The Development of the Nucleus of Amoeba proteus. Pallas (Leidy) [= Chaos diffluens (Schaeffer)].

By

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With Plate 28 and 2 Text-figures.

A VERY thorough knowledge of the nuclear phenomena in an organism so largely employed as is Amoeba proteus by investigators in many branches of science, no less than by teachers of biology, is greatly to be desired. That there are, at present, gaps in this knowledge is quite evident from an inspection of the literature. One such gap was responsible for a question asked of me by Professor Grégoire while I was working in the Carnoy Institute, Louvain, in 1924. This question emphasized the fact that, in my paper on 'Nuclear Divisions in A. proteus', I had, as had Doflein (**8**) before me, left it open whether or no the portions of the karyosome which stain more deeply than the rest consist of chromatin. When reading over the manuscript of my paper Professor Graham Kerr had said that he saw no reason for their not being chromatin, while fully sympathizing with my reluctance to decide the question from observation made on the nucleus of the adult. Grüber (**11**) had no hesitation in describing these portions of the karyosome as 'chromatin-containing'.[1] While I was working out the pheno-

[1] In most of my previous work on A. proteus, as well as in this present paper, I have made little reference to papers published before 1916 because it is not always possible to be quite sure which of the two large free-living amoebae is referred to in the said papers. In the case of Grüber (**11**), however, it is quite easy to see from his figures that he worked on the same amoeba that I have used, i.e. the A. proteus of Pallas (Leidy). In view of the recent work on Protozoan nuclei by Bělař (**1**) it may be well to remind the reader that in 1916 Schaeffer (**19**) showed, quite conclusively, that two distinct species of amoebae had hitherto been called A. proteus,

mena connected with the formation of encysted young, the karyosome of the fully mature individual had appeared to me to be nothing more than a portion of the nuclear reticulum where the meshes were not quite so large as in the surrounding areas. In contradistinction to this condition was that which obtained in those young A. proteus which I had been able to examine before the publication of my paper (**25**, p. 137, Text-fig. 10) and of which a preliminary account was given. In these the karyosome appeared much more solid and less reticulate, being quite the most conspicuous structure in the nucleus.

A subsequent and much more extensive series of observations extending over three years has confirmed this (cf. also figs. 71 and 77, Pl. 28). What events are responsible for this change in the density of the karyosome is a question that naturally arises.

It will be remembered that the nucleus of A. proteus presents different appearances as it is rolled about by the streaming movements of the endoplasm. It is mobile. The disc-shaped

viz. (*a*) the true A. proteus Pallas (Leidy) and another (*b*) which he named A. dubia (Schaeffer). (See also Carter (**3**).)

I have sent cultures of A. proteus to Kansas, where they arrived in good condition. Professor Schaeffer has informed me that these amoebae differ in no respect from those he obtains in America.

In his recent monograph on amoebae (**20**) Schaeffer suggests that these two free-living amoebae should be placed in the genus Chaos, and that A. proteus should henceforth be known as Chaos diffluens.

Bělař (**1**) has evidently not consulted the work of Schaeffer (**19**) and Carter (**3**). His descriptions are therefore vitiated by the fact that he has not made clear to his readers which of the two large free-living amoebae he has used. The figures which he gives on p. 296 are those of A. dubia, not A. proteus.

His want of knowledge of this fact may also be responsible for his statements on p. 560, that my observations on the division of the nucleus in A. proteus can be dismissed, because made on pathological material. I should suggest to him that before making a priori statements he should verify the accuracy of the figures in Pl. 2 (**24**). Such an investigation on his part would reveal to him the highly refractory and unaccommodating character of A. proteus (= Chaos diffluens), and its quite unsuitable character for inclusion into the beautiful schemes shown on p. 538 of the same work (**1**).

