

während das Absterben unter normalen Umständen eine solche bewirkt. Hierzu kann ich mitteilen, dass sicher eine maximale Expansion der Chromatophoren eintritt, wenn man eine Fischlarve im ausgehöhlten Objektträger unter dem Deckglase dem Erstickungstode nahe bringt, während andererseits die maximale Kontraktion, besser Ballung oder Attraktion des Pigments erfolgt, wenn der Tod infolge zu großer Wärme im offenen Wasserglase eintritt. Diese postmortale Zusammenballung erinnert, wie schon Verworn hervorhob, an die postmortale Abkugelung der Amöben und nicht minder an die postmortale Kontraktion aller Muskeln (Totenstarre), und überhaupt zeigen die mesodermalen Pigmentzellen in allen soeben erwähnten physiologischen Eigenschaften, wie ich (l. c.) zeigte, eine ganz auffällige Analogie mit dem Verhalten der ektodermalen, pigmentierten Muskelzellen im Sphincter der Selachieriris, wofern man die wohl berechtigte Annahme macht, dass der Sphincter hier an Kraft den Dilatator überwiegt. Es verdient aber hervorgehoben zu werden, welche Schwierigkeiten in diesen Analogien zwischen Pigmentzellen und Muskelzellen liegen, da die Ballung der Pigmentzellen zunächst nicht auf einer echten Kontraktion, sondern auf einer intrazellulären Körnchenströmung beruht.

Zusammenfassung.

1. Der Ballungsvorgang der Pigmentzellen beruht auf intrazellulären Pigmentkörnchenströmungen.

2. Die plasmatische Radiärstruktur der Pigmentzellen besteht in einem intrazellulären Skelett, dessen Vorhandensein wegen der regen intrazellulären Körnchenströmungen genügend erklärt ist, dessen Bau in einigem an Acantharienskelette erinnert.

Some Remarks upon the „Autogamy“ of *Bodo lacertae* (Grassi).

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(Aus dem Zoologischen Institut München.)

The following comments have been called forth by my observation of some curious organisms occurring in the gut of the frog, *Rana temporaria* L. I will first briefly recapitulate the „autogamy“ which Prowazek has described in *Bodo lacertae*¹⁾, and then describe these organisms and their development, pointing out the bearing of the one upon the other.

Bodo lacertae is said by Prowazek to display two different sexual processes — autogamy and heterogamy. The former takes

1) „Untersuchungen über einige parasitische Flagellaten“ in: Arb. kaiserl. Gesundheitsamte, Bd. XXI, Heft I, 1904.

place as follows: A number of *Bodos* become united at their posterior ends, forming a kind of „Agglutinationsstern“. Each animal later undergoes a shortening and finally rounds itself off and forms a delicate cyst membrane. The autogamy-cysts thus occur together in little heaps. Inside each cyst an autogamy is now enacted. At first the nucleus increases in size. Then small bladder-like chromidia are extruded from it into the cytoplasm. Their maximal number is 8. They soon come together and fuse, forming the sexual nucleus. A stage is thus reached in which the cyst contains two nuclei — the pale (poor in chromatin) somatic nucleus, and the darker (richer in chromatin) sexual nucleus. The sexual nucleus now divides by amitosis; each daughter nucleus again dividing similarly, so that four nuclei are formed. Of these, two divide once more, so that six small nuclei come to lie round the somatic nucleus. Four of these are reduction nuclei and degenerate: the other two are the gamete-nuclei, which increase in size, approach one another, and fuse. Thus once more the cyst contains two nuclei — the old somatic nucleus and the new synkaryon, formed by the fusion of the gamete nuclei. In this stage the cyst remains for some time: then finally the new nucleus increases in size and forms a new karyosome, while the old nucleus degenerates²⁾. A thick cyst membrane is formed, and we thus reach the final stage — the yellow, durable cyst.

