I

THE NATURE AND RELATIONS OF THE INTRACELLULAR INCLUSIONS PRESENT IN THE MOSAIC OF TOBACCO

(WITH AN APPENDIX AND 2 PLATES)

BY

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II

CYTOLOGICAL ASPECTS OF THE VIRUS DISEASES IN PLANTS

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I.

The Nature and Relations of the Intracellular Inclusions present in the Mosaic of Tobacco.

by

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The association of amoeboid bodies with the tobacco-mosaic is now too well known to require any explanation. After their first discovery by IWANOWSKI (1903) their presence in the diseased plants was confirmed by DELACROIX (1906), DICKSON (1922), KUNKEL (1922), PALM (1922), RAWLINS and JOHNSON (1925), GOLDSTEIN (1924—1926), SMITH, F. F. (1926), HOGGAN (1927), and KLEBAHN (1926). Along with these bodies certain crystal inclusions are also present in the diseased cells of the plant. The nature of all these intracellular inclusions has remained to be solved for the greater part. The present work was undertaken to clear up this question to a certain extent.

METHOD.

It was thought necessary to avoid the use of certain fixing ingredients such as alcohol, ether, acetic acid &c., which have proved to be fatal to these inclusions. The Kolatchev method was found to be extremely suitable for the purpose. Small pieces of healthy and diseased tobacco stem and of certain other plants such as Petunia, Tomato, Hyoscyamus and Solanum nigrum, on which tobacco mosaic was transferred by grafting were fixed in a solution of equal parts of 6 % potassium bichromate, 1 % chromic acid, and 2 % osmic acid for from 24 to 48 hours. They were then washed for about twelve hours in running water, and being passed through distilled water several times, were put for a week at 30° C. in 2 % osmic acid. Being again washed for about twelve hours in running water, they were dehydrated and embedded in paraffin as usual. Sections were cut from 8—10 μ thick. Mounts were made directly without any further staining.

In this way the protoplasm and nuclei were stained uniform pale yellow, the crystalloid material deep yellow and the vacuolate bodies dark. The inclusions were found to exist only in the affected plants.

THE VACUOLATE BODIES.

GOLDSTEIN (7) has distinguished these bodies as x-bodies. By other workers they have been known as vacuolate, or amoeboid bodies. Most of the observations made so far were limited to the leaves or epidermal tissue of the diseased plants. With modifications in their form they can be easily found in any of the tissues of the diseased plant. They were found in all the six types of tobacco mosaic distinguished by GOLDSTEIN (8); and though no special mention of them is made in the sixth, the irregular narrow-nervisequum type, they seem to be present in it. In her „pale, definitely blotched type" (the fifth) „these bodies were found to be present in the companion cells, phloem and xylem parenchyma cells of the small anastomosing veins of the leaf". The only exception made is that of cells between two vascular bundles. KLEBAHN (13) did not find them in the older cells of the epidermis, mesophyll and the vascular tissue, neither in certain mosaic tobacco plants. The only explanation that can be offered is that the mosaic on these plants might have been of some other type. My observations have led me to the conclusion that they can be found in any diseased part of the plant.

These vacuolate bodies can be easily seen under living conditions (7). They are hyaline in nature, granular in structure, formed of a denser protoplasmalike substance, rounded or slightly oval, more generally pear-shaped and very susceptible to nuclear dyes. A confusion with a degenerated form of nucleus is very likely to occur (12). Under fixed and stained conditions they are very easy to be distinguished from the nucleus especially by their vacuolar structure. They are deeply stained by the osmic acid and contain numerous vacuoles of different sizes within them. The whole body is stained homogeneously blueish dark by the osmic acid with the exception of the vacuoles. Sometimes a granular substance lightly stained is found to be present in these vacuoles.

The usual size of these bodies (figs. 13, 19, pl. 2) found in the inner tissue, becomes smaller and smaller towards the peripheral tissue of the epiderm, hairs &c. and they appear finally as minute, deeply stained globules (fig. 1, pl. 1). Same gradation in form was observed by GOLDSTEIN (7). PALM (34) has supposed these extremely small bodies to be the causal agency. I have found them present in the packets of crystals (fig. 2, pl. 1). They are figured in the striated crystals by KLEBAHN (13) and named „miculae". In a little larger form they have one vacuole (fig. 1c, 3, pl. 1) in the granular substance. Their substance is not of the same nature as the plasm of the host as it proves to be greatly osmiophile. In this respect these bodies approach the mitochondria or certain other cell constituents, known to be greatly osmiophile in their nature.

They have an autonomous movement. Blunt pseudopodia are often thrown out by the smaller bodies and when this is the case the vacuole may be extended into it (fig. 3a, 6, pl. 1). In forms of ordinary size, more than one pseudopodium of fine threadlike nature are given out (fig. 4a, b, 5, pl. 1). GOLDSTEIN (7, 8) has observed the blunt pseudopodia and attributes the movement of these bodies partly to them, and partly to the currant of the protoplasm. SMITH (41) signifies it only by the protoplasmic currant. A fish in the currant of water may be carried by the force of medium in which it lies, but to deny its autonomous movement would be rather hazardous. The presence of the pseudopodia finally settles this question in favour of the autonomous movement of these bodies. It will be difficult to see the movement of these pseudopodia under living condition as they are of a fine nature. Occasionally a whole body may be seen to be prolonging itself into a long pseudopodiumlike protuberance with more vacuoles and granular structure in the front end (fig. 6, pl. 1). The corpuscles can render themselves flexible enough to permeate through the cellwall. In figure 7, pl. 1 can be seen a body thus traversing through the cellmembrane; instances of such sort are very common to be met with.

The vacuolate nature of these bodies is unanimously settled. Commonly one but often several vacuoles, giving a foamlike appearance to the body are present (8). I have never observed only one vacuole except in smaller forms; the increase in size is invariably accompanied by the addition of vacuoles, which become so numerous that they can hardly be counted. I have observed them on the periphery of these bodies (fig. 10, pl. 1) contrary to the remarks of GOLDSTEIN. In a granular, slightly stained form, there sometimes occurs a dark lining membrane around these vacuoles (fig. 8a, b, pl. 1) as has already been observed (8). A limiting membrane is also present and can only be rendered visible when the body being in a degenerating condition (fig. 9, pl. 1) is lightly stained. SMITH (41) and GOLDSTEIN (8), have seen it, the body being under plasmolytic conditions during the observations of the former.