karyosome, on this account, presents alternately its plan and elevation appearance to the observer. If a nucleus happens to be ' fixed ' while in the act of rolling over from its ' plan ' to its ' elevation ' position or vice versa, a somewhat complicated picture is the result. It will also be remembered that the picture is still more complicated when a dividing nucleus is being rolled over, especially when it is a case of division into four or more products. Yet, allowing for all these sources of complexity there exist additional complexities of structure in some figures drawn from carefully fixed and stained preparations which needed further investigation. A complete answer to Professor Grégoire's question ' What is the karyosome in the nucleus of A. proteus ? ' was impossible in 1924. It seemed reasonable to suspect that the data for the answer must be sought, as in so many other cases in cytology, from a study of the development of the nucleus.

In Sedgwick volume i (**21**) Professor Dunkerly, summarizing my work, writes of A. proteus (Pallas), ' large, with large nucleus dividing amitotically '. A renewed search for any trace of true mitosis occurring in the developmental stages, or among specimens of adults incidentally observed while procuring the necessary numbers of young amoebae was a duty especially in view of such work as that of Phelps (**17**) and others, because an exact knowledge of the normal behaviour of the nucleus is a first essential for any progress in experimental work on A. proteus.

The long and rigid examination of cultures necessary for this investigation on the growth of the nucleus has failed to reveal any trace of syngamy in the life-history of A. proteus under cultivation (see ' Nature ', **26**). Indeed the very phenomena to be described appear to me to be of so primitive a character, and to exhibit such a lack of anything approaching ' differentiation ' as to preclude any necessity for, or utility of, syngamy. I have no further contribution to make to the summary of the life cycle given in Sedgwick (**21**), i. e. agametes are formed from secondary nuclei which have been derived from ' chromatin blocks ' escaping from the nucleus of the mother amoeba.

Before proceeding to describe the development of the nucleus I am glad to have an opportunity of discussing in the full light of my present knowledge a paper entitled 'A contribution to the Life History of A. proteus (Leidy)' by Hausman (**12**), to which my attention was directed by Professor Grégoire and which I had not read before writing 'A. proteus: some new Observations on its Nucleus, Life History, and Culture' (**25**).

(1) All my observations confirm his hypothesis that A. guttula, A. radiosa, Dactylosphaerium are merely stages in the life cycle of A. proteus, and not true species. Wallich quoted by Hausman included 'radiosa' in the life cycle of A. proteus, while Cash (**4**) says Dactylospheria can with difficulty be separated from young A. proteus. I cannot help thinking that if Graff, who recorded the absence of A. proteus and the presence of A. guttula (**10**), had 'planted' out some of the material from the source, a crop of A. proteus would have been forthcoming in about three months' time, especially if the experiment were carried out on sufficiently extensive lines.

(2) Metcalf quoted by Hausman states, that possibly the life cycle of A. proteus may require a year for its completion, and may exhibit during its course three or even more modes of reproduction. The hypothesis that the life cycle may require a year for its completion I can substantiate. That the long period is necessary because A. proteus may exhibit more than one mode of reproduction I have no evidence whatsoever, as already indicated. The data from my field-book readings show that, on an average, a period of six months elapses before a culture which contains no adult amoebae becomes once more densely crowded with adults. Often that period is greatly prolonged. I have records of cultures in which no adults could be found during the course of a whole year and upwards, and which, after this long delay, became crowded with fully developed, mature, adult amoebae. I attribute this long delay in the attainment of maturity to an environment unsuitable for the developing young. In spite of many trials I have not yet succeeded in perfecting a technique for the successful

