5. Some remarks on the behavior of the kinetonucleus in the division of Flagellates: With a note on Prowazekia terricola, a new Flagellate from sick soil.

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(With 8 figures.)

eingeg. 26. Dezember 1912.

Of recent years the question of the behavior of the kinetonucleus (= the blepharoplast of some authors) seems to have become acute. In 1909 Rosenbusch in a paper "Trypanosomenstudien" in the Archiv für Protistenkunde Vol. 15, came to the conclusion that the division of the blepharoplast (= kinetonucleus) in Trypanosomes was a mitosis with a spindle, centrosomes and chromosomes, (p. 265). Jollos in 1910 put forward a similar view as to the division of the kinetonucleus of *Trypanoplasma helicis*.

As regards the behavior of kinetonucleus in Trypanosomes there seems to be very strong recent evidence against the view that the division of the kinetonucleus shows any indication of a mitosis, (see especially "Cytologische Studien an Trypanosomen" by Alfred Kühn and W. V. Schuckmann Zoologische Jahrbücher, Supplement XV, 2 Band. 1912).

As regards the Trypanoplasmas Jollos in his paper "Bau und Vermehrung von *Trypanoplasma helicis*" in Archiv für Protistenkunde Vol. 21, 1910, says: —

»Die Teilung des Blepharoplasten erfolgt bald vor, bald nach der des Kernes und, wie erwähnt, gleichfalls auf mitotische Weise; klare Bilder der verschiedenen Stadien sieht man allerdings erheblich seltener. Die Spindeln (Fig. 10—13) ähneln denen bei der Kernteilung sehr und gelangen auch beim Blepharoplasten noch innerhalb der alten Membran zur Ausbildung; auch hier kommt es schließlich vor der Trennung der beiden Hälften zu Phasen, die an Amitose oder richtiger »Promitose« erinnern (Fig. 13).

Die Vermehrung des Blepharoplasten ist also eine Querteilung auf mitotischem Wege. Andre Vermehrungsmodi konnten ebensowenig wie beim Kern festgestellt werden.«

The figures which Jollos publishes in support of his view certainly do not appear to me to form a complete series of division figures, and I am rather inclined to question whether some of the figures have anything to do with division at all. Whether *Trypanoplasma helicis* is a true Trypanoplasma is still I believe an open question. vid. (M. Kuhn, Die Trypanoplasmen und deren Verbreitung in einheimischen und ausländischen Schnecken. Schr. Phys.-ökonom. Ges. Königsberg. III. p. 63—89. 1911). In the Quarterly Journal of Microscopical Science Vol 55, 1910, I published an account of the division of what I regard as a true *Trypanoplasma* from the stomach of a conger, and in this division although I found a fairly complete series I could find no evidence for the view that the division of the kinetonucleus is a mitotic one. In a forthcoming paper I hope to deal with the division of the other two flagellates from the stomach of fishes which have been described as Trypanoplasmas, and in both of these cases again, although I do not regard these forms as Trypanoplasmas, I have not been able to find any evidence of a mitotic division of the kinetonucleus. From the work of Rosenbusch and Jollos it would appear that the chief difficulty in seeing the spindle in the dividing kinetonucleus depends largely on the compact nature of the kinetonucleus and its intense capacity for nuclear stains. I had long wished to find some form with a more vacuolar kinetonucleus, and such a form I believe I have found in some cultures of a sick soil the opportunity of studying which I owe to the kindness of Doctor Russel, of Rothamsted. I have decided provisionally to describe this animal under the name of *Prowazekia terricola.*

Though it seems to differ rather markedly from all the *Prowazekia* that I have found in my cultures in the fact that during movement the two flagella as far as could be observed are constantly directed forwards. At rest both flagella may be directed backward across the body.

Prowazekia terricola in life is a more or less spindle-shaped organism measuring, roughly, 12 by 6μ, with two flagella arising near its anterior end. Near the base of the flagella there is a small contractile vacuole and a not too well marked cytostome. In life the trophonucleus can be made out as a rather large vacuolar area containing a distinct karyosome. This flagellate is rather an active form, moving with a curious wriggling motion with both flagella directed forwards. In stained forms the two flagella can be seen to arise from as far as I could make out two closely approximated blepharoplastic granules lying near the anterior end of the body. Lying constantly posterior to these granules there is a large vacuolar structure which stains intensely with nuclear stains This structure having regard to its position and to its behavior in division I am inclined to regard as a kinetonucleus. The kinetonucleus is somewhat variable in structure but as a rule it presents the appearance of a more or less elliptical vacuole containing a number of darkly staining granules. The trophonucleus is a rather large spherical structure containing a rather lightly staining karyosome and with a number of very fine chromatin granules lying between it and the nuclear wall (figure 2).

I have been able to examine a large number of dividing forms, and as it is so frequently the case in these rather complicated flagellates the order of the division of the various structures does not seem to be absolutely constant. Attention has been drawn to the same point in a paper Miss Robertson and I published recently in the Quarterly Journal of Microscopical Science on the Coecal Parasites of Fowls. (Q.J.M.S.)

