish to say that Rennie's treatment is not helpful; we state, however, that it is probably only an alleviative, and not a cure.

Some of the "cures" are substances which are slightly toxic to the bees, and by giving the *coup de grâce* to badly diseased and weakened individuals lessen the incidence of infection of the newly-emerged bees. So far as we are aware none of these substances does more than alleviate the disease: this, however, is a step in the right direction, but does not prevent the spread of the disease, and may leave the stock in a weak condition. The danger of such half-measures is that these stocks are a constant menace to clean hives in the neighbourhood.

At Trinity College, Dublin, we set up an apiary for diseased bees, and have had six stocks under continual observation. Besides this we have been able to examine and observe at intervals many other stocks, both diseased and healthy.

We arranged our experiments with the object of trying to affect the haemocoel fluid of the bees, and thus to make the conditions unsuitable or toxic for the mites. Two stocks were used as controls, one being at Trinity College. The winter months, during which the experiments were carried out, were very favourable for working out percentages of infection, for noting fluctuations if such occur, and so on, because no new bees were emerging.

Every week we examined large numbers of bees, and were able to establish the fact that very little, if any, infection of healthy bees occurs during winter within the hives at the winter temperature, and during the special period we observed the hives. This result is in accord with the fact that during winter the mites breed very slowly, and in many cases not at all.

On the time taken for bees to become infected.

It was important to find out how soon infection of the hive may take place after a parasitized bee has gained access to a clean stock. From our experiments we have been able to gain a clear idea as to this.

In April, a frame containing comb with drone brood, capped, was put into

a White's queen cage, and the wooden top replaced by a piece of excluder zinc allowing free access for diseased bees, but preventing the drones getting out. It was examined on the 8th day, so that drones were seven or less days old. The drones were infected. Each time the drones were removed and the frame was returned to the White's cage. The experiment was repeated on six occasions, i.e. on the 7th, 6th, 5th, 4th, 3rd, and 2nd days, and diseased drones were found. In the tracheae of a drone two days old or less, one mite and two eggs were seen (Pl. XVIII, fig. 3). In the trachea of a drone less than twenty-four hours, one mite alone was seen. This seems to prove that the gravid female gains access to the trachea very early and rapidly commences to lay eggs.

Experiment to show that the brood is free from infection.

The Aberdeen workers concluded that the brood is not diseased before emergence. Miss Harvey carried out a number of experiments, and our results confirm her findings. The following is taken from our notes on one of our experiments in this department.

(August 8th.) A frame of brood from a native stock infected with Acarapis was put into a "White's" queen cage, and a fertile Italian queen placed on the comb; this frame was then put in the hive from which the brood was taken, but no bees were able to get access to the comb. (14th.) Removed the cage. Some young bees had emerged from the cells and the queen had commenced laying. Two more frames of brood now added—one from the same stock, the other from a stock of Acarapis infected bees, to which a Carniolan queen had been introduced on July 3rd. The young bees from this queen had been examined on August 10th and found infested with Acarapis. (19th.) These three frames were now placed in an upper brood chamber above a strong colony (diseased) with a screen of wire gauze between the chambers. (20th.) Added three more combs of capped brood from diseased colony. (25th.) Put the six frames of brood into a nucleus hive, and transferred them to a fresh locality and allowed young bees to have their first flight. (September 7th.) Transferred frames to a permanent hive. Queen laying well; brood in all stages. (November 22nd.) Examined bees, no trace of Acarapis: shut up for winter.

1923. (February 27th.) Examined bees, no trace of Acarapis. (March 28th.) Examined bees, no trace of Acarapis. (April 11th.) Spring cleaned hive; bees doing well; brood in all stages; plenty of stores. (July 7th.) Stock swarmed (a very large swarm). Removed surplus honey (about 100 lb.); this in a poor honey season. At the present date (April, 1924) this stock is still doing well.

