These experiments show, that the blood from the pancreatic vein at first exalts the N. metabolism and then lowers it again. Under these circumstances it would not be proper to dwell any longer on these contrary effects, as we are unable to account for them satisfactorily.

It also appears that the blood favours the permeability of the kidney tubules for sugar.

This experience throws light on the contrary results in our preceding series of experiments in which the glucosuria was disproportionate to the sugar content in the blood. It would seem then that the secretum of the pancreas subserves the function of the kidney as well as the glycogenesis of the liver. Indeed, this has also been admitted by DE MEYER and others, however, on a different basis and just the other way about. DE MEYER held that the internal secretum of the pancreas *prevented* sugar from passing through the kidney. It must be borne in mind, however, that DE MEYER experimented with artificial renal circulations, which readily lead to paradoxical phenomena.

Summary.

1. Secretin decreases the amount of sugar in the blood.

2. The blood that has passed through the pancreas, is capable of neutralising the action of levorotatory suprarenin on the sugar content in the blood. In this study no effort has been made to detect whether this action is due to a diminished splitting of the glycogen in the liver or perhaps to an increase in the formation of glycogen. Presumably the activity of this blood (internal secretum) is furthered by the injection of secretin. The secretum is thermostable and is soluble in alcohol. These results are perfectly concordant with DE MEIJER's experience.

3. Our experience that the secretum favours the permeability of the kidney for glucose instead of lessening it, clashes with the results of DE MEIJER's investigations.

Rotterdam, Dec. 1912.

Botany. — "*On the nucleolus and karyokinesis in Zygnema*". By Prof. C. VAN WISSELINGH. (Communicated by Prof. J. W. MOLL).

(Communicated in the meeting of April 25, 1913).

Whilst *Spirogyra* has very often been used for the investigation of the nucleus and nuclear division, *Zygnema* has so far as I know, up to the present only been studied for this purpose by two investigators. It should be no cause for surprise that the latter alga has

generally been neglected. The dimensions of the cells and nuclei are so much smaller than those in the larger species of *Spirogyra*, that one would expect to meet with still greater difficulties in a karyokinetic investigation than would arise in the case of *Spirogyra*. Such is indeed the case and in studying karyokinesis I have not been able to trace the details of the process to the same extent as in different species of the genus *Spirogyra*.

MABEL L. MERRIMAN [1]) was the first to study karyokinesis in *Zygnema*. She could not with certainty identify the species studied, because she had no zygospores at her disposal. The chief results of her inquiry were as follows.

She found in the nucleus of *Zygnema* no body that corresponds to the nucleoli of higher plants. There is in the middle of the nucleus a central body that is composed of the greater portion of the cromatin-granules, whilst the rest of the chromatin-granules is situated in the peripheral network between the central body and the nuclear membrane. During karyokinesis the central body splits into many small ones, whilst the granules in the network increase in size. In this way there are formed 20 or more mostly loose chromosomes. A spirem is not formed. The chromosomes come to lie in a ring round the centre. The nuclear membrane dissolves. Then the chromosomes approach one another, and unite into 4 to 6 tetrads or groups of four, which become arranged in two parallel planes, lying close together. The chromosomes of these two planes separate. No longitudinal splitting takes place. The groups of four now divide into smaller groups, which form two rings. Thereupon the central body is formed, composed usually of the greater number of the chromosomes. A nuclear membrane also appears again. Daughter nuclei with many tetrahedral granules, with several masses and with a single mass are observable.

EUD. ESCOYEZ [2]) investigated the nucleus and karyokinesis in a species of *Zygnema*, which he believes to have been different from that studied by Miss MERRIMAN, but which he could not identify.

His results are entirely different from those of Miss MERRIMAN. He states that in the resting nucleus, there can be distinguished a network, an ordinary nucleolus and a nuclear membrane. Rarely there are two nucleoli in the nucleus. The nucleolus, according to ESCOYEZ, lies in a cavity (cavité périnucléolaire) which is surrounded by a

[1]) MABEL L. MERRIMAN, Nuclear division in Zygnema, Reprinted from The Botanical Gazette, 41, Jan. 1906, p. 43—53.