rearing of a quantity of newly hatched amoebae to the adult stage. The numerous successes have been outbalanced by the still more numerous failures, and apart from such occurrences as the sudden appearance of a crop of bacteria, ciliates, &c. I have not yet discovered the cause of the failures. As will be indicated later, it is a much easier task to rear young A. proteus when adults are present. The environment seems to remain much more stable when they are present. I attribute those cases of the long absence of adults from a culture which is yet continuously peopled with young developing amoebae to the fact that encysted young, of which millions must be produced, are continuously hatching out, developing for a time, and then being killed off by something untoward in the environment. Once that environment becomes suitable the young attain maturity, and fission divisions ensue. I have called the period that ensues when the culture contains no adults or very few adults the Developmental Period. The period which follows, when the adults are increasing rapidly in numbers because of fission, may be prolonged almost indefinitely by sub-culturing, but without such interference it lasts about six months, at the completion of which there is an onset of the phenomena described fully in (**25**). These time values are by no means always the same. They depend wholly on the environmental surroundings of the amoebae.

(3) The observations of Miss McGuire, Sister Carmela, and myself on newly hatched amoebae are in complete accordance with those of Hausman, although we find that very shortly after hatching the young tend to become star-shaped. The floating star-shaped individual may always be made to assume the creeping form when put on to a slide.

(4) My observations on the mother amoeba, in contradistinction to those of Hausman, revealed no sluggishness in behaviour during the phenomena attending the (later stages at any rate) formation of encysted young, nor have I ever observed the extrusion of encysted young from the posterior end of the mother amoeba while the anterior end was actually moving, such as Hausman describes in the case of ' hyaline spheroids ' (= ? en-

cysted young). I hope later to publish an account of a parasite in A. proteus which is responsible for causing the infected amoebae to look very black, to refuse to ' grip ' the slide on which they are placed or to reveal any ectoplasm (cf. Parsons, **16**), in short to appear very sluggish. In some cases this parasite exists along with the encysted young in a mature specimen. It is possible that Hausman's material was of this latter kind. One sentence in his account is significant, ' Others [i. e. hyaline spheroids] synchronous with their emergence disintegrated '. In my infected material I have observed the parasite move to the ectoplasm of the amoeba, escape, and immediately ' disintegrate ' into numbers of flagellate organisms. I feel convinced that these observations were made on parasites and not on gametes of the amoeba from a long experience of keeping pedigree cultures of A. proteus. Cultures containing a large proportion of these black amoebae are never healthy, and often die out.

Cultures containing Cryptodifflugia (= Allogromia) while holding their own, as they do in some cases, never attain the luxuriance in numbers which characterizes non-infected cultures at their optimum.

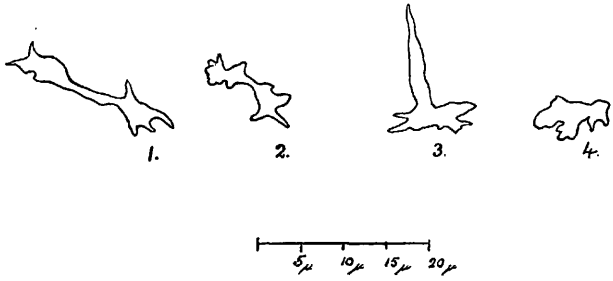
Leidy (**14**, Pl. 4, fig. 25) describes half a dozen darkly granular balls the contents of which exhibit lively swarming movements. From a study of the nucleus depicted I think the reference is to A. dubia, but I have observed a similar phenomenon fairly often in A. proteus. The ' balls ' are about the same size as a contractile vacuole at full diastole, and were full of myriads of short rod-like bodies ' dancing ' vigorously. The culture containing these specimens became depressed and has never recovered.

Unfortunately Hausman gives no scale to his figures, therefore it is difficult to compare his young amoebae with mine. Although he makes no reference to Schaeffer and Carter I think his work was done on A. proteus and not A. dubia.

With regard to the conjugation of helizoan-like forms alluded to by Minchin (**15**) I should like to record that bizarre shapes (Text-fig. 1), while suggesting conjugation or division, are merely

due to change of shape, and are frequently to be met with. Although many writers have referred to the fission of the cytoplasm in A. proteus, and although Phelps (17) has given a verbal description of the process, yet in my experience few people have actually witnessed the phenomenon. Yet it is easy of observation in any laboratory where cultures are kept. If a quantity of adult amoebae from a culture nearing its optimum be put in a solid watch-glass with as little weed as possible,

TEXT-FIG. 1.