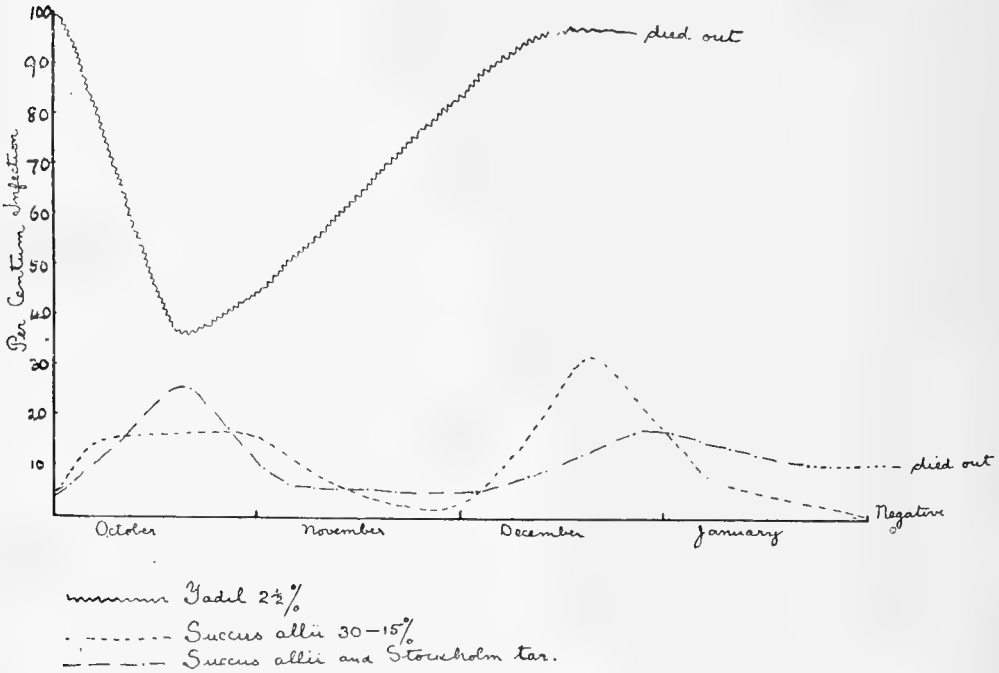
Experiments on Diseased Stocks.

Four stocks were treated with drugs in food during the winter months of 1923–1924. As is well known, during the colder period of the year the stocks are usually fed on some type of commercial candy. The drugs used by us were mixed with the latter, and by this means the bees were treated in four different ways. 1. Succus Allii of about 15 per cent., in water, sugar added as necessary, according to thickness of food desired. 2. Yadil candy of 2½ per cent. 3. Succus Allii of 15 per cent., followed by a treatment of Stockholm tar in candy of 5 per cent. 4. Yadil, as above, followed by Stockholm tar as above. We found that the bees would take candy syrup containing as much as 30 per cent. of Succus Allii, but not so freely as when the strength of the latter was reduced to 15 per cent. Consequently we worked with 15 per cent. strength.

Of the four hives the three treated as in paragraphs 2, 3, and 4, all died

after some weeks. In the graph in the text figure the Yadil-treated hive is shown in a wavy line. It was 100 per cent. infected at the end of September and the beginning of October. By the middle of October the infection had gone down to about 40 per cent., this probably being due to the hatching out of new bees. Despite the treatment infection steadily crept up, and the stock ultimately died out before the end of the year 1923. Possibly the Yadil was not strong enough, but this was the strength (2½ per cent.) sent by the makers.

In the case of the two hives treated with Succus Allii, we had more encouraging results. One stock marked thus ---- in the figure, began at 10 per cent. infection, which remained fairly uniform till December when it rose suddenly, but thereafter fell till all the samples of bees examined were negative. This stock is still negative, and healthy.



Graph showing percentages of infection of three hives treated respectively with Yadil, Succus Allii, and Succus Allii followed by tar, for four months of the year.

The other hive marked - - - -, gave much the same results, but in this case, Stockholm tar candy was given at the end of December; the stock died out about the end of January, poisoned, we believe, by the Stockholm tar. Nevertheless, we consider that if the tar had not been given, there would have been some ground for believing that this stock might have been cured.

In the other case where Yadil was given the percentage of infection at the beginning of the experiment was only about 10 per cent., and never rose above 50 per cent., but this stock was the first to die out.