The average dimensions of these bodies are 6 $\times$ 4 μ which corresponds exactly with those found by KLEBAHN (13). The largest body found measured 10 $\times$ 7 μ (fig. 10, pl. 1). A body of almost the same size curved in the middle is shown in figure 11, (pl. 1).

In the woodvessels these bodies have their vacuoles much enlarged and these vacuoles then occupy most of their part. Their component structure becomes so thin that here only they have more resemblance to a foamy mass (fig. 12, pl. 1).

A cluster of these bodies is seen surrounding the nucleus from time to time (fig. 13, pl. 2) and they even seem to pass over this apparent obstacle in their way as can be made out from their traces left; but no influence of degenerating nature can ever be found exerted by them on the nucleus. Nor were they strictly in association with the nucleus, an observation contrary to that of RAWLINS and JOHNSON (38). My observations here coincide with those of GOLDSTEIN (8) and SMITH (41). It may be that the nucleus may show a temporary distortion on account of the pressure of an adjoining body; but it can replace itself as soon as this foreign body is removed from it.

In their movements these bodies may leave behind them a certain part of their plasma (fig. 14, pl. 1, fig. 15, pl. 2). When this is the case the detached portion of the plasma seems to regenerate itself into a new body. Figures 16a and b, represent different sorts of fissions in these bodies. Regeneration through fission, therefore, seems also to take place. Division by constriction is very common and the bridging plasma thread joining the two individuals may remain attached for a long time (fig. 18 a, b, pl. 2). Figure 18a shows a third animalcule developing from the middle of such a linking plasma. GOLDSTEIN (8) gives a hardly convincing figure (N°. 7) of fission in her plate 29. SMITH (41) never seems to have observed it.

Conjugation was observed to be common. Two bodies approach each other (fig. 19a, b, pl. 2) and finally fuse together (fig. 20a, b, pl. 2). A rather abnormal mode of fusion is shown in figure 21 (pl. 2). To what effect their conjugation leads, I was unable to decide; but sporulation may seem most likely to occur from it. Figures 22a and b (pl. 2) show small granules formed in a body. They seem to be also formed in vacuoles (8). In the traces of the movement they are often seen to be left behind (fig. 15, pl. 2). It is very likely that these granules are spread all over the epidermal tissue. In figure 17 (pl. 2) a fission can be said to be occurring in an encysted form. These encystments are found occasionally and the new forms may be said to be regenerating from their pieces through fissions.

Every part of the bodyplasm or the granules such formed can give rise to a new animalcule under favourable conditions, and infection is very easy to occur.

THE CRYSTALS.

In their movements these bodies secrete certain substances (fig. 23a, b, pl. 2). Under living conditions I have occasionally seen a body in connection with small acicular crystals. Contrary to the observations of RAWLINS and JOHNSON (38) in very young epidermal tissue of the leaf, showing pale patches, the amoeboid bodies appeared prior to the formations of any crystalloid substances. The most probable chance is, therefore, that these secretions, first taking acicular form, go finally to construct the polyhedral plates, presenting striated nature under a different vision. In very old cells again these crystals break up and small fine fibrils are formed joining in a crescent shaped crystal (fig. 2, pl. 1). All these crystals can seperate themselves into their fundamental acicular shape if acted upon by certain acid or basic compounds (41). It is clear, therefore, that whatever their form may be the component substance of all the crystals is the same.

These crystals are so closely associated with this type of Tobacco mosaic that in fact it would not be out of place here to suggest them as the criterion for this disease (provisionally termed Tobacco Virus 1, after JOHNSON).

In my experiments, Tobacco mosaic was transferred by grafts on *Lycopersicon esculentum, Petunia hybrida, Solanum nigrum* and *Hyoscyamus niger*. When distinct external signs of shrinking, islet formations &c. had appeared on the leaves a cytological examination of the epidermal tissue and hairs exhibited the presence of these crystals. In the summer of 1928 a few tubers of potato variety Rural New Yorker infected with Tobacco mosaic and sent by Dr. K. M. FERNOW of the Cornell University, Ithaca, N.Y. were growing in the experimental fields of this laboratory. The shoots from these tubers were extremely dwarfed (not higher than a few inches); the leaves were very small and showed islet formation and shrinking. All the types of the crystals were found to be present in the affected cells.

Some of the types of these crystals (polyhedral and striped) have been observed in Tomato, Petunia, Datura, Physalis, Capsicum and several other Solanaceous hosts (5, 8, 10, 13).

The fibres of the crescent shaped crystals were discovered by GOLDSTEIN (8) and studied in detail by KLEBAHN (13). Both of them are unanimous in declairing their presence in old cells from which other inclusions have disappeared. The last or crescent shape of these crystals is found only in old, yellowed leaves. But it is not rare to find all these sorts of crystals in the same cell of an approximately young age.

The nature of these crystals had remained a mystery for a long time. IWANOWSKI (12) and DICKSON (5) have called them waxy plates and they were explained (after EWART) as the product of chlorophyll degeneration combined with the changed plastid protoplasm (5). DICKSON further remarks that there is a great reduction in the amount of protoplasm, leaving a larger central vacuole than usual after the appearance of the platelike crystals. RAWLINS and JOHNSON (38) find them always in contact with the nucleus. On account of this close connection between the two they were supposed to be the product of the diseased cell or the effect of the causal agent on the cell plasma.

My observations have convinced me that they are secreted by the vacuolate bodies. Much light has been thrown on their chemical nature recently by KLEBAHN (13) and consequently they prove to be of albuminoid material. Crystals of the same nature in spindle form have been also found in mosaic and intercostal mosaic of potato and have been proposed to be taken as particular signs of these diseases (13). Such crystals in striated form have already been figured in *Cecropia peltata* and *Prosimum macrocarpum* and the crescent shaped ones have been found in Epiphyllum and Opuntia species (31). Their relation to mosaic diseases if any in these plants has not yet been ascertained.

I have tried the following tests for the reactions and my results confirm those arrived at by KLEBAHN (13).