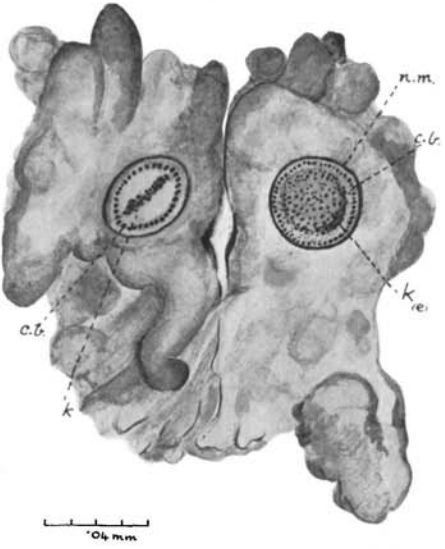


Successive forms assumed by young A. proteus suggesting fission, or syngamy. Appearances similar to these are constantly met with.

and kept under observation upon the stage of a Greenough binocular, one or other of the individuals is sure to be seen undergoing division. Professor Graham Kerr has drawn my attention to the parallel that exists between the process and the first segmentation division of a zygote. As viewed through the binocular over a dark background, the amoeba about to divide is highly reminiscent of a zygote, except that the periphery of a zygote is generally smooth, whereas that of the amoeba is broken with a quantity of numerous, short blunt pseudopodia regularly disposed on the periphery. The plane of division can be gradually traced through the poles and the equator (Text-fig. 2). When division is complete the two daughter amoebae gradually creep away from each other. Fixed and stained specimens of amoebae

in fission are best obtained by removing as much as possible of the weed and debris from a large number of adults, and then allowing them to settle before pouring over the fixative.

TEXT-FIG. 2.



Fission of cytoplasm. Drawing made from a stained preparation of A. proteus. Method described in text. *cb*, chromatin block in periphery; *k*, karyosome, seen in elevation; k_e, ditto, seen in plan; *nm*, nuclear membrane.

MATERIAL AND METHODS.

As already stated, the field-book readings of some one hundred cultures extending over a period of many years have been available, as well as material for fixation from these cultures. The temperature conditions have been those of the Notre Dame Training College Laboratory and therefore seasonal, including winter vacations when artificial heating was a negligible

quantity. There was no lack of material, even for the very large numbers of observations an investigation such as this demands ; but the slow development of A. proteus was responsible for intervals when one had, so to speak, to sit down and wait for the creatures to grow up. This was especially the case in experiments made to time exactly the rate of growth. Many stages can, of course, be obtained by fixing material, in large quantities, during the ' Developmental Stage ' of the culture. I have already referred to the many vicissitudes that attend the development of A. proteus. Over and over again promising cultures, perfectly free from adults, have been killed off by an epidemic of a large ciliate, or by a bacterial infection. Especially is this the case when the culture has been treated with tartaric acid or ferrous sulphate. Both these reagents, so useful for killing off adults, and therefore for facilitating the observation of the hatching out of the young, have this disadvantage, that they favour the growth of harmful bacteria.

In the beautiful work on the ' Cultivation of Entamoeba histolytica and some other Entozoic Amoeba ', Dobell and Laidlaw (7) have demonstrated the existence and inimical effects of a starch-splitting bacterium. Some such organism in my cultures is responsible for low pHs. As already indicated, the presence of adults in a culture of developing young is a great help in keeping the environment healthy for the latter. Rotifers, too, are useful scavengers in the developmental period, while for the adult amoeba they form an excellent diet. Under artificial conditions of rearing A. proteus there is no doubt that compensation for the delicacy of the developing amoeba is to be found in the hardiness of the adult. The production of copious numbers by the numerous fission divisions that follow the attaining of maturity results in the formation, at the optimum period, of a white carpet of amoebae.