[2]) EUD. ESCOYEZ, Le Noyau et la Caryocinèse chez le Zygnema, Extrait de la Revue "La Cellule", t. XXIV, 2d fasc. 1907, p. 355—367.

very thin membrane. He thinks it possible, however, that this cavity is formed in the fixing. He describes the nucleolus as mostly spherical and homogeneous; in some cases it shows an irregular, very aberrant form.

ESCOYEZ states that during the prophase of karyokinesis thicker parts arise in the network which shows a looser structure. Finally 30 to 40 chromosomes are formed which resemble rodlets.

The chromosomes arise directly from the network which does not first form a spirem. The nucleolus plays no morphological part in the formation of chromosomes. Its shape undergoes modification and finally it completely dissolves. ESCOYEZ states that the nuclear spindle penetrates into the nuclear-cavity and that the chromosomes subsequently form an equatorial ring. Then longitudinal splitting takes place, the chromosome halves take up a position near the two poles of the nuclear spindle, which are found near the chromatophores. They crowd together into plate-shaped bodies Later they again become visible to the number of 30 to 40. Gradually a network forms which corresponds with that of the resting nucleus. ESCOYEZ says that the nucleolus is first a small body which gradually increases in size. Its formation is independent of the chromosomes.

The object of ESCOYEZ's investigation was not only to control Miss MERRIMAN's results, which diverge greatly from those generally obtained in karyokinetic inquiry, but he wished also to answer the question whether *Zygnema* so far as the nucleolus and karyokinesis are concerned, agrees with *Spirogyra* where according to ESCOYEZ J BERGHS [1]) has established that the twelve chromosomes arise exclusively derived from the nucleolus. As is already evident from the above, ESCOYEZ's investigation yielded negative results on both points. His results differ widely from those of Miss MERRIMAN and also from those of BERGHS obtained with *Spirogyra*.

With regard to the latter, I remark, that the opinions of investigators on the nucleolus and karyokinesis of *Spirogyra* are very divergent and that weighty objections can be advanced against the conclusions of BERGHS in particular [2]).

The object of my own inquiry was to answer the question concerning the agreement of the two genera in respect of the nucleoli and karyokinesis, of which I had already made a complete study in five species of *Spirogyra*. The results obtained with three thick

[1]) J. BERGHS, Le Noyau et la Cinèse chez le Spirogyra, Extrait de la Revue "La Cellule", t. XXIII, 1ei fasc. 1906, p. 55—85.

[2]) C VAN WISSELINGH, Ueber die Karyokinese bei Oedogonium, Beihefte zum Botan Centralblatt. Bd. XXIII (1907), Abt. I. pag. 152 and foll.

species have been already described [1]), those with two thinner ones are still to be published.

The species of *Zygnema* which I examined and of which I also had the zygospores, I identified as *Zygnema cruciatum*.

The method of investigation was mainly the same as that which I had previously used with good results in the case of *Spirogyra* and other plants, namely, fixing with FLEMMING's mixture and treating with chromic acid. I modified the method slightly so as to facilitate the investigation of *Zygnema*. The many globules of fat in the cytoplasm sometimes greatly hinder the investigation of the nuclei. For this reason I fixed with absolute alcohol, then left the material for some days in ether, transferred it again to absolute alcohol and replaced this by distilled water. Finally the material was placed in FLEMMING's mixture, in which it remained some days, until the treatment with chromic acid yielded the desired result, namely, slow solution of the cytoplasm and chromatophores and isolation of the nucleus. The latter afterwards gradually dissolves, during which process some of the more resistant parts become very clearly visible. No contraction or coalescence of the protoplast should occur. Should this happen as the result of a faulty application of the method, the material is useless. The strength of the chromic acid solution, which I applied was 10 or 25%. Sometimes the chromic acid, when it had acted sufficiently was washed out with distilled water and the preparations were stained blue by means of "Brilliantblau extra grunlich".