Fresh and fixed material was used. Fixed material was prepared in absolute alcohol preferably mixed with 5 % tartaric acid, which dissolves oily, resinous and other material, and helps to coagulate the albuminoid one. Formol-acetic-alcohol was also used. The advantage of this latter is that it renders the material soft, so that the epidermis can be easily scraped out. Material put in alcohol-tartaric acid is extremely brittle.

If the epidermis of the fresh material is first fixed by dropping into absolute alcohol saturated with picric acid for some hours, then washed in absolute alcohol, put into saturated solution of eosine in alcohol, for a few minutes, again washed in absolute alcohol and at once mounted with a drop of Canada balsm, the crystalloid substances take a deep red colour.

A blue stain is obtained by these crystals if the sections are mordanced for an hour in acetic-potassium-ferrocyanide solution (3 gr. potassium ferrocyanide, 10 c.c. acetic acid, 80 c.c. water), then washed in water, and dipped into a solution of ferric chloride. (The Prussian blue test of Zacharias).

Lastly by the action of potassium iodine (5 gr. potassium iodine, 1 g. iodine, 100 c.c. water), phosphomolybdic acid (90 gr. distilled water, 5 gr. concentrated nitric acid, 1 gr. sodium phosphomolybdat, left for a week and then filtered) or concentrated nitric acid, they are stained yellow.

FREIBERG (6) has found that it is probable that an excess of albuminoid material exists in the lighter areas of the mosaiced tobacco plants.

Such albuminoid material occurs also in the nuclei; and it seems probable that the square and oblong intranuclear plates (10), found associated with mild tobacco mosaic on several solanaceous hosts, bleaching mosaic on pepper, tomato and tobacco mosaic on henbane may prove of the same nature. The association of these intranuclear crystals with the mosaic disease is rather doubtful as they are already described in many plants such as *Melampyrum arvense, Russelia juncea, Candollea adnata, Polypodium caespitosum, Campanula trachelium* etc. (31). Holmes (11) observed some conspicuous spheres in the bodies in *Hippeastrum equestre.* He has compared them with similar structures in the nerve cells in rabies and the intranuclear ones in human small pox. It will be of interest to know if these prove to be of protein type as well.

THE INTER-RELATION OF THE VACUOLATE BODIES.

Such vacuolate bodies have been found in several other plants affected with mosaic or similar diseases. Distinction has been tried to be made according to the monocotyledonous or dicotyledonous type of the plant. The general nature of these bodies is so strikingly similar in both these types that such a distinction is in my opinion unnecessary. To give a general idea about their existence in different plants, the following list is made according to these two types of the phanerogams in which they have been observed. In the monocotyledons they were studied in sugarcane (2, 3, 15, 20, 30), Hippeastrum (2, 11, 16, 27), corn (2, 14), wheat (28), Eucharis (2), sorghum (2), and Soudan grass (2); among the dicotyledons they were observed in tobacco (4, 5, 7, 8, 10, 12, 13, 16, 34, 38, 41), potato (10, 42), Dahlia (9), *Evonymus japonica* (41), *Phytolacca decandra* (41), Chinese cabbage (16), strawberry (35), sugarbeet (38), grapevine (36), sandal and red periwinkle (32). However in the following discussion no such distinction will be followed.[1])

Although in the beginning, the study of these corpuscles was limited to the pale yellow blotches of the leaves of affected plants and the gall tissue of the Fiji Disease of sugarcane, their existence, later on, was found by no means restricted to these parts. I have found them present in all the tissues of the tobacco plant and Mc KINNEY *et al* of the wheat. They were observed in leafsheaths, stem phloem, and adjoining tissue, procambial strands, tracheids (sugarcane) in parenchymatous tissue of the stalk, outer portions of the cob and its pith and certain fibrovascular bundles (corn), stomatal cells, trichomes, and epidermal tissue of almost all the affected plants. In the growing parts of Hippeastrum, KUNKEL found them and in those of Dahlia, GOLDSTEIN studied them. HOGGAN has found them all over the leaf lamina except the fibrovascular bundles. COOK fails to find them in roots while Mc KINNEY *et al*, GOLDSTEIN, RAWLINS and PLAKIDAS have observed them in those parts in wheat, tobacco, sugarbeet and strawberry respectively. KUNKEL saw them dividing within the cells in the process of division while COOK never finds such a thing. BREMER found them present in the E K 44 and Hawaii 211 varieties of sugarcane affected with the stripe disease but lacked them completely in eight varieties of sugarcane and one corn plant affected with sugarcane mosaic. They may or may not be found in heavily diseased plants of Hippeastrum (11). Only in one instance namely in Dahlia, GOLDSTEIN found such a body intranuclearly present.

From the above statements one is confused about the exact position of these bodies in the plants. More exact studies are required to clear up all these points. The lack of these individuals in certain cells or

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[1]) Miss ECKERSON in her paper on „An organism of tomato mosaic" (Contr. Boyce Thomp. Inst. for Plant Res. 1 : 3, 109—114, 19—22 pl.) represents some flagellate organisms found in Hippeastrum Johnsonii, Dahlia, and Squash. I have not considered them here as they seem to be of a different type, though GOLDSTEIN seems to think them as like organisms under crushed conditions.

parts can only be explained for the present by their not being affected. However, their complete absence in certain plants giving external signs of disease remains yet inexplicable. It may be due to defective fixing or „to some lack of conditions sufficient to cause the mottling but not enough for the formation of inclusions" (26).

They are one or more in a cell (Mc WHORTER quotes from 1 to 6 in Fiji disease of sugar cane) and their position is not at a fixed point; they may lie anywhere in the cell. Often they are said to be in close contact with the nucleus and in corn they are found only so. If closely attached to the nucleus, by their pressure the nucleus naturally gets distorted. In corn and *Hippeastrum Johnsonii* they have been found clustered round the nucleus. I have equally observed them so in tobacco. But this position is rather accidental. There does not seem any special attraction between them and the nucleus. Sometimes they completely surround the nucleus (corn, wheat), when they are larger than the nucleus. In Petunia, yellow mosaic of tobacco produced bodies considerably larger in size (10). This Petunia mosaic lacked in the crystalloid material and therefore is considered to be of another type than the tobacco number I. The bodies in potato (42) are evidently larger than the nucleus and HOGGAN points them out as twice the size of the host nucleus. Their passing over the nucleus, apparently a temporary obstacle in their way, can be traced by the marks left on it.