Although the point needs much further investigation I am of opinion that large numbers of these specimens either do not produce encysted young or produce very few (cf. Dobell and Laidlaw).

Most of the observations were made on material which was

placed on a slide, fixed and stained under a cover-slip, Bouin's fluid (formula of Duboscq Brasil, 1905) being the fixative mostly employed, Ehrlich and Delafield's haematoxylin, light green being the stains employed.

Material was also sectioned. I am indebted to Professor Debaisieux for giving to me the slides he had prepared by a method which had already been described to me by Dr. Muriel Robertson. Large numbers of amoebae, separated as completely as possible from debris, are transferred to a centrifuge tube where they are fixed, dehydrated, stained, cleared, and embedded in paraffin. The centrifuge tube being broken, a block is prepared and the sections are cut in the usual way. I am also indebted to Sister Carmela for preparing many slides by the same method.

Lastly, slides were prepared by taking material, fixed, stained, dehydrated, cleared in the centrifuge tube and mounting in the usual way.

Observations and Results.

Surveying the results as a whole, a fact which stands out very clearly is that the diameter of the karyosome does not bear a constant ratio to that of the nucleus. Even when every care has been taken to allow the amoebae to spread on the slide, and to fix the specimens carefully, this ratio varies, as can be seen by an inspection of the table on p. 249.

Grüber (11) had noticed this phenomenon in adult amoebae.

Professor Grégoire, whose cytological experiences are of so varied a character, is impressed with the small size of the nucleus of A. proteus compared with the bulk of cytoplasm with which it is associated, and this in spite of its conspicuous appearance in stained specimens of the animal. In the developing A. proteus, too, the nucleus looks small, especially when compared with such forms as A. limax and A. villosa.

The diameter of the nucleus can be taken as a rough gauge of the age of the amoeba, at least in nuclei so situated in the preparation as to be capable of being studied in plan. An

TABLE SHOWING THE RELATIONSHIP BETWEEN THE DIAMETER OF THE NUCLEUS AND THAT OF THE KARYOSOME IN A. PROTEUS.

(The value of the unit employed is 1·6 μ.)

Karyosome.	*Nucleus.*	*Karyosome.*	*Nucleus.*	*Karyosome.*	*Nucleus.*
1	4	3	7	4·5	10
1·3	4	3·5	7	6	10
1·5	4	4	7	4·5	10·5
2	4	3	7·5	5	11
1·5	4·5	2·8	8	6	12·5
1·5	5	3	8	11	16
2	5	3·2	8	10	17
3	5	3·5	8	14	20
2	5·5	4	8	15	21
1·5	6	4·5	8	18	21
2	6	3	9	19	25
2·3	6	3·2	9	20	26
2·5	6	3·5	9	22	27
3	6	3·5–4	9	21	28
3·1	6	4	9	19	29
2	6·5	4·5	9	21	30
2·5	6·5	4·8	9	24	30
2	7	5	9	24	32
2·5	7	4	9·5	15	37

appreciation of the enormous disparity in size that exists between the young amoeba and the adult can be obtained by an inspection of figs. 1 and 78, Pl. 28.

Briefly described the nucleus of the developing amoeba is disc-shaped, and consists of a more or less centrally placed karyosome in a reticulum of nucleoplasm, the interstices of which are filled with nuclear sap. Chromatin in varying amount is to be found on this reticulum. The nuclear membrane is well marked and lies contiguous to the circumference of the reticulum. In badly fixed specimens the reticulum contracts away from the membrane. In the young amoeba the nucleus is not rolled about by the endoplasm as is that of the adult, possibly because the bulk of the cytoplasm is not sufficiently great to admit of this. Like the adult it is mobile, as can be gathered from the fact that food vacuoles sometimes indent the rim of the nucleus and sometimes the rim is folded inwards (fig. 70, Pl. 28). In all such cases, however, the karyo-

some never appears in elevation, i.e. never looks like a band, diametrically placed. Judging by the appearance of the folds one concludes that the disc-like nucleus has very little depth and is very thin. As has already been stated, the karyosome is a much more conspicuous object in the developmental stages of the amoeba possibly because in the adult the chromatin blocks under the nuclear membrane arrest the attention (cf. figs. 65 and 78, Pl. 28).