These results, meagre as they are, nevertheless lead us to believe that an efficient treatment of Acarine or Isle of Wight disease might be found along these lines.

Garlic can easily be grown by bee-keepers, the bulbs can be dug up, and the juice expressed and mixed up to a strength of 15 per cent. with sugar.

This treatment should be tried during the winter months when the bees are clustered.

It should be pointed out that our experiments have not been numerous enough to enable us to claim that we have discovered a cure for Acarine disease. But our experience leads us to consider that the method might with benefit be tried by bee-keepers.

DESCRIPTION OF PLATES XVIII AND XIX.

All figures are from microphotographs. Fig 1, × 400; figs. 2–8, × 120.

Fig.

1. Adult male Acarapis woodi.
2. Clear non-infected tube.
3. Early stage of infection, one female (♀) and two eggs, l^1, l^2.
4. Discolouration (*d*) of the tube caused by mites.
5. Later stage of infection.
6. Multiplication of parasites at its height (summer).
7, 8. Winter tubes showing discolouration and very few mites.

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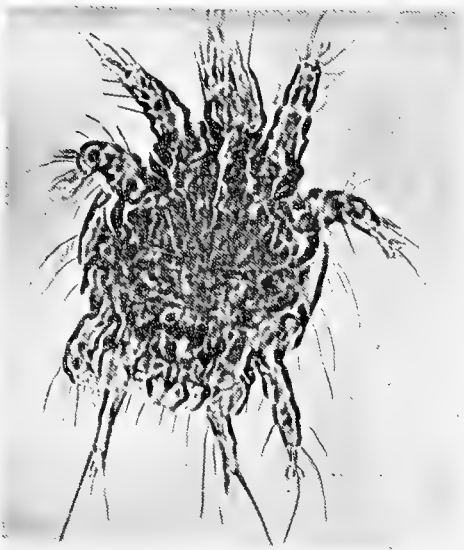

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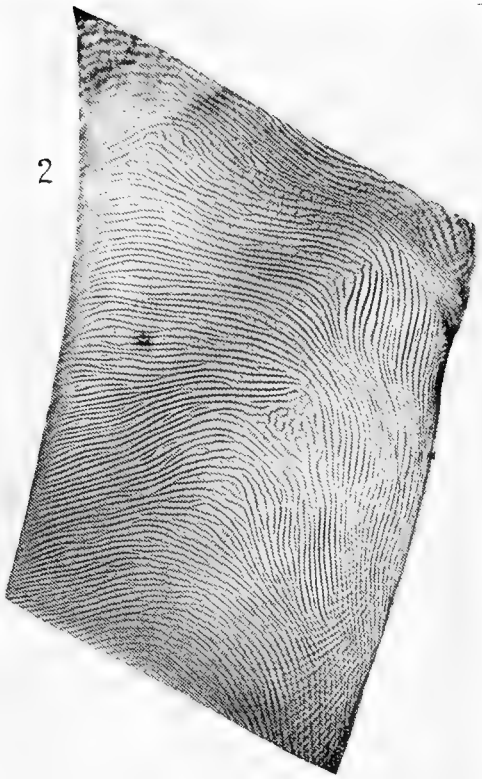

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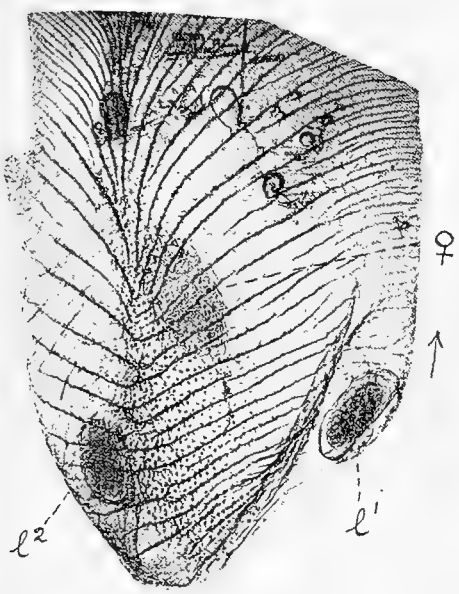