It has been suggested that they influence the degeneration of the host nuclei (10). I do not think it probable that they exert any direct influence on the nuclei. The affected cell may get degenerated owing to old age. Had they any such effect on the cell nucleus, it would in my opinion have appeared in the very beginning. When studied in growing points no change in mitosis has consequently been observed (9).

The form of these bodies is very variable and differs according to the nature of disease as it seems. They are larger than the nucleus in corn, wheat, Hippeastrum, Petunia and smaller in tobacco, and sugar cane.

Generally irregular, oval or round, pearshaped, spherical but never so in corn (see KUNKEL 14), coarsely reticulate, minute in early stages but growing larger in severely diseased cells and more or less amoebae-like has been defined as the structure of these corpuscles. I have found them in all these shapes in tobacco. Unfortunately attention is not always given to the measurement of these bodies. In tobacco they are found to be of $6 \times 4\ \mu$, and in sugar cane $25-30 \times 5\ \mu$. The bodies in wheat as measured from the figures proved to be of $12 \times$

10 μ dimensions (28). The longest was 110 μ in length and the smallest only 3 $\times$ 3 μ. The form of these bodies varies according to the nature of the disease and if care is taken to measure them, it will prove of much help to ascertain the nature of similar diseases in plants of resembling characters. KUNKEL finds the bodies in corn larger than those of sugar cane and consequently thinks that disease to be different from that of sugarcane.

They have got a dense plasma having granular structure and can thus be very easily distinguished from the surrounding cell contents under living conditions. Old bodies are less dense. They get deeply stained. A distinguishing character of these bodies is that all of them contain vacuoles. Their number may differ from one to many and they are scattered all over their plasma. Some deepstaining rings are occasionally seen round these vacuoles along with deepstaining granules with radiations. The nature of this plasma seems to me very similar to that of the mitochondria. POLITIS (36) may have very likely taken the brown corpuscles found in the grape vine affected with the „browning (brunissure) disease" for simple transformations of the granular mitochondria. As no figures are given, nothing could be said definitely about the bodies described by him.

A membrane is often present, although on account of the deepstaining property of the plasma it can be detected only when the degeneration in the body proceeds on account of the age.

A veil-like structure was found to be attached to some of these bodies in corn (14).

An autonomous movement is present in them as long fine pseudopodia with a wavy outline and more hyaline structure than the rest of the body are shown to be thrown out; the body itself may get into a long pseudopodiumlike protuberance (wheat, sugar cane, tobacco and dahlia). Mc KINNEY has compared the movement in the wheat bodies with that of mitochondria.

Some deeply staining granules are always found existing within the plasma of these bodies, but they may or may not be evident (14). These are especially visible, when the bodies are lightly stained. Their affinities to nuclei are very doubtful and workers on the line have never taken them so. The absence of nuclei has long served as a strong argument against their being living organisms. The plasma of these bodies is so densely chromatophile that it would be very likely to think such material scattered all through it. Certain lower organisms have been known to lack the nuclear material or to have it mixed up completely with the cellular contents of the organism. Recently a special search was made in the bodies found in *Hippeastrum equestre* to find out the presence of such material if any (11). Only fat globules and some chondriosomes could be recognized. BREMER (2) has equally found fat globules in Hippeastrum bodies he studied.

Although not observed by some workers, division seems to be very commonly present. It has been for certain observed in tobacco, sugar cane and dahlia. Along with the cell division it takes place and this seems to be one of the ways of providing infection to the new cells. Mc WHORTER has observed amitotic division in the bodies of sugar cane (30). Clefts are produced in the bodies of sugar cane and tobacco. Breaking of the body into several chromidial filaments is common in sugar cane (30). The particles thus given out may seem to regenerate and produce new organisms.

Encystment does take place. A rather thick membrane is produced round the body and the plasma gets concentrated into it. There are few vacuoles in the encysted bodies as compared to the free ones. Mc WHORTER seems to have got new organisms through germinations from these cysts. An interesting sort of encystment is described in corn by KUNKEL. In this case a long body was divided into several cysts, kept separated by the shrinking of the plasma in the middle places.

I have found sporulation in tobacco. Innumerable minute globules are formed within the body which getting loose, are spread all over the host and serve for new infection.

PALM (34), MATZ (23, 24) and REINKING (39) have classed these granular corpuscles under chlamydozoa, and BEWLEY (1) has found some small granular forms in tomato mosaic cultures. These have been defined by them as causal organisms. PLAKIDAS (35) in strawberry xanthosis says „in what appears to be the final stage in the process of degeneration, the remnant of the nucleus and the bodies disappear and the entire cell is filled up with amorphous, somewhat granular material, staining black with haematoxyline". HOGGANS (10) found it possible „to trace back the x-bodies to very small forms which appeared always in close contact with the host nucleus, irregular, different shaped and without any vacuoles". All these granular bodies seem to have arisen from the sporulation of the vacuolate bodies.

Accompanying these bodies BREMER (2) observed another type of spindle shaped, striped bodies, single or grouped together in a mass.

PLAKIDAS (35) finds definitely spherical bodies along with the x-ones, in strawberry cells affected with xanthosis. In addition these extra-bodies seem to have a membrane. He has named them y-bodies after RAWLINS (37) who has distinguished them so in the root-tips of beet plants affected with curlytop disease. We are not for the present in a position to decide the nature of these extra corpuscles found in the cells of the affected plants.

HOGGANS has observed another type of material in all the virus diseases studied except calico, leaf roll and streak. It is brownish in nature and sometimes occurs in the cells containing the bodies. It seems like degenerated cytoplasm from the figures. No connection between this material and the vacuolate bodies can as yet be said to be convincing.

So far as is known to the present writer the crystalloid material has been found accompanying these bodies in the tobacco mosaic (number 1). It proves to be a special secreting capacity of the specific bodies, confined to that mosaic. It is seen in connection with yellow tobacco mosaic and medium tobacco mosaic (10) but these diseases may prove exactly similar to the ordinary tobacco mosaic (number 1) as external symptoms can hardly be trusted in such diseases.

NATURE OF THE VACUOLATE BODIES.