To facilitate description it may be well to state here at the outset that the results of this renewed study of the nucleus have led me to conclude that the deeply staining portions of the karyosomes of both young and old amoebae are chromatin-containing, that they are of precisely the same nature as the chromatin blocks described in (**25**). Henceforth they will be called chromatin, although this conclusion was only arrived at after long investigation, as described below.

While conforming in principle to the general description given above there is a great variety in detail in different individuals, as can be seen by a brief inspection of Pl. 28. I have grouped together nuclei of about the same size to facilitate an appreciation of this diversity in spite of an underlying unity. I found this diversity very perturbing in the earlier stages of my investigation. Figures such as fig. 44, Pl. 28, are very strongly reminiscent of the nuclei of A. limax and A. villosa. Only that I had the histories of the cultures I was dealing with, and knew that they contained no amoebae of another species, I should have rejected them as not being A. proteus. However, this very variety of appearance, as will be explained later, has thrown light on obscurities in the behaviour of the adult nucleus and also on the method of growth.

The staining capacity of the karyosome varies very greatly. Sometimes it appears to be an almost homogeneous body (figs. 1, 2, 3, 21, 38, 55, 71, Pl. 28), at other times it is distinctly coarsely reticulate (figs. 33, 47, 65, Pl. 28), the threads of the reticulum staining deeply and being rich in chromatin. In still other specimens the chromatin has segregated almost entirely from the underlying substance forming a thick rim to the karyo-

some (figs. 12, 15, 28, 58, 61, 75, Pl. 28). In yet other cases masses or blocks of chromatin can be distinguished (figs. 46, 48, and 54).

In A. lacerta Dobell (**6**) distinguishes three types of karyosome—homogeneous, reticulate, and granular (cf. also Entz, **9**).

That constant changes are going on in the karyosome, elaboration of a viscous liquid into a more solid substance can be gathered from the fact that in some cases Brownian movement has been observed in the karyosome. I concluded that the specimens showing this phenomenon were sickly individuals, but to my mind the fact is interesting as throwing light on the physical and chemical changes taking place in the karyosome of a normal individual.

There is great variety, too, in the reticulum. In some cases the threads are so fine as to give the impression that the karyosome is immersed in liquid (figs. 1, 2, 15, 65, 74, Pl. 28). Variety, too, exists in the amount of chromatin on the reticulum and its distribution in the nucleus. In very young individuals the chromatin gives one the impression of consisting of five to eight or more chromosomes (= blocks) situated in the periphery (figs. 6, 7, 9, 13, 14, 20, 40, Pl. 28). Indeed, I described them as such (**25**). At other times the periphery is apparently devoid of chromatin or almost so (figs. 8, 12, 49, 50, 59, 63, 74). When this latter condition obtains the young nucleus forms a strong contrast to the adult nucleus (figs. 74 and 78, Pl. 28) with its well-marked chromatin blocks under the nuclear membrane.

The meshes in the reticulum are sometimes very irregular (figs. 24, 42, 54); at other times they are quite symmetrical, the threads of the reticulum being radially arranged (figs. 66 and 67, Pl. 28). It will be remembered that some of the preparations were made by placing specimens on a slide and running the fixatives, &c., under the cover-slip. It might therefore be contended that perhaps these varying appearances are due to faulty irrigation of the fixative. That they are not due to this cause I feel convinced. Two contiguous individuals necessarily under precisely similar conditions of fixation and staining may show the differences described. Nor can they be ascribed to

the condition of the culture from which the specimens were taken. In each case they have been compared with controls and with material freshly gathered from ponds.