The preliminary treatment with alcohol and ether was advantageous and unaccompanied by any drawback. The troublesome fat was got rid of and it seemed to me that the fixation of the nuclei in *Zygnema* was even better than by the direct action of FLEMMING's mixture. I did not see the cavity round the nucleolus, which ESCOYEZ named cavité périnucléolaire, and regarding the existence of which in the living object there are also differences of opinion in other cases. Therefore I assume that no such cavity occurs in the living material and this agrees with the results I obtained previously with other plants.

Resting nucleus. The nucleus is situated in the middle of the cell between the two chromatophores and is stretched longitudinally. The following parts can be distinguished in the resting nucleus: the nuclear membrane, the network composed of small granular bodies

[1]) C. VAN WISSELINGH, Ueber den Nucleolus von Spirogyra, Bot Zeitung 1898, Heft XI/XII, p. 195. — Ueber Kernteilung bei Spirogyra, Flora, 1900, 87. Bd. 4. Heft, p. 355. — Untersuchungen über Spirogyra, Bot. Zeitung, 1902, Heft VI, p. 115. — Ueber abnormale Kernteilung, Bot. Zeitung, 1903, Heft X/XII p. 201.

united by fine threads, and the nucleolus. I never saw a resting nucleus with two nucleoli. There is nothing special to say about the nuclear membrane and the nuclear network. With regard to the latter there is here as little reason as in other cases for assuming that the granules and the connecting threads are chemically different.

The nucleolus calls for special attention. Superficial observation would lead to the assumption that it is an almost spherical body, about which nothing special can be said. More exact observation, even before the action of chromic acid, shows that sometimes two small points on the nucleolus can be distinguished. During the action of chromic acid they become much more visible and are seen to be small bodies which sometimes resemble rodlets. They are situated on the periphery of the nucleolus, usually opposite one another and seem often half immersed in the principal mass. According as a more concentrated or weaker solution of chromic acid is used, the nuclear network or the main mass of the nucleolus dissolves first. In either case, however, the two small bodies show a longer resistance. During the process of dissolution it can be seen that the two small bodies are united by a thread which generally runs across the nucleolus and is straight or slightly bent, but which may also be much curved. When the nuclear network has dissolved and the chromic acid has also had a strong solvent action on the main mass of the nucleolus, the thread which unites the two small bodies can be distinguished, and when the preparations are further stained with "Brilliantblau extra grünlich", all is still more clearly and more easily visible. The two corpuscles are stained dark-blue, the thread, which unites them, is paler and the rest of the nucleolus is light blue. After more prolonged action of the chromic acid the thread with the two corpuscles alone is still present; after a still longer action only the latter are found and finally these also are seen to have dissolved. This can all be seen with special clearness after staining with "Brilliantblau extra grunlich."

In every preparation that I made, the above observations were confirmed dozens of times, so that I am in no doubt that the nucleolus in *Zygnema* differs in type from that of the higher plants, and resembles the nucleolus of *Spirogyra* in having a peculiar structure. In *Spirogyra* there are two convoluted threads or a threadwork or network, in *Zygnema cruciatum* there are two short corpuscles united by a thread or indeed a thread with two thickened ends.

I consider the main mass of the nucleolus in *Zygnema* as identical with the substance which occurs in *Spirogyra* together with the threads or the thread- or network in the nucleolus. I have not been

able to answer the question whether the nucleolus in *Zygnema*, like that of *Spirogyra*, possesses a membrane.

A few nucleoli did not seem to correspond to the above description, although I cannot definitely state, that these were aberrant. I have never met with very abnormal nucleoli such as ESCOYEZ observed in the resting nucleus so that the question occurs to me whether such nucleoli do not arise in fixing. like the perinucleolar cavity or whether perhaps they may be of a pathological nature.