About the nature of the vacuolate bodies found in plants affected with mosaic or like diseases, there has been much difference of opinion. IWANOWSKI (12) introduced the idea about their arising as the effect of the virus on the diseased cytoplasm or nuclei. Several later investigators on different plant diseases of the same type have been closely adhering to this theory, their principal argument being (a) the want of nuclear material in these bodies, (b) their scarcity or complete absence in certain affected plants, (c) their movement being only due to cytoplasmic currant. The want of nuclear material cannot prove the invalidity of an organism. Several plants and animals of the lower type have been known to lack such material completely or have it scattered all through the cytoplasm. Scarcity as discovered by COOK and complete voidity must be mostly depending on the nature of infection of the plant or of the organism existing under another form. Much work is required on this line to be able to come to some final conclusion. About the nature of their movement there remains hardly any more to be said.

It is time now to remind the scientists interested that this *effect of the cause* theory has been our long acquaintance. Time was there when fungi were taken as not the cause but the effect of the disease. It is opportune now to change the idea about these bodies as effect as there can be no doubt about their animal nature on account of (a) their presence (in large number in certain tissues) in the affected parts of the plant, (b) movement, (c) reproduction, and (d) encystment.

This article is restricted to the plant diseases; similar diseases are known to affect animals and man. Certain inclusions have also been reported as belonging to small pox, rabies, foot and mouth and other diseases. I have seen certain of these preparations through the kindness of Dr. J. P. BIJL Head of the department of bacteriology in the State Central Laboratory for Public Health (Utrecht) and his staff. No similarity could be detected in the nature of these inclusions. Nor as yet have been seen in the animal diseases several phases of these organisms as discovered in plants. MAGROU (22) has denied the causal nature of the so called chlamydozoa on account of their being found in the intoxicated nerve cells of the animals. No evidence has yet come forward of such findings in the intoxicated tissues of plants.

It has already been said that every portion of the plasma of these animalcules is capable of regenerating under favourable conditions. Recent investigations go to favour this theory as certain bacterial solutions are found to pass through very minute filters (19) and keep their power of regeneration. The dense plasma or the minute granular form can render itself so elastic as to pass through these filters.

Attempts have been made several times to name these organisms. PALM (34) calling the minute granules chlamydozoa, named the forms in tobacco *„Strongyloplasma Iwanowski"*; MATZ (23) later on considered the granular organisms in sugarcane of the same nature. This nomenclature cannot be accepted as it refers to a phase in the life history of the principal organisms — *the vacuolate bodies.*

LYON (20) has named the bodies found in the Fiji galls of sugar cane *Northiella sacchari.* No diagnosis was given and the genus was created simply because he had obtained the material from Mr. NORTH. It is out of question to accept this name as well.

Mc WHORTER (30) has been more systematic in diagnosing and suggesting the name of Phytamoeba for the genus. But the bodies although resembling amoebae in their form are different from them by the deficiency of nuclei, by the diffusion of chromidial material all through their plasma and by the much higher resistance for heat, certain chemicals and drying. Nor are the amoebae filterable. Besides amoebae are found in plants (33). The name *Phytamoeba* is considered therefore to be misleading. On account of the uniform vacuolate nature (which is the chief characteristic feature amongst them) I suggest for the genus the name:

Vacuolarium n. gen.:

Parasitic, intracellular, organisms with dense, hyaline plasma of the nature of mitochondria; membrane present but may be invisible under certain conditions; one or several vacuoles; round, oval, pear or irregular shaped; no nuclear structure detectable; minute chromidial granules sometimes present; producing pseudopodia; regenerating by *division, fission, encystment, sporulation* or from any part of the plasma; of a filterable nature, and having a particular thermal death point for the whole organism or its component particles.

Animalcule occurring in several plants, producing chlorosis, stripe, gall and other severely infectious diseases, commonly known as virus diseases.

Vacuolarium Iwanowski n. sp.

Round, pear shaped or oval organisms; one to many vacuoles; pseudopodia very fine; reproduction by division, fission, encystment, sporulation; size $6 \times 4\ \mu$, and secreting albuminoid material, forming crystals of polyhedral, striated or crescent shape. The thermal death point is at 90° C for 10 minutes.

In tobacco and other solanaceous plants.

SUMMARY.

1. Vacuolate bodies have been found in all the phases of their life in the tobacco stem affected with tobacco mosaic number I. They were also found in Tomato, Petunia, Hyoscyamus and Solanum nigrum affected with the same mosaic.
2. Crystals found accompanying these diseases are formed from the albuminoid material secreted by the vacuolate body. They vary from polyhedral, striated to crescent form according to the age of the diseased part.
3. No nuclear material was found present in the vacuolate bodies.
4. These bodies prove to be of mitochondrial structure and regenerate by division, fission, encystment and sporulation. They are considered to be of animal nature.

5. The term *Vacuolarium* is proposed for the genus; *V. Iwanowski* is the name given to the specific organism causing tobacco mosaic number I.

I wish to express my great indebtedness to Professor H. M. QUANJER, without whose constant encouragement this work would have been impossible.

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APPENDIX.

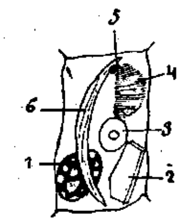
A TABULAR INDEX TO VACUOLATE BODIES FOUND IN DIFFERENT PLANTS.

I. MONOCOTYLEDONS.

Host.	Variety.	Year.	Dimensions.	Author.	Remarks.
1. Saccharum officinarum . .		1910		Lyon	Fiji Disease of galls. The first figure of the bodies in sugar cane was given. Called it *Northiella sacchari.*
		1919 1922	less than 1 μ	Matz	Mosaic and yellow stripe: these are minute granular bodies and seem to be the last phase of the organism.
		1921		Reinking	Fiji Disease.
		1922	seldom more than 12 μ	Mc. Whorter	Calls it *Phytamoeba sacchari.*
	Demerara 117 " 625 " 1135 New Guinea 111 " 118 Badila yellow Caledonia	1924	25 — 30 × 5 μ	Kunkel	Fiji Disease and Mosaic.
		1925		Cook	Mosaic. Very scarce.
	E K 44 Hawaii 211	1926		Bremer	Stripe. Not found in E K 28 D T 52 247 B S W 3 Tjeweng Payaman Soerat Payaman Ratoe II Bali Betoeng I Rokan Kapoer I Rokan Mia Bau Mia Tim.
2. Zea Mays		1921		Kunkel	As the bodies are larger than those