Areas containing much chromatin are to be seen sometimes quite near the karyosome (figs. 15, 19, 21, 22, 38, 70, 75, Pl. 28), at other times midway between the karyosome and the periphery (figs. 26, 48, 57, Pl. 28). When such conditions are viewed in conjunction with the condensation of chromatin round the rim of the karyosome (fig. 54, Pl. 28 ; cf. also figs. 70 and 75, Pl. 28, where the outer rim of the karyosome is preparing to separate itself from the main mass), it seems reasonable to deduce the fact that chromatin-containing material from the karyosome passes out at intervals to the nucleo-reticulum, gradually making its way to the periphery as the nucleus grows in size.

The differences in the ratios of diameter of karyosome to diameter of nucleus have already been commented upon. These differences receive an explanation in the hypothesis just set forth, i. e. that material from the karyosome is constantly being given to the periphery.

I should further suggest that the chromatin in the periphery is concerned with the elaboration of food for the nucleus which, stored up in the karyosome, is gradually elaborated into chromatin, as the nucleus grows larger.

One effect of division of nuclei is to increase the volume of nuclear material. I see a certain analogy between the events that take place in the growth of the nucleus of A. proteus and the events which occur in karyokinesis. Thus, I should suggest that the large karyosome, more or less homogeneous in its staining capacity, is analogous to the telophase mass of chromatin in the metazoan nucleus. There is a rapid absorption of fluid into the nucleus on the completion of telophase associated with the increase in volume of the daughter nuclei as it attains the normal proportions of the particular metazoan nucleus under consideration.

So a large homogeneous staining karyosome in a finely divided and pale-staining nucleoplasm with a thin nuclear mem-

brane reminds me of the condition that obtains in a daughter nucleus just after telophase (fig. 74, Pl. 28). Miss Jepps has shown that in the telophasic condition of Gromia oviformis (**13**) nuclei, there is no differentiation into karyosome and peripheral chromatin. The whole forms a deeply staining sphere which gradually becomes converted into the resting nucleus (**13,** figs. 32–57, Pl. 39). Similarly in A. proteus a nucleus like that figured in figs. 12 and 50, Pl. 28, gives rise to the condition illustrated in figs. 13, 55, Pl. 28. In the nuclei of both animals chromatin is emerging from a densely staining body. In short the whole cycle of changes by which chromatin is supplied by the karyosome to the reticulum in A. proteus is suggestive to me of the changes that follow after the telophase stage of division of a nucleus when the synizetic mass of chromatin is gradually resolved into a reticulum-like condition characteristic of the resting nucleus (**2,** figs. 1–7, Pl. 21 ; **22,** figs. 4–7, Pl. 27).

When the nucleus is fully adult the chromatin of the periphery becomes separated out into patches, large and not regular to begin with, and eventually the appearance of the adult is reached. In the light of the preceding account it seems quite evident that the ' blocks ' in the periphery are really ultimately bits of the karyosome which in turn give rise to the chromatin blocks that escape into the cytoplasm of the ' agamont ' to form there the rudimentary karyosomes of the ' agametes '. The rudiment of the nucleus of the encysting young amoeba was shown in (**25**) to consist of chromatin on an underlying substance, a nutritive substance. It was also shown that the chromatin is much more perfectly segregated from the underlying substance when division into two of the ' block ' is about to take place. Many of the drawings made of the karyosome in the developing nucleus are highly reminiscent on a larger scale of those made from the developing rudiment of nucleus (**25,** Text-fig. 6). Indeed, this study has led me to conclude that the rudiment of the nucleus in a developing A. proteus is wholly karyosomic, that it remains so during all the encysted condition, and that it is converted into the form illustrated in figs. 79, 80, Pl. 28, by the

absorption of liquid, by much the same process as a sperm-head is converted into a pro-nucleus. The fully formed 'chromatin blocks' of the adult nucleus are therefore miniature rudimentary karyosomes. Once the blocks have differentiated out, the amoeba is mature and capable of giving rise to young amoebae.