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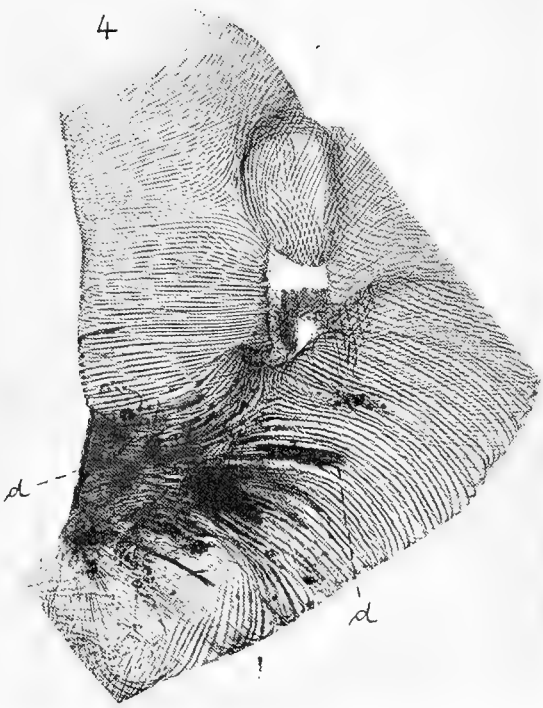


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No. 47.

NOTE ON A PHYSICAL METHOD OF SEPARATING THE FATS IN BUTTER-FAT.

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(Read April 29. Printed August 21, 1924.)

The texture of butter, an important quality in its marketing value, is controlled primarily by the character of the butter-fat and the churning temperature and also by the conditions incident to the churning, washing, setting, and working of the butter. The most suitable conditions have been arrived at empirically, but our knowledge of the actual constituents of butter-fat and their physical properties is yet insufficient to give a full scientific explanation of the process of butter-making. The work described in this note indicates a method of carrying out a physical analysis of butter and separating to some extent its constituent fats, and so studying their physical properties. As the process is slow and tedious, it has been considered desirable to give this account of some preliminary observations before undertaking a more extended investigation.

In the course of an examination of the texture of different butters, it was noticed that those butters which had the firmer texture gave a smaller grease ring on filter-paper. No information on the nature of this oily diffusing substance was found in the extensive literature on the butter industry, though the question of the wrapping-paper for butter is discussed. Apparently the subject has never been investigated. Probably as it was assumed that the fatty acids were present in butter as triglycerides, it was concluded that this substance must be olein M.P. – 4° C., or butyrin M.P. – 76° C. It is impossible, however, to separate butyrin from butter-fat (1) by solution in hot alcohol in the same way as from an artificial mixture of triglycerides. This and other experiments have led to the view that the fatty acids are combined with glycerine in butter mainly as mixed glycerides. Amberger (2) showed by examination of the portion of the hydrogenated butter-fat soluble in alcohol that the original fat contains butyrodiolein, butyro-palmito-olein, and oleodipalmitin. Of these oleodipalmitin has a melting-point of 38° C., and solidifies about 28° C. The butyro-compounds do not appear to have been isolated. Amberger concluded that the butter examined did not contain more than 3 per cent. of olein. This result is confirmed by the work described in this paper. The oil which diffuses into filter-paper is mainly a substance which solidifies between 10° and 12° C., and liquefies about 19° C. From these constants it cannot be identified with any of the glycerides recorded in Beilstein.

The initial experiment was made with unpasteurized Irish butter several weeks old. It was distributed in small pats on Whatman filter-papers previously

