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Whether blood is also formed in the placenta of other mammals must be carefully looked into. Corpuscles are figured, mixed up with maternal blood-corpuscles, by NOLF for the bat's placenta, by MAXIMOW for that of the rabbit, by SIEGENBEEK VAN HEUKELOM for that of man, with which I feel inclined to identify my „haematogonia” and of which the first and last-named author decidedly state that they are distinguished by certain characters from polynuclear leucocytes.

Neither of them, however, refers what he has observed to haematopoeisis.

In point of fact MASQUELIN and SWAEN (1880) and FROMMEL (1888) have already stated that blood is formed in the placenta respectively in the rabbit and in the bat. Their observations have up to now convinced but few and do not correspond in their details with my own. What I have myself observed in the rabbit, the hedgehog, the shrew and the mole has never emboldened me to conclude to the existence of haematopoietic processes in the placenta: it was not until I had examined the *Tarsius*-placenta in which the phenomena are so extraordinarily lucid that I was forced to draw the conclusions of which a rapid sketch was given above, but which is in no way meant to be a generalisation. Ungulates and Lemurs, certain Edentates (and probably also the Cetacea) undoubtedly miss a similar haematopoeisis. Its strong development in *Tarsius* is perhaps connected with the unfavourable relation in which the small and delicate mother finds itself placed with respect to the comparatively large foetus, while moreover each parturition is generally immediately followed by a new pregnancy, a circumstance which however exhausting its effect may be upon the mother is decidedly most favourable to the collector of embryological material.

This short account will soon be followed by a full description with plates and figures, which will appear in the Report of the Zoological Congress that was held at Cambridge in 1898.

A discussion followed in which Prof. MAC GILLAVRY and Prof. HUBRECHT took part.

Botany. — „*On a Contagium vivum fluidum causing the Spot-disease of the Tobacco-leaves*”. By Prof. M. W. BEIJERINCK.

The spot-disease of the tobacco plant, also called mosaic-disease, consists in a discoloration of the chlorophyll, spreading in little spots over the leaf and afterwards succeeded by the partly or entirely dying away of the tissue which originally composed the spots. Com-

monly the discoloration first manifests itself near the nerves of the leaf by a considerable increase of chlorophyll; afterwards the interspaces between the dark green spots are affected by a bleaching-process, which mostly does not go farther than to give a yellow hue to the sickened parts, but which in some cases, causes variegation. The dark green spots grow in the beginning more intensely than the rest of the leaf, thus becoming blown-up protuberances rising from the upper surface of the leaf. This, however, occurs oftener with artificial infection experiments than on the tobacco fields, where the diseased leaves remain flat. The third phase of the disease consists in the locally dying of little spots irregularly spread over the leaf; they get soon brown, are very brittle and even the culling of the leaf may change them into holes. They make the leaves valueless as wrappers of cigars.

Professor ADOLF MAYER pointed out in 1886 that this disease is contagious. He pressed the sap out of diseased plants, filled with it little capillar tubes, put them into healthy plants and after two or three weeks he found these to be likewise attacked by the disease.

In 1887 I endeavoured to solve the question whether any parasite might be found as cause of the disease. It was clear that, if this should really be the case, there could only be thought of bacteria, for microscopic observations had not indicated the least traces of microbes. The bacteriologic culture-methods proved that aërobic bacteria could not come into consideration for they failed as well in the tissue of the healthy as of the diseased plants, and the same holds good regarding the anaërobics. So, it was certain that here was an instance of a disease caused by a contagium fixum. This consideration induced me in 1897 to conduct new infection experiments in order to become better acquainted with the characteristics of the contagium. The chief results of these experiments are the following.

In the first place it was proved that the sap pressed from diseased plants and filtered through very dense porcelain was absolutely devoid of bacteria, without losing of its virulence. Attempts made to point out in the filtrate aërobics or anaërobics again did not give any result.

In order to answer the question whether the virus ought to be considered as corpuscular or as dissolved or liquid, some parenchyma of diseased plants rubbed fine was spread over agar-plates and then, left to diffusion. A virus, consisting of discrete particles, would needs remain on the surface of the agar and consequently be in the impossibility of rendering the agar virulent; a virus, really

dissolved in water would, on the contrary, be able to penetrate to a certain depth into the agar.

After about ten days' diffusion, a time which I considered sufficiently long in accordance with that wanted for the diffusion unto a considerable depth of diastase and trypsin, the surface of the agar-plate was first cleaned with water and then with a solution of sublimate; then the upper layer was removed by means of a sharp platinum spatula. In this way the inner part of the agar might be reached without its having any contact with the particles adhering to the surface. Infection experiments performed with these deeper layers caused as well the disease as the porcelain-filtrate. So, there seems no doubt left but the contagium must be fit for diffusion and consequently considered as fluid.

