1000 or less of methyleneblue, the pigment penetrates through the seed coat into the germ, which partly colours blue. The germroot takes up the colour the earliest; then follows a triangular field on the outer of the two seedlobes, which lie folded up in the seed. The base of the triangle, which colours first and most intensely, lies at that margin of the cotyledo, which is turned towards the germroot.

Obviously the pigment has very quickly penetrated through the micropyle of the seed, and only later through the seed coat. With stronger methyleneblue solutions the experiments do not succeed much better, because then the pigment accumulates so much in the seed coat, that even water can only enter with difficulty. After 24 hours such seeds are but imperfectly swollen but, somewhat later, the germination takes place as well. The coloured germs swell at 30° C. so vigorously, that many soon burst out of the seed coat. When the partly blue germs, freed from the seed coat, germinate on filterpaper, they yield part of their pigment to it, but especially in the meristem of the germroot it continues to show for several days and disappears only at length, by the dilution which accompanies the growth. It is then easy to see how the part near the rootmeristem grows the most rapidly whilst the region of the roothairs grows no more at all.

That the pigment, without killing the cells, has penetrated into the inner part of the tissues, is not only shown by the germroots, but also by the coloured spots of the seedlobes, whose phloembundles even have taken up the colour.

Botany. — *"On Karyokinesis in Eunotia major Rabenh."*. By Prof. C. VAN WISSELINGH. (Communicated by Prof. MOLL).

(Communicated in the meeting of November 30, 1912).

LAUTERBORN'S [1]) detailed investigation on Diatomaceae suddenly brought about in 1896 a complete change in our knowledge of the karyokinesis of these organisms. This investigator studied the process in Surirella calcarata, Nitzschia sigmoidea, Pleurosigma attenuatum, Pinnularia oblonga, and Pinnularia viridis. He came to the conclusion that the nuclei always divide karyokinetically. The karyokinesis here is not less complex than in higher plants. It shows an important deviation. For LAUTERBORN found that in all cases during karyoki-

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

nesis a body appears, which plays an important part, namely the central spindle (Zentralspindel), a body which does not occur in higher plants, but is specially found in Diatomaceae. During karyokinesis the nucleolus and the nuclear membrane disappear. The network forms a skein (Knäuel) and by segmentation the chromosomes arise out of it. They are long and well-formed. In Nitzschia 16 appear and more in Surirella. In the middle the chromosomes form a ring round the central spindle. By division of this ring there arise two rings which separate from each other along the central spindle. Each of these rings consists of the halves of the chromosomes. The daughter nuclei develop from the rings.

Shortly after LAUTERBORN a paper was published by KLEBAHN [1]) on karyokinesis in Rhopalodia gibba (Ehrenb.) O. Müller. He describes the diaster stage and mentions the central spindle and the chromosomes which to the number of 5 or 6 are placed in a circle and are granular in shape.

Some years later KARSTEN [2]) described in detail the karyokinesis of Surirella saxonica. In general his results agree with those of LAUTERBORN; in one point however they disagree greatly, for KARSTEN found the chromosomes in Surirella calcarata and other Diatomaceae short and of irregular shape in complete contrast with the observations of LAUTERBORN.

In October 1903 I found an Eunotia in a ditch near Steenwijk. After further examination and consultation of the descriptions and drawings of the various species [3]), I assumed that the specimen found was Eunotia major Rabenh. In the healthy, although not plentiful, material I saw numerous stages of karyokinesis, and since this phenomenon had not yet been described in Eunotia, I determined to utilise this opportunity of studying it.

The living object was first investigated, and then material which had been fixed with FLEMMING's mixture. In order to study the karyokinetic figures better I treated the fixed material with a solution of chromic acid of 20°/₀. Various constituents of the cell-contents successively dissolve in it and finally there remains inside the siliceous skeleton of the cell-wall, when the cells contain no fatty oil, only the nuclear network, or what results from it. The prepa-

[1]) H. KLEBAHN, Beiträge zur Kenntnis der Auxosporenbildung, I. Rhopalodia gibba (Ehrenb.) O. Müller, Pringsheim's Jahrb. f. wiss. Bot. Bd. 29, 1896, p. 595.

[2]) G. KARSTEN, Die Auxosporenbildung der Gattungen Cocconeïs, Surirella und Cymatopleura, Flora, 1900, Bd. 87. p. 253.

[3]) L. DIPPEL, Diatomeen der Rhein-Mainebene, 1905, p. 125.
VAN HEURCK, Traité des Diatomées, p. 298.

rations can easily be washed with water and stained, for example, with Brillantblau extra grünlich. The nuclear network, the nuclear plate or its halves which all fall over during the action of chromic acid are then stained a fine blue whilst the siliceous skeleton is not stained. I will not enlarge on the method followed. I have already earlier stated the advantages which it possesses and which must be borne in mind in its application [1]).

Like other Diatomaceae Eunotia major has but one nucleus, situated in the centre and surrounded by cytoplasm, which sends out strands in various directions. As seen from the side of the belt it shows an oval shape and seen laterally it is round. It is provided with a membrane and consequently shows a sharp outline. The nuclear network consists of grains which are united by threads of protoplasm. In the centre of the nucleus is the nucleolus. The latter dissolves in chromic acid more readily than the network. Special filamentous organs, such as occur in the nucleolus of Spirogyra, I have not been able to distinguish and to separate by the use of chromic acid in the case of Eunotia. The nucleolus agrees with that of the higher plants.