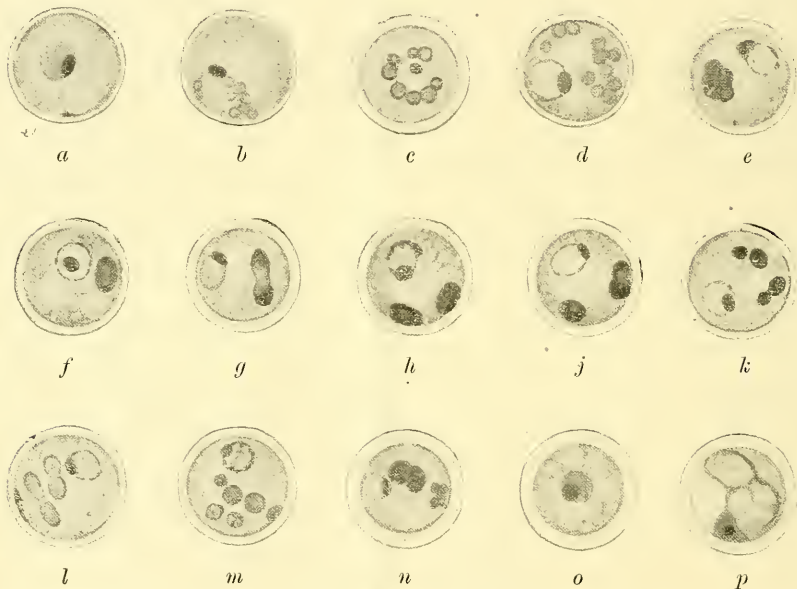
Whilst I was working at the life-histories of the protists in the gut of the frog, I came across some small cysts which bore a very close resemblance to those described by Prowazek as belonging to *Bodo lacertae*. It appeared to me that they were the cysts of some small protozoon which went through an autogamic process almost identical with that of *Bodo*. For some time I imagined that the cysts belonged to the *Octomitus* in the frog's gut, but I was unable to confirm, this opinion. I was able to find practically every stage described by Prowazek (See text-fig. 1, *a—p*).

First of all, there were cysts containing a single large nucleus, usually with a conspicuous karyosome (*a*). Then there were stages showing the bladderlike „chromidia“ emerging from the nucleus (*b, c*). And a very large number of the cells had an appearance like that shown in *d*, where the „old, somatic nucleus“ and the „chromidia“ are completely separate. I also found stages in which

2) Prowazek's description is not clear on this point. He says that the „old nucleus“ decreases in size, becomes dense, uniformly darkly-staining with iron-haematoxylin, and sometimes shows a central pale space. He then describes and figures (Fig. 81, plate III) how the two nuclei may come together. No figure of a uni-nucleate cyst is given, nor is it definitely stated that the „somatic nucleus“ completely disappears — though in one place it is referred to as „den alten der Degeneration anheimfallenden Kern“ (p. 27).

the „chromidia“ appeared massed together (*e*), forming the new „sexual nucleus“ — which, when fully formed appeared beside the „somatic nucleus“, thus giving rise to a binucleate cyst (*f*). I further found stages — though these were few — in which the „sexual nucleus“ was dividing (*g*), and more frequently cysts in which the „sexual nucleus“ appeared to have given rise to two „daughter nuclei“ (*h*). Quite a number of cysts presented appearances which could be interpreted as subsequent divisions of these nuclei. For example, in *j* one nucleus alone is apparently in division, whilst in *k* both „nuclei“ have divided, forming the first pair of „reduction nuclei“. In *l* a somewhat similar stage show is. After

Fig. 1.



searching for them, I was able to find cysts (*m*) which showed the formation of the „second reduction nuclei“. In all these stages the somatic nucleus is still present. It usually has an annular appearance, with a large peripheral karyosome. It is interesting to compare this with the „somatic nucleus“ in *Bodo*. During these stages Prowazek describes this as the . . . „alten Kern, der blass und chromatinarm ist und nur ein weitmaschiges achromatisches Gerüstwerk, dem seitlich der oft zerfallene Innenkörper anliegt, besitzt . . .“ And further he remarks. „Der alte Kern blasst stetig ab und das Chromatin verklumpt öfters in Brockenform an seiner Peripherie.“ In the cyst depicted in *n* we apparently see the „autogamy“ of the „reduced nuclei“, while the four little „reduction

nuclei“ lie grouped together and in course of absorption. Nor were the last stages of all wanting — the uninucleate, thick walled, yellowish cysts (*o*), which sometimes had the appearance of enclosing a single monad (*p*).