extracted with ether. After twenty-four hours the pats were removed, and the paper scraped clean of adhering butter, and then extracted with ether in a Soxhlet extractor. (It will be convenient to refer to the process by which the butter passes into the filter-paper as diffusion, though obviously diffusion, capillarity, and viscosity all play a part in it.) 3 grams of a yellowish oily liquid were obtained, which did not solidify when allowed to stand overnight at 15° C., but became solid when placed in a room at 10° C. The solidifying-point was determined by a cooling curve. The extract was heated to 30° C. and placed in a test-tube with a thermometer. The test-tube was separated by an air-space from a boiling tube to ensure more regular cooling. The latter was immersed in a bath containing melting ice constantly stirred. Solidification, or more precisely the clouding of the liquid, began at the bottom at 12° C. and was completed at 10° C. The cooling curve gave a well-marked stationary temperature at 10·2° C., indicating that the extract consists mainly of a single fat or a group of fats having this solidifying-point. A mixture such as butter gives no stationary temperature. The quantity of the extract was too small to make any complete examination by chemical methods. Its iodine value was found to be 41 grams per 100 grams of fat. From this it may be concluded that this extract contains oleic acid in much the same proportion as the original butter. For comparison it may be remarked that a mixed triglyceride such as butyro-palmito-olein would give an iodine value of 38 and butyrodiolein of 73.

A more systematic investigation was then instituted. Comparison of the diffusion of butter with that of butter-fat showed only a slight difference. The water and the texture of the butter have, therefore, very little influence. As it is desirable to obtain the constituents of butter as far as possible unchanged by subsequent heat-treatment, butter was used throughout these experiments. The diffusion was carried out on Whatman No. 1 filter-papers 15 cm. in diameter, which had previously been extracted with ether. On each filter-paper were placed 15 grams of butter in eighteen small cylinders 1 cm. in diameter and about 1 cm. high. After varying intervals these butter cylinders were removed, and the paper scraped and extracted with ether in a Soxhlet apparatus.

One set of observations was carried out at a temperature of 20°–21° C. which was available in the germinating chamber of the Seed Testing Division of the Department of Agriculture. At this temperature the butter became very soft, and diffusion took place rapidly. An exhaustive experiment to determine the approximate quantity of oil in the butter could, therefore, be carried out in a reasonable time. 75 grams of butter in ninety small cylinders were placed on five sheets of filter-paper, and allowed to remain in the germinating chamber for twenty-four hours. 4 grams of extract were obtained. The residue again placed on fresh filter-papers for twenty-four hours gave 3 grams of extract. Repetition of the process gave 2 grams. The time was then extended to forty-eight hours, and for the fifth and sixth extract to seventy-two hours, and later to ninety-six hours and one hundred and twenty hours. The increased time was necessary to obtain a workable extract. 20 grams of extract, representing nearly 30 per cent. of the original quantity of butter, were obtained in the course of three weeks. The solidification of this series of extracts, or rather the attainment of a solid-like rigidity with its accompanying opaqueness, took place between 11° and 12° C. The cooling curve gave no stationary temperature. On standing at 15° C., these samples solidified into a gel-like condition, and the colouring matter present became bleached. A progressive change was shown in the iodine values. The following values were obtained :—

first fraction, 37·5; third fraction, 34·8; sixth fraction, 34·0; butter-fat 32. This approximation towards the value for butter may be due in part to the incomplete removal of the butter from the paper by scraping. Assuming the presence of several substances with different rates of diffusion, it is also possible, though not immediately evident, that the composition of the extract may depend on the time of diffusion. These considerations suggested some modifications in the method of working.

The next series of experiments was accordingly arranged to obtain a more uniform extract. The time for diffusion was now reduced to three hours. The paper on which the cylinders rested was cut out of the filter-paper before extraction so as to obtain only the substance which had diffused. This extract was a golden-brown liquid which set to a butter-yellow gel-like solid on standing at 15° C., and gave a stationary temperature on the cooling curve at 11·7° C. The substance was still moist in appearance below this temperature, and when placed in a room at 10° C. it yielded an oil to filter-paper. The solidifying-point of the residue removed from the filter-paper was found to be 11·6° C. The other extracts obtained between 20° and 21° C. were also allowed to diffuse into filter-paper at 10° C., so as to obtain these extracts as free as possible from lower melting products previous to analysis.

The presence of at least two diffusing oils with different solidifying-points explains the observations made at 16° C. At this temperature 3 grams of extract were obtained in three days. It remained liquid at room temperature. On taking a cooling curve it gave a pasty mass between 12° and 10° C., and solidified completely about 5° C. The two oils were evidently present in nearly equal proportions. No chemical analysis was made on this sample.