					of sugar cane. Failed to find the bodies in one of the three plants on which the disease was transferred.
3. Andropogon sorghum . .		1926		Bremer	Stripe.
4. Andropogon halepensis . .		1926		Bremer	Stripe.
5. Triticum sativum	Harvest Queen	1923		Mc. Kinney, Eckerson and Webb	Mosaic and Rosette diseases. Both the diseases due to one causal agent.
6. Hippeastrum equestre . .		1922 1924		Kunkel	Probably the same disease as found in tulips, narcissi, and hyacinths.
		1926		Bremer	In addition found some striped bodies.
		1928		Holmes	A search for nuclear material, which was not found.
7. Hippeastrum Johnsonii . .		1923		M. Kinney, Eckerson and Webb	
8. Eucharis amazonica . .		1926		Bremer	Stripe.

II. DICOTYLEDONS.



1. Nicotiana Tabacum . . .		1903		Iwanowski	Index cell to tobacco mosaic (number I).
		1906		Delacroix	1. Vacuolate body.
		1922		Palm	2. Polyhedral crystal.
		1922		Dickson	3. Nucleus.
		1924		Kunkel	4. Striated body.
		1924 1926		Goldstein	5. Micula. 6. Crescent crystal.

Host.	Variety.	Year.	Dimensions.	Author.	Remarks.
		1925		Rawlins and Johnson	All these three types of inclusions found in my graft-transmitted mosaic on Tomato
		1926		Smith, F. F.	Petunia
		1927		Hoggans	Henbane and Solanum nigrum
		1928	$6 \times 4\ \mu$	Klebahn	Potato Rural New Yorker.
					The other sorts of inclusions were found by other workers in the following plants in addition to the above ones.
					Capsicum annuum.
					Datura.
					Physalis alkekengi.
					" pubescens.
					" franchetti.
					Nicandra physaloides.
					Solanum aculeatissimum.
					" laciniatum.
					" miniatum.
					" atropurpureum.
					" cabiliense argentum.
					" marginatum
					" pyracanthum.
					" tuberosum variety Green Mountain.
					It is proposed to take these bodies as an index to this sort of mosaic, called Tobacco Mosaic I.

	Green Mountain				Yellow tobacco mosaic.
	" "				Crinkle mosaic.
	Green Monntain Bliss Triumph Early Ohio	1927		Hoggans	Rugose mosaic.
	Bliss Triumph				" "
	American Wonder				Supermild mosaic.
	" "				Mild mosaic.
	Rural New Yorker				Montana crinkle.
	Green Mountain				Leaf rolling mosaic.
					No such material was found with calico and leafroll on Burbank and streak on Bliss Triumph.
4. Vitis sp.		1921		Politis	Browning disease. Thinks them to be transformations of mitochondria.
5. Brassica pekinensis . . .		1924		Kunkel	Mosaic.
6. Beta vulgaris		1926		Rawlins	In addition other sorts of more regular bodies, named as y-bodies are mentioned.
7. Evonymus japonica . . .	1. Argentio-variegata 2. Mediopicta	1926		Smith, F. F.	Infectious chlorosis.
8. Phytolacca decandra. . .		1926		Smith, F. F.	In several other chloroses which were not of an infectious nature, no bodies were found.
9. Fragaria sp.		1927		Plakidas	Xanthosis (yellows) y-bodies of Rawlins were found.
10. Dahlia sp.		1927		Goldstein	Dwarfing and mosaic.
11. Santalum album		1928		Narasimhan	Spike disease.
12. Vinca rosea					Mosaic.

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REFERAAT.

DE AARD EN BETEEKENIS VAN DE INTRACELLULAIRE LICHAAMPJES, DIE VOORKOMEN BIJ DE MOZAIEKZIEKTE DER TABAK.

Door IWANOWSKI en verschillende andere auteurs, genoemd in de inleiding, zijn amoebeachtige, soms ook op kristallen gelijkende lichaampjes in de cellen van mozaiekzieke tabak gevonden. De schrijver onderzocht zieke Tabak en Tomaat, Petunia, Hyoscyamus en Solanum nigrum, die met tabaksmozaiek door enting waren geïnfecteerd, na fixatie met kaliumbichromaat, chroomzuur en osmiumzuur, waarbij deze lichaampjes een zeer donkere kleur aannemen. Hij vond ze in alle duidelijk zieke deelen der plant; niet in gezonde planten. In de weefsels aan den omtrek, zijn zij kleiner dan in het centrum; de allerkleinste zijn dezelfde lichaampjes, die KLEBAHN „miculae" noemde. De lichaampjes bewegen zich door middel van pseudopodia, zij kunnen zich daarmee door celwanden heenboren (P. 1, fig. 7). Zij zijn van vacuolen voorzien; de kleinere lichaampjes hebben slechts één vacuole, de kleinste geen enkele. De afmetingen der grootere lichaampjes zijn $6 \times 4\ \mu$, soms iets grooter (Pl. 1, fig. 10 en 11); die, welke in de houtvaten voorkomen, zijn grooter en van wijder vacuolen voorzien (Pl. 1, fig. 12). De lichaampjes zelf, zoowel als de vacuolen, hebben fijne membranen (Pl. 1, fig. 8 en 9). Soms raken zij de celkern (Pl. 2, fig. 13) of liggen er om heen, evenwel zonder die aan te tasten. Bij hun bewegingen laten zij fijne deeltjes achter (Pl. 1, fig. 14, Pl. 2, fig. 15), die weer tot nieuwe lichaampjes schijnen te kunnen uitgroeien, soms vormen zij cysten (fig. 16), soms scheiden zij zich daarbij in twee lichaampjes (Pl. 2, fig. 17 en 18). De niet geëncysteerde lichaampjes deelen zich ook en kunnen daarbij door een plasmadraad verbonden blijven (Pl. 2, fig. 18). Conjugatie komt voor (Pl. 2, fig. 19 en 20). In de lichaampjes en de vacuolen worden zeer kleine korreltjes gevormd (Pl. 2, fig. 21 en 22).