Karyokinesis. In investigating karyokinesis in *Zygnema* the first question concerned the behaviour of the thread with tickened ends in the nucleolus, for in *Spirogyra crassa* I was able to determine without interruption the karyokinetic changes in the two nucleolar threads. In *Zygnema* I have not succeeded in doing this. Observation in this case must necessarily be so much more minute and is accompanied by so many more difficulties, that after a few futile attempts I was obliged to abandon the study of the changes in the nucleolar thread. I am unable therefore to give any further information about this important point.

At the beginning of karyokinesis the nuclear network has a somewhat coarser and looser structure; everywhere there arise by aggregation portions which are much thickened, whilst the meshes become wider. The nucleolus acquires an irregular shape and seems to dissolve completely. By further aggregation of the nuclear network threads are formed, resembling strings of pearls. The nuclear-wall is then still visible. In later stages it is dissolved and the network has formed a number of short, thick corpuscles, which are connected together by thin filaments. Meanwhile there arises from the cytoplasm gathered round the nucleus a well developed nuclear spindle, whose pointed poles extend to the chromatophores. The nuclear network now moves back more and more into the equatorial plane, so that finally there is in the centre of the nuclear spindle a flat round disc surrounded by the spindle-fibres. This is the nuclear-plate. It is composed of a number of small bodies resembling short thick pieces of thread or lumps which are joined to each other by fine threads, or they may be intimately connected or completely united. Their number cannot be determined. Clearly visible and well-formed chromosomes, such as occur in some *Spirogyra* species to the number of 12 or 6, are not found in *Zygnema*, but there is no great objection to calling the small, short bodies of the nuclear plate chromosomes, in agreement with the usual nomenclature. The mass out of which the nuclear plate is composed appears noticeably smaller than that of the network of the resting nucleus.

The nuclear plate of *Zygnema cruciatum* is not ring-shaped. Miss MERRIMAN and ESCOYEZ believe that they have seen annular nuclear-plates in *Zygnema*, but I think this in a visual delusion. When a nuclear-plate is seen edge-ways it may appear as if it were a ring, but if looked at afterwards from the side, as is possible in using the chromic acid method, all doubt immediately vanishes. I came to the same conclusion with *Closterium* [1]), in which LAUTERBORN [2]) had described a ring-shaped nuclear-plate.

The nuclear-plate divides by longitudinal splitting into two halves which separate. At first their structure becomes more dense. When, by the use of chromic acid, they have been isolated and fall over, they somewhat resemble round discs which appear spotted in consequence of local differences in density. These halves of the nuclear-plate develop into daughter-nuclei which acquire a membrane and consequently show a sharp outline. It is difficult to say when the nuclear membrane reappears. The dense structure of the halves of the nuclear plate again gives place to a looser one and finally there is again spread out within the nuclear-membrane a fine network, which resembles that of the resting nucleus. During the entire process of karyokinesis the nuclear network forms a coherent whole. When the structure becomes looser again, the nucleoli also quickly appear. At first there can be distinguished in the network many small masses more or less globular and irregular, which gradually unite into several larger masses and finally form one single spherical mass in the centre of the nucleus. This representation of the origin of the nucleolus differs very much from that given by ESCOYEZ, but it agrees with what has been observed in *Spirogyra* where also many nucleoli flow together into a single one.

There is a further point to be noted concerning the development and position of the daughter nuclei in *Zygnema cruciatum*. In *Spirogyra* and other cases the halves of the nuclear plate take up a position near the poles of the spindle where they develop into daughter-nuclei. In *Zygnema cruciatum* the development takes place earlier. Before the poles have been reached, the daughter-nuclei have already a membrane and consequently show a sharp outline, whilst the nucleoli have already united into several larger masses. The spindle-fibres lie immediately against the daughter-nuclei. Between the halves of the nuclear plate, the spindle greatly increases in

[1]) C. VAN WISSELINGH, Ueber Kernstruktur und Kernteilung bei Closterium, Beih. zum Bot. Centralbl., Bd. XXIV (1912), Abt. I. p. 429.