An experiment on diffusion was also made in a cold room whose temperature varied between the limits 9° and 10° C. The process was very slow, and not more than 1 gram was obtained in three weeks from 74 grams of butter on five filter-papers. To avoid the possibility of extracting any butter particles, the paper on which the cylinders rested was cut away before insertion in the Soxhlet apparatus. A determination of the iodine value gave 82. The iodine value for olein is 86. We may infer, therefore, as one might expect, that the oil diffusing in the cold is olein, but that, in agreement with Amberger's results, the quantity present is small.

The authors are indebted to Mr. Brownlee, Agricultural Analyst of the Department of Agriculture, for an examination of the butter used in these experiments, and of the fat diffusing from it at 20° C. A sufficient quantity of the latter was obtained by combining all the extracts together, and they were partially purified by cooling them to 10° C., and absorbing the liquid present at that temperature in filter-paper. The comparative determinations include the Reichert-Wollny number, indicating the volatile fatty acids soluble in water, and the Avé-Lallemant number, which is calculated from the difference between the insoluble and soluble barium salts of the acids present. Mr. Brownlee (3) has shown that the Reichert-Wollny number has a seasonal variation for Irish butter, reaching its peak value between March and June. As the sample of butter examined was made in March, the figures obtained in the above two determinations are consequently above the average.

	Butter-fat.	Extract.
Reichert-Wollny number	31·37	35·03
Avé-Lallemant number	−37·175	−46·75
Koettstorfer's saponification value	237·02	235·62

A more complete examination was made of the extract. It gave 2·74 for the Polenske figure, which estimates the insoluble volatile fatty acids, and 29·9 for the Kirschner value, which is stated to correspond to the butyric acid. The average values for butter of the above Reichert-Wollny number are: Polenske 3·1, and Kirschner 26·0. When the diffusion had gone on for two months there were 27 grams of residue. Accordingly, allowing for 16 per cent. of water, 48 per cent. of the butter diffused into the paper at 20° C., and thus the extract constituted nearly 60 per cent. of the butter-fat. Correspondence between the two analyses is therefore to be expected. The numbers show, however, that the percentage of the butyric acid is higher than in butter, though they do not indicate that all the butyric acid is contained in the extract. In combination with the iodine values already given, they would accord with the view that the diffusing oil consisted in a great part of fats of a constitution similar to the butyro-palmito-olein mentioned by Amberger.

The interest of these experiments does not lie so much in the information they yield on the actual fats present in butter, which cannot be in any degree exact, but more in their relation to the churning temperature and consequent texture of butter. According to Hunziker the churning temperature may vary within wide limits, possibly from 5°–24° C. In the northern and central part of the dairy region in the United States the variations are confined within much narrower limits, 9°–12° C. in summer, 13°–21° C. in winter. In Ireland it is found desirable to cool the cream as low as 2·2° C., and begin churning at 6·7° C. The presence in Irish butter of a large quantity of a constituent solidifying only when cooled below 12° C. indicates the importance of low-cooling in Irish creamery practice to give the fat globules sufficient firmness before churning. Experiments require, however, to be made on a much larger scale, and over a long period to carry out the separation of the fats in sufficient quantity to determine their physical properties or their chemical composition.

Summary.

The grease ring formed around butter when placed on paper has been investigated, using fat-free filter-paper. The fat which diffused into the paper was extracted with ether. When the diffusion took place at 20° C., the extract was an oil solidifying between 10° and 12°, and liquefying about 19° C. In comparison with butter it contained a higher percentage of butyric acid, and also of unsaturated fatty acids. Almost 50 per cent. of the content of a sample of Irish butter diffused into paper at this temperature. At 10° C. the diffusion from butter was extremely slow, and the oil obtained had an iodine value corresponding to triolein. The small amount obtained and the slowness of the diffusion are in agreement with the work of Amberger, who estimated the percentage of olein present in butter as not more than 3 per cent.

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4. On a Phytophthora Parasitic on Apples which has both Amphigynous and Paragynous Antheridia; and on Allied Species which show the same Phenomenon. By H. A. Lafferty and George H. Pethybridge, Seeds and Plant Disease Division, Department of Agriculture and Technical Instruction for Ireland. (Plates I and II.) (June, 1922.)
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