Ook worden andere op kristallen gelijkende lichaampjes bij het tabaksmozaiek gevonden. Deze „kristalloiden" zijn eerst langgerekt (Pl. 2, fig. 23); later vindt men polyedrische plaatjes, die van de kant gezien een gestreepte massa vormen; in zeer oude cellen zijn deze kristallen weer tot fijne vezeltjes uiteengevallen, die zich vereenigen tot een sikkelvormig voorwerp. Wanneer er op de kristalloide complexen zuren of alcaliën inwerken, vallen zij tot de langgerekte vormen uiteen. Deze kristallen zijn voor het tabaksmozaiek (Tobacco virus, 1 JOHNSON) typisch, bij de meeste andere mozaiekziekten komen zij niet voor. Men kan ze vinden bij de Solanaceae, die met dit virus besmet zijn, zoodra de uitwendige symptonen zichtbaar worden. Volgens KLEBAHN zijn zij opgebouwd uit albuminoid materiaal. De schrijver kan dit bevestigen.

De van vacuolen voorziene lichaampjes zijn gevonden zoowel bij viruszieke exemplaren van monocotylen als van dicotylen (opsomming in de tabel, zie appendix). De schrijver vond ze bij mozaiekzieke tabak niet alleen in de bleeke, maar ook in de groene plekken en in alle weefsels.

De schrijver toont op grond van gegevens, aan de literatuur ontleend, aan, dat er kleine verschillen o. a. in grootte bestaan tusschen de intercellulaire lichaampjes bij verschillende plantensoorten; zoo zijn zij b.v. grooter bij mais, Hippeastrum en Petunia en kleiner bij tabak en suikerriet. Maar alle bevatten zij vacuolen. Cysten kunnen gevormd worden. De fijne korreltjes worden als voortplantingslichaampjes beschouwd, de kristalloïde lichaampjes schijnen zich te vormen uit de afscheidingsproducten der vacuolen-lichaampjes.

Het ontbreken van kernen in de vacuolen-lichaampjes heeft er aan doen twijfelen, of zij organismen zijn; de schrijver beschouwt ze als kleine dieren op grond van hun plasticiteit en hun vermogen zich voort te bewegen en zich te reproduceeren. Hij wijst op de gelijkenis met de lichaampjes gevonden bij pokken, hondsdolheid, mond- en klauwzeer en andere dierlijke ziekten, welke door de welwillendheid van Dr. BIJL en zijn assistenten bestudeerd konden worden aan het Rijks Centraal Laboratorium voor de Volksgezondheid. In den vorm van de fijne korreltjes moet het zijn, dat het organisme van de mozaiekziekte der tabak bacteriefilters kan passeeren. De naam *Strongyloplasma Iwanowski* Palm kan niet geaccepteerd worden daar hij gegeven is naar de afgescheiden koreltjes. De diertjes zijn bij alle virusziekten, waar men ze aantreft, in de eerste plaats gekenmerkt door vacuolen, reden waarom de schrijver de geslachtsnaam *Vacuolarium* invoert, die beter is dan de door Mc WHORTER voorgestelde naam *Phytamoeba*. Het organisme toch is geen amoeba. Het artikel eindigt met de diagnose van het geslacht en van de soort *V. Iwanowski* n. sp., het „Tobacco virus 1, Johnson".

EXPLANATIONS OF PLATES.

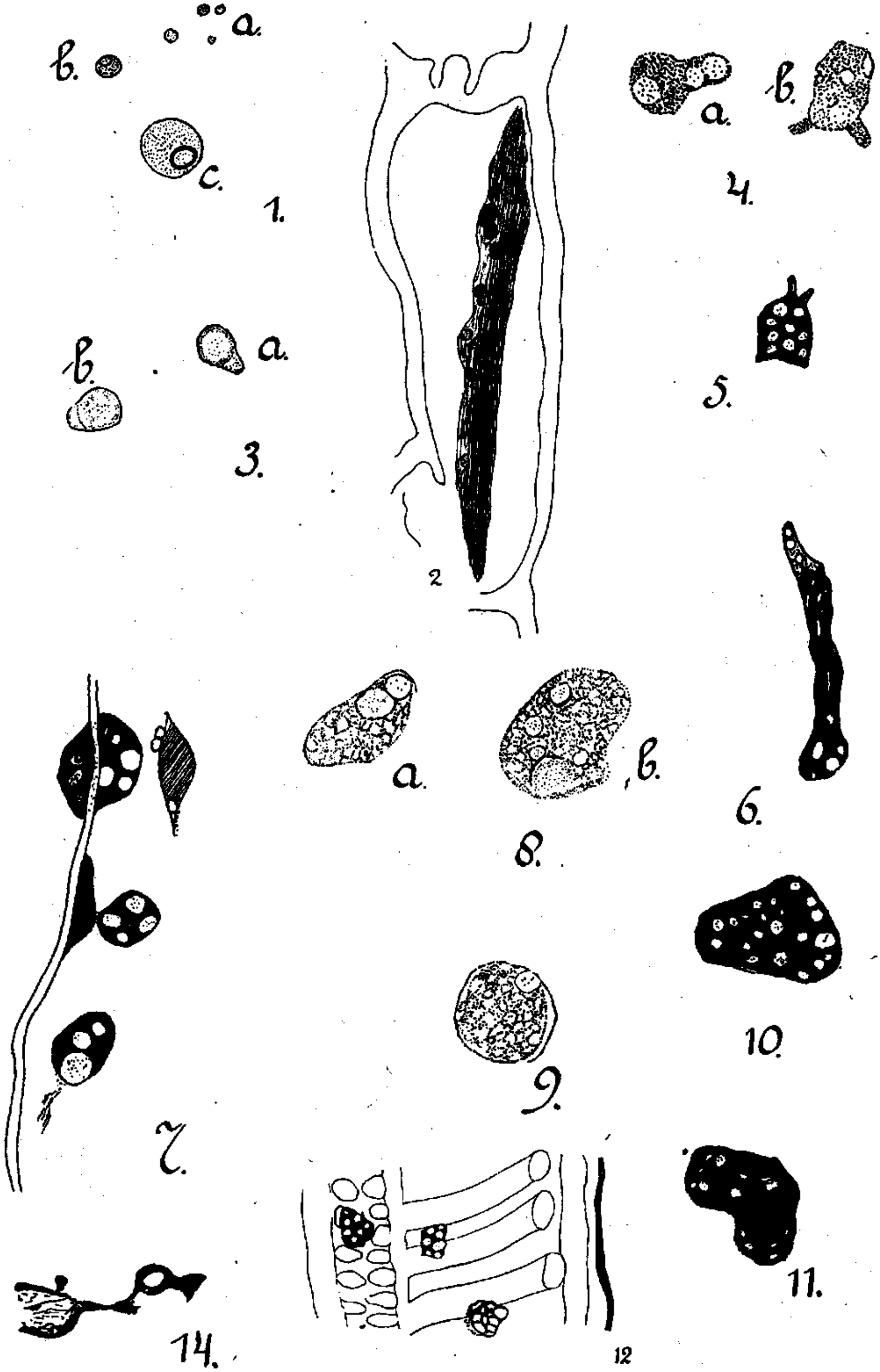
All figures were drawn with a camera lucida and the magnification is 1350. Figures 2 and 12 were reduced to half their size.