[2]) R. LAUTERBORN, Untersuchungen über Bau, Kernteilung und Bewegung der Diatomeen, 1896. Fig. 68.

circumference through its fibres becoming longer and bending outwards. At the end of karyokinesis the spindle moves away and often turns so that its longitudinal axis makes an angle with that of the cell. The daughter-nuclei move against the chromatophores which divide into two. They take up a position between the new chromatophores of the daughter-cells. The transverse wall develops in the same way as in *Spirogyra*. It arises at the longitudinal wall and grows inwards until the cell is divided into two. The division of the chromatophores generally occurs after cellular division, but it is not limited to the latter, for cells with 3 or 4 chromatophores are sometimes met with.

Summary of results.

1. In *Zygnema cruciatum* as in *Spirogyra* the nucleolus has a peculiar structure. It contains a thread with two thickened ends or indeed two corpuscles, which are united by a thinner thread.

2. The nucleolus dissolves when karyokinesis begins. I cannot say with certainty whether, as in *Spirogyra*, there remain behind morphological elements, which play a part in karyokinesis, but I consider this probable.

3. There is no perinucleolar cavity (cavité périnucléolaire of Escoyez) in living specimens of *Zygnema cruciatum*.

4. In *Zygnema* the chromosomes, short threadlike pieces or lumps, arise from the nuclear network, as Escoyez also assumes and not from the nucleolus, as Miss Merriman imagines.

5. During karyokinesis the chromosomes remain continually united.

6. In *Zygnema cruciatum* the chromosomes do not form tetrads as Miss Merriman claims to have established in this genus.

7. The nuclear membrane dissolves, but this is not accompanied by a penetration of the spindle-fibres into the nucleus or nuclear cavity.

8. The halves of the nuclear-plate arise, as Escoyez also assumes, through longitudinal splitting of the nuclear-plate, and not through the grouping of the chromosomes in two parallel planes without splitting, as Miss Merriman maintains.

9. In *Zygnema cruciatum* the nuclear plate is disc-shaped and not annular as Miss Merriman and Escoyez believe they have seen in *Zygnema*.

10. The network of the daughter-nuclei develops from the halves of the nuclear plate.

11. The nucleolus is formed by the coalescence of many smaller ones to a single body.

12. In *Zygnema cruciatum* the halves of the nuclear plate develop already to daughter-nuclei within the nuclear spindle, and before they reach the poles. That part of the spindle which lies between the daughter-nuclei increases in circumference, so that the spindle becomes peculiar in shape.

The results obtained by Miss MERRIMAN, ESCOYEZ and myself differ very greatly. This may partly be ascribed to the circumstance that different species of *Zygnema* were investigated. To a much greater extent the differences must be assigned to other causes, in particular to a different interpretation of observations. The observations themselves however, are also sometimes different and perhaps not always complete. Also the fixing agent and the method of investigation may contribute to the divergence between the results of different investigators. ESCOYEZ, for example, observed a perinucleolar cavity, whilst I, using an other method of fixation observed no such cavity. Miss MERRIMAN and ESCOYEZ both believe they have seen ring-shaped nuclear-plates, whilst I came to a different conclusion, using a method by which the nuclear-plates could be observed edgeways as well as sideways.

It seems to me desirable that the various investigators should endeavour to complete their observations on *Zygnema*, and extend them to more species and also should apply different methods of investigation. Exchange of material might also be very useful. Some such action would be conducive to agreement, which will not be readily obtained by other means.

Physiology. — *"On the change in the permeability of the red blood corpuscles (also in man)". (A contribution to the knowledge of chlorine-retention in fever* [1]). By I. SNAPPER. (Communicated by Prof. HAMBURGER).

(Communicated in the meeting of April 25, 1912).

It has been known for a long time that in a number of febrile diseases an important change in the excretion of chlorine is to be observed. Under normal circumstances, all the chlorine which is taken up with the food leaves the body within 24 hours, not so, however, in the above mentioned diseases; though the patients take daily 5 or 6 grammes of NaCl, only some hundreds of milligrammes

[1]) A detailed account of these researches will be published in the Biochemische Zeitschrift, and in the Zeitschrift für Klinische Medizin.