The appearances thus seemed in perfect harmony with Prowazek's discoveries. The striking resemblance between the „autogamy cysts“ of *Bodo* and those just described can easily be seen by anyone who will take the trouble to compare my figures with those of Prowazek. One slight difference will at first be apparent — namely, as regards the actual cyst itself. For although Pro-

Fig. 2.

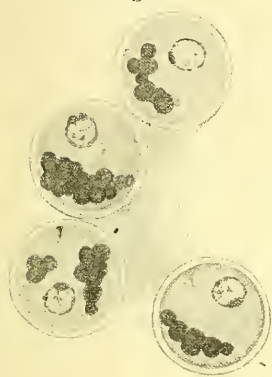


Fig. 3.



Fig. 4.

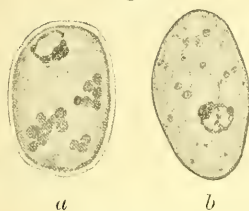
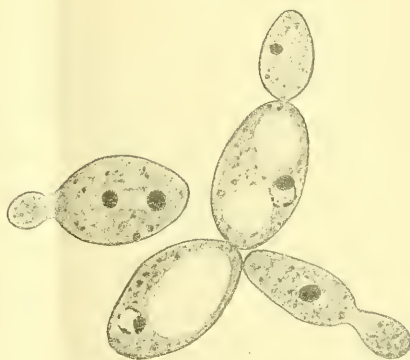


Fig. 5.



wazek constantly refers to the cysts as such, in no case (save the very last stages) does he figure a cyst-wall.

A further remarkable resemblance is to be seen in the way in which the cysts are nearly always to be found in little clusters (see text-fig. 2). Most commonly they occur in groups of 4, 8, 12 or 16, though often also in groups of 6 as shown in Prowazek's Fig. 67, Pl. III, which bears a really remarkable likeness to many of the cysts I encountered. My cysts were almost always united by a granular connective substance, as in Fig. 2, where we see 4 cysts — each containing apparently a „somatic nucleus“ and a mass of „chromidia“.

In not a few cases I found darkly staining strands lying in the cyst (see text-fig. 3). These I imagined to be the degenerating

remains of the two axial rods of the *Octomitus* to which I then attributed the cysts.

Now all these results were obtained by staining with iron-haematoxylin, after sublimate-alcohol fixation — just as were Prowazek's. On staining with more reliable nuclear stains (Delafield's haematoxylin, borax carmine), I was surprised to find that only the „somatic nucleus“ was coloured, the „chromidia“ and their derivatives remaining quite unstained. This fact might perhaps also appear to be in harmony with Prowazek's description. For although he does not say whether or not the „chromidia“ in the „autogamy-cysts“ are coloured by any other stains than iron-haematoxylin, he records that the „chromidium“³⁾ in the free-living form („gametoid“ individual) „mit den gebräuchlichen Kernfarbstoffen, wie Grenacher's Hämatoxylin, Pikrokarmin und Boraxkarmin . . . färbt sich sehr schlecht, nur mit EH kann man ihn gut zur Darstellung bringen.“ In addition to their peculiar reactions to nuclear stains, the „chromidia“ appeared during life to be much more highly refractive than chromatin usually is.