In this connexion it is interesting to note that Davis (**5**) has shown that the peripheral chromatin (in blocks) of the trophozoite gives rise to the karyosome of the cyst nuclei in Schizamoeba salmonis.

In the absence of the knowledge that the karyosome provides the periphery with chromatin, many phenomena exhibited by the living nucleus were difficult of interpretation. In the living amoeba the nucleus is constantly being rolled about in the endoplasm. If the nucleus happened to be precociously divided into daughter nuclei, and if in addition to this complication the karyosome was also providing the periphery with chromatin, it was hitherto wellnigh impossible to interpret. As already hinted, this investigation has therefore thrown light on the whole question.

Although not so easy to follow in the adult as in the young amoeba because of the presence of the peripheral blocks, the outflow of karyosomic material into the reticulum to reinforce the periphery does not discontinue when the adult stage is reached. Hence the inconstancy in the ratios existing between the diameters of nuclei and karyosome. An inspection of figs. 77 and 78, Pl. 28, will show the process at work in the adult. These masses of karyosomic material being differentiated into blocks (figs. 77 *a*, 78 *a*, Pl. 28), especially when near the periphery, quite account for those 'over and above' complexities which could not otherwise be accounted for, especially in nuclei incipiently divided into two, four, eight daughter nuclei.

The karyosome eventually becomes exhausted of its chromosomic material. In adults ready to form encysted young, as already explained, the karyosome appears to be nothing more or less than a nucleoplasm. The solid-looking structure of the young stage has given place to a vacuolated reticulum in which are to be seen a few chromatin blocks.

Why the general metabolism of the amoeba cannot replenish the karyosome, as hitherto, is a point which needs further investigation. The whole cycle of events seems to point out the truth of Minchin's hypothesis (**15**) that the emission of chromidia is due to the exhaustion of the nucleus.

Lastly, this investigation has convinced me that, just as a nucleus can grow very quickly and thus become incipiently divided into four or eight in good cultures, so also the chromatin blocks can be divided incipiently into more than two daughter blocks (as was explained in (**25**) blocks that are dividing can be distinguished by their deeper staining capacity). Hence the want of regularity in the size of the chromatin blocks of some nuclei.

Summary.

1. The structure of the nucleus of the young A. proteus has been described.

2. The development of the nucleus of A. proteus has been traced out.

3. Light has been thrown on some points of difficulty in the interpretation of the structures in the nucleus of the adult A. proteus, both in the resting and in the dividing stages.

4. Comparisons with other forms have been made, and some criticisms have been answered.

5. A method of obtaining stained preparations of fission of the cytoplasm has been given.

6. The investigation has failed to reveal any trace of syngamy in the life-history of A. proteus.

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EXPLANATION OF PLATE 28.

Illustrating Dr. Monica Taylor's Paper on 'The Development of the Nucleus of Amoeba proteus. Pallas (Leidy) [= Chaos diffluens (Schaeffer)]'.

All the figures are of A. proteus and are drawn to the same scale, which is indicated on the plate.

LETTERING.

a, chromatin blocks condensing out of karyosomic material which is losing its attachment to the main mass; *k*, karyosome; *cb*, chromatin blocks; *rnp*, reticulum of nucleoplasm; *nm*, nuclear membrane.

Each division of the scale is 1 μ, the whole scale is 10 μ.

Figs. 1–78.—Nuclei in successive stages of growth from one just hatched to adult.

Figs. 79 and 80.—Amoebae just ready to hatch. *n*, nucleus before hatching. The nucleus has become fully differentiated at this stage.