The cells in which karyokinesis is about to occur are broader than the others and possess four large flap-shaped chromatophores. When the cells are viewed from the side of the belt, the nucleus is seen in the midst of the four chromatophores, two of which lie in the epitheca and two in the hypotheca. When a cell has divided, two chromatophores lie in each daughter-cell. These change their shape and position. They become twice as long and place themselves opposite each other in the epitheca and the hypotheca. A constriction then occurs in the middle and finally each chromatophore has divided into two. This process, the division of the two chromatophores, therefore precedes the division of nucleus and cell.

The first phenomena of karyokinesis show agreement with those observed in other plants. The nuclear network becomes more and more roughly granular in appearance. In a number of places it comglomerates and forms lumps, which unite into larger masses which more or less resemble short threads. I have not been able to determine the number of these thicker parts in the network. They always remain united to each other by slender connections.

[1]) Ueber den Nukleolus von Spirogyra. (Bot. Zeitung. Jahrg. 56. 1898. Abt. I. p. 199). — Ueber das Kerngerüst. (Bot. Zeitung. Jahrg. 57. 1899. Abt. I p. 155). — Ueber die Karyokinese bei Oedogonium. (Beih. z. Bot. Centralbl. Bd. XXIII. 1908. Abt. I. p. 138 ff.). — Ueber die Kernstructur und Kernteilung bei Closterium. (Beih. z. Bot. Centalbl. Bd. XXVII 1912. Abt. I. p. 414).

The thicker parts are comparable to chromosomes. Well-formed chromosomes, such as are met with elsewhere in the vegetable kingdom, do not occur in Eunotia. The nuclear wall dissolves and consequently the nucleus loses its sharp outline; the nucleolus also gradually disappears.

To this point karyokinesis in Eunotia presents nothing peculiar, but the further course of the process is wholly different from that in higher plants. In the centre of the mass of protoplasm in which the nucleus is found, the central spindle can soon be distinguished. It is a strand of protoplasm of which the outer ends are turned towards the two shells. At first I could distinguish the central spindle as a short rod embedded in the protoplasm, but in later stages of karyokinesis I observed it extending right across the whole mass of protoplasm; the two ends were seen to be club shaped and thickened. I was unable to study the origin of the central spindle, since the amount of material at my disposal was insufficient.

The nuclear network contracts around the central spindle, and in this way the ring shaped nuclear plate is formed in Eunotia. The latter divides into two halves which are likewise annular and separate from each other along the central spindle, until they are finally quite at the spindle ends. Together with this, there occurs division of the mass of protoplasm in which the nuclear plate lies. It divides into two parts, which send out strands of protoplasm in different directions just as did the whole mass and at first they are also connected with one another by strands of protoplasm. The whole figure very much resembles the diaster stage in higher plants, although I have never been able to distinguish a nuclear spindle. Meanwhile the primary division-wall has developed; it broadens out more and more and approaches the nuclear figure; the protoplasmic links between the halves of the nuclear plate and the central spindle are divided into two. The central spindle disappears. The daughter-nuclei are now very close against the division-wall, then separate again from each other, move into the neighbourhood of the epitheca and hypotheca and finally take up a position in the middle of the daughter-cells.

With the development of the annular halves of the nuclear plate into daughter-nuclei the same phenomena appear as in the formation of the nuclear plate from the resting nucleus, but in reverse order. The rings divide into lumps or short thread-shaped pieces which remain connected with each other by fine threads of protoplasm; the division proceeds to a point at which the nuclear network agrees again with that of the resting nucleus. In fully-developed nuclei I always saw one nucleolus and in less-developed ones often there were two. Probably also in Eunotia the nucleoli which appear in the daughter-nuclei gradually coalesce.

The primary division-wall, of which mention has been made, is a lamella easily soluble in dilute chromic acid. The siliceous shells are formed later. I have not found a centrosome in Eunotia.

Conclusions.

In Eunotia major Rabenh. the nucleus divides karyokinetically just as in other Diatomaceae, a fact established by LAUTERBORN and KARSTEN. In Eunotia major a central spindle (Zentralspindel) also occurs, a body which plays an important part in karyokinesis, as the above authors have also shown in other Diatomaceae. Well-developed chromosomes are not found in Eunotia major. The nuclear network forms short bodies of indefinite shape, which crowd round the central spindle and form an annular nuclear plate, which divides into two annular halves; these separate from each other along the central spindle and develop into daughter-cells.

With regard to the chromosomes, I may say that my results agree with those of KLEBAHN and KARSTEN, but not with those of LAUTERBORN. He found in Surirella calcarata and other Diatomaceae, in the mother-nucleus as well as in the daughter-nuclei, well developed long chromosomes, whose number could be ascertained (16 or more). KLEBAHN has not been able to see such chromosomes in Rhopalodia gibba nor KARSTEN in Surirella saxonica, but as I did in Eunotia major they found only a few short thick bodies of various shapes which could not be accurately described, and whose number was indeterminate. It must be remembered that the results which differ were obtained with different species.

Physiology. — *"On a shortening-reflex"*. By Prof. J. K. A. WERTHEIM SALOMONSON.

(Communicated in the meeting of December 28, 1912).

By the expression shortening reflex I propose to indicate the contraction of a muscle, the ends of which are passively brought nearer together. I shall try to prove this contraction to be a real reflex; though the primary shortening of the muscle may not be the direct cause.

We shall first consider what happens when any part of an extre-