The foregoing facts made me exceedingly sceptical of my original interpretation of the phenomena, and it was obvious that the only way of deciding the matter lay in careful observation of the living organisms. I therefore directed special attention to the living cells, and observed the following phenomena. — For many hours — sometimes for days — the cysts remained quite unchanged. The first change which then occurred was quite unexpectedly a change of shape. The cysts, which had previously been spherical, became elongated (Fig. 4*a*). Meanwhile the „chromidia“ appeared smaller. Later, I found that the cyst wall vanished, and the cells assumed an oval form (Fig. 4*b*), the „chromidia“ becoming very small and finally dissolving in many cases. Still later, the „old nuclei“, instead of degenerating, divided, and the cells began to multiply by budding (Fig. 5), and vacuoles made their appearance. Multiplication proceeded very rapidly, so that large masses of cells were soon formed. After growing in this manner for a few days (in moist chambers), some of the cells began — as a rule — to form long tube-like outgrowths (Fig. 6), probably as a result of the anaerobic conditions. Many of these elongated forms subsequently divided into chains of long, brick-like cells (Fig. 7). In old cultures, the refractive bodies („chromidia“) again became conspicuous in the cells.

3) From the reactions and general behaviour of this structure, it is not at all obvious why it should be called a „chromidium“. There is no proof that it corresponds with other structures usually so designated. Prowazek himself does not seem clear about its real significance. It does not consist of chromatin, but of „eine — sit venia verbo — platinartige . . . Substanz (oder Substanzen) . . .“

Beyond this stage I did not follow the growth of the organisms.

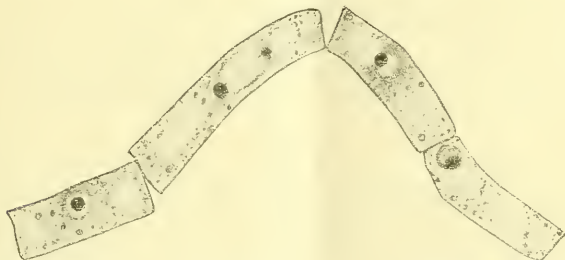
There can be little doubt that these cysts have nothing whatever to do with the Protozoa living in the frog's gut — that they are on the contrary, yeasts, or vegetable organisms allied to the yeasts.

What, then, are the „chromidia“? From their general behaviour and reactions I think there can be little doubt that they are some kind of reserve material, which is largely — if not entirely — used up in germination. Though the material varies in form — being sometimes in strands or rodlets, sometimes in large lumps, etc. — its arrangement in the cell appears to me to be entirely adventitious. When it is absent, uninucleate cysts are presented — the first stage in our arbitrary arrangement of the cysts (Fig. 1*a*). When the granules lie round the nucleus, an appearance suggesting the for-

Fig. 6.



Fig. 7.



mation of chromidia is seen (Fig. 1*b, c*). When the granules are in pairs — a not uncommon condition — we see the formation of „reduction nuclei“ (*k, l, m* etc.). And so on: all stages are to be found if one looks for them.

When I had reached these conclusions, the conviction forced itself upon me that Prowazek had really committed the error of describing similar organisms as stages belonging to the life-cycle (autogamy) of *Bodo*. The resemblance, amounting almost to identity, appears to me to be too close to be accidental. Dr. Prowazek himself would, I am sure, be one of the first to acknowledge the ease with which such a mistake might be made. The difficulties of singling out the stages in the development, of one organism from a great number are often very great.

The following points now require to be considered from this new point of view.

1. My organism was, of course, observed in the frog, while Prowazek's was in the lizard. I do not claim that they are identical, but merely urge that similar forms exist in both. Prowazek has himself recorded (l. c., p. 3), the presence of yeasts in the lizard's gut, and I can confirm this observation. I have also been able to observe⁴⁾ — in the living organism — the development of yeasts like those in the frog from very similar cysts. These cysts bear a strong resemblance to rounded-off *Bodos*, being about the same size. The „chromidia“ are less refringent than those in the frog's yeast.

2. Prowazek gives no indication of the size of the „autogamy cysts“ of *Bodo*. I am therefore unable to say how far this is in agreement with the cysts I have observed. These are, on an average, between $4\ \mu$ and $6\ \mu$ in diameter.

3. The reason why the cysts occur together is to be found by searching for them in the upper part of the frog's intestine. Here the cysts are often to be found enclosed in an asc-like capsule. This disappears when they reach the large intestine, only a trace of the investment being there found (Fig. 2). How this capsule originates, I am unable to say, having followed only a part of the life-cycle of the organism.