The infection experiments were performed with the expressed sap by injections with the syringe of PRAVAZ. The most proper place for injecting is the stem, and in particular the youngest parts which are still in growth. The nearer the place of injection is to the terminal bud, the sooner its consequences show themselves. This is evident from the experience that only those leaves are susceptible of infection which are still in growth and in the phase of cell-multiplication, meristems being by far the most susceptible. By making use of this fact and injecting the virus cautiously quite near to active meristems, I was of late enabled, three days since the injection, already to observe the first symptoms of the disease, whilst otherwise they must be waited for two or three weeks longer. Full-grown leaves, and even leaves whose cells are still in the phase of elongation but no more in that of multiplication, are unfit for infection.

As the quantity of virus, sufficient to produce a large number of diseased leaves, is extremely small, and as the juice of these leaves will serve to infect an unlimited number of plants, it is clear that the virus must increase within the tissues. In accordance with what is said before, this increase occurs in and with the dividing cells, the full-grown tissues of the plant not allowing any such increase. This quality of the virus reminds, to a certain extent, of the action of the cecidiogenous substances, which likewise exert their influence in those parts of plants only, which are still in a state of growth and cell-multiplication. Out of the plant it seems impossible to bring the virus to increase. This conclusion must be drawn from the fact that bougie-filtrate mixed and long kept with a certain quantity of the filtrate of juice of a healthy plant, not only does not increase but even loses in virulence in the same degree as if it had been

diluted with pure water. It is not difficult to convince oneself of this, for the quantity of the virus employed is of great influence upon the symptoms and the course of the spot-disease. If a considerable quantity of virus is at once introduced into the plant, the first diseased leaves, which develop from the bud, not only show, besides the usual symptoms, a remarkable relaxation and suspense of growth, by which they remain much smaller than normal leaves, but also deep, irregular pinnate or palmate incisions in the margin in consequence of some lateral nerves remaining short. As the chlorophyll-tissue thereby develops very imperfectly and as, especially near the nerves, the formation of chlorophyll may be quite deficient, these leaves get a peculiar striped appearance and by their shape belong to the true monstrosities¹⁾. When a small quantity of the sap is used such deformations don't appear at all, so that it seems to me that an increase in virulence of the sap under the said circumstances would not have escaped me.

Consequently I consider it as certain that the virus can only be reproduced in the manner described, with and through cell-multiplication of the plant. In my opinion this fact must be related to the fluid or dissolved state of the virus, for, with regard, to a contagium fixum, were its particles ever so small that they escaped all microscopic observation, there is no plausible reason why it might not augment, like parasitical bacteria, out of the fosterplant. It does not even appear impossible that a microscopically invisible, but notwithstanding corpuscular contagium, might occasion visible colonies on culture gelatine. A fluid virus, fit for diffusion like that of the spot-disease, would penetrate into the gelatine or the agar and, if it were fit for reproduction, it would then alter the chemical nature of the nutritive substances, which might perhaps be observed by a change of colour or of refrangibility in the plates. When „sowing” the virus on malt-extract gelatine, and on plates obtained from some plant-infusion with 2^o/_o canesugar and 10^o/_o gelatine, — according to my experience excellent culture masses for bacteria commonly occurring on plants, — such alterations could in no way be observed. Moreover, though reproduction or growth of dissolved matter is not quite inconceivable, yet, it is difficult to imagine in what way such a process might be achieved. A division-process in the molecules causing their multiplication, meets, to my mind, with great difficulties; even the conception of „molecules which take food”, would seem to me vague, if not inconsistent. The increase of the contagium now, is

¹⁾ On one of my plants such a leaf had taken the form of an ascidium.

on the contrary, partly explained by the circumstance that it must first be bound to the protoplasm of the living cell itself, and so carried on into the reproduction. In any case, two enigmas seem by this fact to a certain degree to be reduced to one ¹⁾).

If the ground in which the tobacco-plants grow is infected with the virus, one sees, after some time, the disease appear in the terminal bud. The time of the incubation varies very much and depends on the size of the plants. In smaller plants I remarked the first symptoms of the disease in the new-formed leaves of the terminal bud two weeks after the infection; in bigger ones after three to six weeks. Root and stem are in this case obliged to convey the virus often to considerable distances. Various observations prove that this conveyance goes, by way of exception, along the xylem, which conducts the water; usually, however, it seems to follow the so-called descending sap, and then it probably goes along the phloem.

That the first mentioned way may be followed, must be concluded from the order of succession in which the symptoms of the disease appear when a great quantity of the contagium is introduced into the stem, for in this case those parts of the young leaves get first diseased which are exposed to the strongest evaporation, such as the tops and margins which reach freely out of the bud. The conveyance of the virus along the phloem may be concluded from the following observation.

If one infects the middle-ribs of full-grown leaves, or of leaves whose cells are in a state of elongation but no more of cell-division, the leaves themselves continue healthy, but the virus turns back to the stem, thence, in the usual way, to infect the meristems of the buds and the youngest leaf-rudiments. This return of the virus from the leaf down to the stem must undoubtedly be by the way of the descending sap, that is along the phloem.