PLATE I.

Fig. 1 a, b.	Minute globular bodies found towards the external periphery.
c.	A larger body with one vacuole.
Fig. 2.	Crescent shaped crystal with 'miculae' in it.
Figs. 3—6.	Bodies giving out pseudopodia.
Fig. 7.	A vacuolate body passing through the cell membrane.
Fig. 8 a, b.	Aged bodies with granular plasma and lined vacuoles.
Fig. 9.	A body surrounded by a cell membrane.
Figs. 10—11.	Large sized bodies.
Fig. 12.	Foamy natured bodies found in the vascular tissue.
Fig. 14.	A body leaving a plasma trace behind.

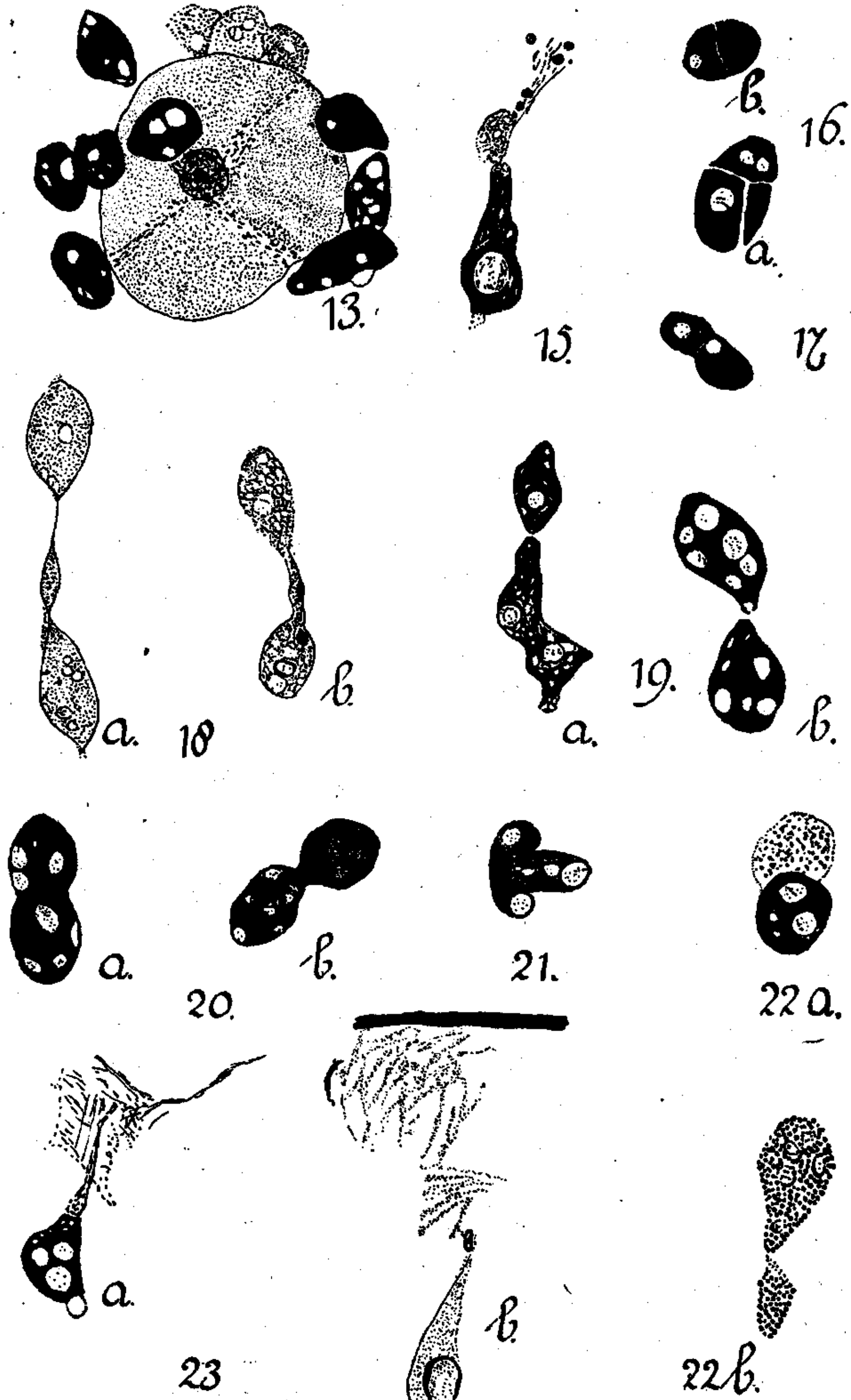
PLATE II.

Fig. 13.	A cluster of vacuolate bodies surrounding a nucleus and while passing over it show traces of their way.
Fig. 15.	Granular bodies left in its traces by a vacuolate body.
Figs. 16 a, b:	17. Fissions.
Fig. 18 a. b.	Plasma linkage between dividing bodies.
Fig. 19 a, b.	Two bodies approaching for fusion.
Fig. 20 a, b.	Fusion.
Fig. 21.	An abnormal fusion.
Fig. 22 a. b.	Sporulation.
Fig. 23 a, b.	Albuminoid secretions left by the vacuolate bodies.



V. LIKHITÉ. del.

Vacuolarium Iwanowski n. sp.



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Vacuolarium Iwanowski n. sp.