4. In the frog, there are at least two different species of yeast — one with a thick cyst (Fig. 1, *o*), giving rise to thick-walled yeasts, and one with a thin cyst (Fig. 1 *a*), giving rise to thin-walled yeasts. Probably more than one kind of yeast also exists in the lizard.

5. Prowazek says that „Die Autogamiecysten kamen auch meistens in der Vergesellschaftung mit den gametoiden Formen vor“ (l. c. p. 25). Yet he figures a cyst with a different kind of „chromidium“ as belonging to the „gametoid“ form (Pl. III, fig. 67). The subsequent history of these cysts is not given. I find it impossible to reconcile his statements.

6. Finally, I may say that Prowazek's figures are not — to me — entirely convincing. For example, the „dividing nuclei“ in figs. 71 and 73 (Pl. III) do not give me the impression of being really nuclei. Again, figs. 68, 74 and 76 are, to my mind, all similar conditions, in which the reserve material is collected about the nucleus. Moreover, no figure is given in which the formation of the second reduction nucleus is clearly shown — figs. 74 and 75 being somewhat doubtful. I may note, also, the remarkable difference in size between the „gamete nuclei“ in figs. 75 and 76. Fig. 80 and 81 are, I believe, degenerate forms (Cp. my fig. 1, *p*, which shows the protoplasm shrunken from the cell-wall).

4) In *Lacerta muralis*, both at Munich and Naples.

In Hartmann's section on „Protozoology“ in „Kisskalt and Hartmann's Praktikum“ (Jena 1907) some of Prowazek's figures are reproduced and to some extent modified. For example, the two „reduction nuclei“ (which Prowazek never shows clearly), are figured quite unmistakably in fig. 8g (p. 117). And a very distinct network is shown in the „sexual nuclei“ in fig. 8e — neither of which is justified from Prowazek's publication. Further, Hartmann states that „Nach der Befruchtung kann direkt im gleichen Wirt wieder das freie Flagellat aus der Cyste hervorgehen oder aber die Cyste wird zu einer Dauercyste und dient der Neuinfektion“ (p. 117). I can find no statement by Prowazek to this effect. Nor can I see how he could have made such an observation, since all his description of the „autogamy“ was based on stained preparations, and not upon observation of the living animal.

I think sufficient has now been said to show that autogamy and chromidia are as yet unproven in the case of *Bodo*. My desire in making the foregoing remarks has been to point out that too much emphasis should not be laid upon this case — which is frequently quoted in recent papers on the chromidia hypothesis — before it receives ample confirmation.

Explanation of Text-figures.

(All figures are drawn from permanent preparations [fixed hot sublimate-alcohol, stained Heidenhain's iron-haematoxylin], with the aid of a Leitz 2 mm oil-immersion apochromat, with compensating ocular 12.)

Fig. 1, *a—p*. Various forms of cysts from the large intestine of the frog — arbitrarily arranged to show an „autogamy“ similar to that described in *Bodo lucertae*.

Fig. 2. Four cysts from the frog's intestine — connected by remains of capsule.

Fig. 3. A cyst from the intestine, showing darkly staining rodlike structures.

Fig. 4, *a* and *b*, stages in the development of the cysts in the faeces.

Fig. 5. Formation of buds by yeasts which have developed from the cysts.

Fig. 6. A yeast forming a long outgrowth — from a hanging-drop slide culture of the faeces.

Fig. 7. Later stage of a similar form, showing segmentation into 4 cells.

Über das Vorhandensein von Tetradenchromosomen in den Leberzellen von *Paludina vivipara*.

Von Methodi Popoff.

(Aus dem zoologischen Institut in München.) Mit 6 Textfiguren.

Die genaue Erforschung der Umänderungen, welche die Geschlechtszellen in der letzten Periode ihrer Entwicklung durchmachen, hat eine Fülle von komplizierten Wandlungen des Kernchromatins dieser Zellen aufgedeckt. Die am Ende ihrer Vermehrungsperiode angelangte Geschlechtszelle fängt allmählich zu wachsen an, und durch das Leptotene-, Synapsis- und Pachytenestadium kommt sie

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