¹⁾ Perhaps, basing on these considerations, one may think of the possibility that enzymes, in a manner agreeing with that of the contagium fluidum, are reproduced in the cells, and might thus, to a certain extent, be considered as independently existing. Concerning this point I wish to observe the following. Pressed yeast, cultivated in nutriment containing diastase, takes from it a notable quantity of diastase which it is difficult completely to remove from it by washing. If, however, this yeast continues growing in a medium free from diastase, then the diastase soon totally disappears. That this might be attributed to incongruence between the protoplasm of the yeast-cell and the diastase-molecule, so that a persistent union might possibly be effected by other microbes or by means of the tissue-cells of higher organisms, is not probable, the yeast-cell being not absolutely free from diastatic substances, containing, for instance, some glucase. For the moment, therefore, I must consider such a conclusion as unavailable.

From out the root, infection is possible even with plants of two or more decimeters in height. If for this purpose woundings of the root are necessary, is not yet clear; probably roots may absorb the virus from the ground even through their unhurt surface. As infection only occurs in the buds and meristems, the number of healthy leaves found at the bottom of the plant, indicates in some way the date of the infection, in case the virus has entered through the root.

Without any loss of virulence the virus may be dried and in that state exist through the winter, for instance in the ground; part, however, gets lost then, just as is the case with many bacteria and yeast species. The leaves, too, keep their virulence when dried, so that the dust of the brittle leaves helps, no doubt, to spread the disease. Precipitating the virus with strong alcohol from its solution and drying the precipitate at 40° C., it remains virulent.

As was to be expected, the virus in moist state is rendered inactive, not only by boiling temperature, but already at 90° C. The lowest deadly temperature I have not been able to fix; I think it will be found between 70 or 80° C.

Above I alluded to the formation of characteristic leaf-monstrosities when a large dose of the virus is injected. Another, but rarer effect of artificial infection, is variegation or albinism. Hitherto I obtained this effect in too few plants, than that I should be able to point out how it may be expected with certainty; but I have some hopes that further experiments will enable to produce it at will.

That albinism, or at least one of the forms in which it appears, shows a certain relation to the leaf-spot disease, may be allowed already at a superficial view of the latter. However, until now, we are obliged to admit that an important difference exists between them as to the way of transferring the infection. In so far as may be concluded from the relatively few experiments concerning this point, infection for albinism requires a direct uniting, by means of grafting or budding of the variegated with the green plant. Infection, on the contrary, of green plants with the crushed tissue of variegated varieties, seems never to produce any results. My above mentioned plants, however, indicate that there must exist another way along which variegation may be called forth, namely by a virus existing outside of the plant.

Probably there are various other plant-diseases, which originate in a manner alike to that of the spot-disease of the tobacco. The disease of the peach-trees in America, described by ERWIN SMITH under the name of „Peach Yellows” and „Peach Rosette” (U. S.

Departm. of Agriculture, Farmers' Bulletin N^o. 17, Washington 1894) are, according to the description, undoubtedly caused by a contagium fluidum, but it is still dubious whether the infection is only transferred by grafting and budding, or, — which is more probable, — also by a virus existing outside of the plant.

Chemistry. — „*On congealing- and melting-phenomena in substances showing tautomerism*”. By Prof. H. W. BAKHUIS ROOZEBOOM.

The latest discoveries on tautomerism, which have shown, that tautomeric substances in the liquid state must be considered as mixtures of two kinds of molecules of different structure, have raised the problem how to explain the complicate congealing- and melting-phenomena of such substances, in case both forms or one of them can appear in the solid state.

Some remarkable investigations on this subject have of late been made by BANCROFT and his disciples, which were a continuation of a theory of DUHEM.

At an attempt to unravel the investigations of CLAISEN on this subject, the reader had come to the same conclusions, which may be united to a perfectly clear graphic sketch.

BANCROFT having already published this, there would be no reason to revert to the subject, if not all examples chosen by him, referred to cases in which all the melting- and congealing-points were found in the region of temperatures in which equilibrium is still obtained between the two forms in the liquid state.

In such a case we generally have the disadvantage of there being no certainty about the mixing-proportion of the two substances at the moment of melting or congealing. Consequently it is impossible to give quantitative representations.

To arrive at a good understanding of the phenomena, it is therefore desirable to begin with a deduction of the conduct of tautomeric substances, the congealing temperatures of which are below the temperature-limit where in the liquid state transformation between two forms is still possible.

If we call the two forms α and β , we may build up a sketch in which the mixing proportion of α and β is measured on the horizontal axis of 0—100 and the temperature on the vertical axis.

According to the supposition made above, the congealing appears in the ordinary and simplest form of the congealing of mixtures of two substances i. e. starting from the melting points A and B of the two modifications, we have two melting-lines AC and BC