As this is the case it seems more convenient to describe the behavior during division of each structure in turn rather than describe the appearances of a series of individuals.

The Flagella: — Early in division one basal granule with its corresponding flagellum moves across to the other side of the animal. At

Fig. 1.

Fig. 2.

Fig. 3.

All figures were drawn at Table level with Zeiss Apt. 1—5 and Comp. oc. 18.
Figure 1. Normal active form of *Prowazekia terricola* showing flagella, kinetonucleus and trophonucleus.
Figure 2. Condensation of the Chromatin in the Trophonucleus preparatory to division.
Figure 3. Early change of division showing division of kinetonucleus.

a slightly later stage it is found that this flagellum has split, so that each of the future daughter individuals will possess their normal number of flagella (see figures 3—5).

The Kinetonucleus: — During the early stages of division the kinetonucleus swells and the contained chromatin has the appearance of a number of distinct granules. During division I have never been able to find any trace of anything corresponding to the mitotic phenomena of an ordinary nuclear division. As will be seen from the figures, all that happens appears to be that part of the kinetonucleus streams across to its apropriate position in relation to the shifted blepharoplastic granule and is then cut off by a simple constriction (see figures 3—5).

The Trophonucleus: — The division of the trophonucleus is rather peculiar, especially in its early stages. It would appear that the karyosome of the active individual does not contain a very large proportion of chromatin since in animal which have been stained with haemalum

Fig. 4.

Fig. 5.

Fig. 6.

Figure 4. Later showing end of kinetonucleus division, the flagellum attached to one blepharoplast has already split.
Figure 5. Later stage both blepharoplasts have two flagella.
Figure 6. Later stage only one flagellum could be traced in one of the daughter individuals, the other one probably wrapped around the body.

or lightly stained with iron haematoxylin and then afterwards stained with eosin it is quite easy to get forms in which the granules of the kinetonucleus are stained as chromatin while the karyosome has taken up the eosin. It would appear in division that the karyosome disappears and that the chromatin contained in the karyosome and the extrakaryosome chromatin condense at one pole of the nucleus, giving rise to the

Fig. 7.

Fig. 8.

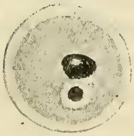

Figure 7. Last stage of division, the connecting zone between the two individuals is occupied by large vacuoles.
Figure 8. The cyst.

very characteristic appearances shown in figure 2. The band of chromatin thus formed becomes stretched out the nuclear membrane at the same time disappears giving rise to such appearances as are shown in figures 5 and 6. During the later stages of these processes the body of the animal becomes rather drawn out and the vacuolar area appears to become concentrated in the zone which connects the two daughter individuals this evidently splits across. The last stages of division when followed on the living animal seem to occupy a considerable period, about half an hour, and during the final stages the animals remain connected by a thin line which I take to be the remains of the periplast around the zone connecting the two individuals.

I have also succeeded in following the encystation of this form. The animals which are about to become encysted become very rounded, the cytoplasm at the same time becomes more dense. The resting cyst is a spherical structure (see figure 8). I have not been able to obtain evidence of conjugation of this form. The chief point of interest in connection with this animal appears to be the structure and behavior during division of the kinetonucleus. Whether the kinetonucleus in flagellates is to be regarded as a true nucleus appears to me to still remain an open question. But the work of Werbitzki has at any rate shown that the old view of the kinetonucleus regulating the organs of movement is no longer tenable and I do not think that any strong evidence for an affirmative answer to this question can be drawn from the behavior of the kinetonucleus in the division of flagellates.

In conclusion I should like to thank Professor Minchin for his kindness in allowing me to work up these results in his Laboratory at the Lister Institute.

6. Beiträge zur Kenntnis einiger Seesterne.

Von Dr. K. Babić, Kustos am kroat. zool. Landesmuseum in Zagreb.

eingeg. 27. Dezember 1912.

Beim Durchsehen der Seesterne des zoologischen Museums in Zagreb, erwies es sich als notwendig, zur Kenntnis der einzelnen Arten einige Ergänzungen hinzuzufügen. Die umfangreiche Monographie H. Ludwigs (Fauna und Flora des Golfes von Neapel, 24, Berlin 1897) diente mir als Grundlage dieser kurzen Publikation. Prof. Ludwig hat in dieser großen Arbeit über die Seesterne des Mittelmeeres auch die adriatischen Formen, welche von Graeffe, Grube, Heller, Lorenz, v. Marenzeller, Olivi, Stossich u. a. angegeben worden sind, berücksichtigt und seinen kritischen Untersuchungen unterzogen. Da alle in der vorliegenden Publikation erwähnten Arten der Seesterne durch die Arbeit Ludwigs gut bekannt sind, beschränke ich mich hier nur auf Abweichungen und Angaben über die Größe, das Vorkommen und die neuen Fundorte, um zur näheren Kenntnis dieser Arten beizutragen.

Von den hier angeführten Seesternarten werden *Astropecten jonstoni* und *Ophidiaster ophidianus*, soviel mir bekannt ist, zum ersten Male für das Gebiet des Adriatischen Meeres angegeben.