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IV.—THE BEHAVIOUR OF THE NUCLEOLUS DURING
OÖGENESIS, WITH SPECIAL REFERENCE TO
THE MOLLUSC PATELLA.*

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TWO PLATES.

INTRODUCTION.

IN a previous paper on the Oögenesis of *Patella* (11)† the relation of the Golgi Apparatus and Mitochondria to the formation of yolk was described, and it was mentioned that during that process there was an extrusion of "solid" nucleolar material from the nucleus into the cytoplasm. The present paper is devoted to an account of the behaviour of the nucleolus and the changes in the chromophilicity of the cytoplasm during oögenesis.

This research, as the former, was carried out in the Zoological Laboratories of University College, London, and my thanks are due to Dr. J. Bronté Gatenby, Lecturer in Cytology, for his kindly interest and helpful suggestions during the course of this investigation.

I have also to thank Mr. H. Graham Cannon, of the Imperial College, for some help with the literature of the subject, and Mr.

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† The figures in brackets refer to the bibliography at the end of the paper.

J. H. Woodger, of University College, for kindly fixing some of the material used in this research, during my absence.

PREVIOUS WORK ON THE NUCLEOLUS.

It is customary to recognize three types of nucleoli within the nuclei of various cells—namely, plasmosomes, karyosomes and amphinucleoli (1). The plasmosomes, or “true nucleoli” are usually spherical bodies, composed of “plastin” whose staining reaction is oxyphil—that is, they take up acid stains such as eosin. The term karyosome is usually applied to nuclear granules whose histo-chemical reactions are similar to those of chromatin; such bodies stain with dyes like hæmatoxylin and methyl green, and are therefore spoken of as basophil. Amphinucleoli are believed to be composed of a mixture of “chromatin” and “plastin”; they occur in many Protozoa. Their staining reactions are intermediate between those of plasmosomes and karyosomes.

Our knowledge of the relationship of the nucleolus to the metabolism of the cell is very fragmentary. Nucleoli at different times have been regarded as storehouses of either albuminous compounds, or chromatin, or both combined, as accumulations of waste products within the nucleus, or as special nutritive organs concerned either with the metabolism of the cell, or else with the elaboration of chromatin from the karyolymph.

Recently, emphasis has been laid upon the importance of the nucleolus by Carleton (4), who has shown that within the nucleolus of epithelial cells of the gut of the cat and frog there is an argento-phil core consisting of several granules. During cell division, while the greater part of the nucleolus disintegrates, these granules persist and pass in about equal numbers to each of the newly forming cells, where a new nucleolus is formed around them. Such intranucleolar bodies have been found associated with both plasmosomes and karyosomes.

Actual fission of the nucleolus can be observed during amitosis of follicle cells in the ovaries of insects. In those of *Dytiscus*, which have been prepared by fixation in Bouin's fluid and stained with Mann's methyl blue eosin, I find a distinct oxyphil nucleolus. This elongates, becomes dumb-bell shaped and eventually separates into two distinct spherical bodies. Meanwhile the nucleus as a whole has elongated, and eventually becomes separated into two parts in typical amitotic fashion.

That nucleoli of somatic cells may stand in nutritional relationship to the cytoplasm has been shown by the work of Schreiner on *Myxine* (15). He found that the oxyphil nucleolus or plasmosome of many of the somatic cells increased in size, became lobulated, and portions broke away and passed in the form of granules from the nucleus into the cytoplasm; there they formed

rodlets which broke up into smaller granules which were converted into fat. Nucleolar extrusion in *Myxine* has been observed in pigment cells, connective tissue cells, blood cells, many gland cells, and in the male germ cells.

Schreiner regards the rodlets formed by the extruded nucleolar fragments as mitochondria, and considers this to be a case of conversion of mitochondria into fat. The transformation of mitochondria into fat globules has been described by Murray in the cells of a transplantable sarcoma of *Cavia* (13), where the mitochondria at first, either moniliform or filamentous, break up to form granules which become swollen and eventually are converted into fat globules. No mention is made as to the nucleolus being concerned in this process, but nucleolar extrusions are described and figured in cells of the same tumour, their ultimate fate being uncertain.

Nucleolar extrusions in somatic cells seem to be specially related to active metabolism of the protoplasm. Instances of this are the considerable nucleolar extrusions which occur in the epithelial cells of the silk glands of insects during the formation of silk, and in the cells of squamous epithelium of Mammals during the formation of keratin.

It is doubtful to what extent the nucleoli of oöcytes are comparable with those of somatic cells. Usually there is a single nucleolus present in the young oöcyte. This is the case in most Echinoderms, many Coelenterates, worms, molluscs and rodents (2). After the oöcyte has undergone the prophases of the heterotypic division, the chromosomes as such generally seem to disappear (5). This seems to be due to their spreading out in order to exert their maximum influence in the growing cell. Confirmation of this is afforded by the work of Maréchal on the oöcytes of Elasmobranchs (12), where after the diplotene stage the chromosomes spread out irregularly to form a structure which has been compared to a cylindrical chimney brush.

During the growth stages when the chromosomes are becoming less distinct in the nucleus of the young oöcyte, the nucleolus makes its appearance, and grows considerably in size. Usually there is one large plasmosome at the beginning. During the early stages of oögenesis in *Periplaneta* and *Libellula* (8), Hogben has recently shown that there is an extrusion of oxyphil material from such a nucleolus, and he concludes that this is connected with yolk formation and that "the chromatic organization of the nucleus retains its integral continuity throughout this phase." This is contrary to the opinion of Schaxel (14), who working on the oöcytes of Echinoderms and Tunicates came to the conclusion that chromatin was emitted from the nucleus and played an important part in yolk formation, a conclusion which is open to considerable doubt in the light of recent work.

In the oöcyte of *Saccocirrus*, Gatenby (6) has found a

complicated case of nucleolar extrusion. "The nucleolus buds off fragments which, passing into the cytoplasm, give rise by fission to numberless granules of a dense proteid nature; these granules form the main bulk of the reserve material of the Saccocirrus egg and constitute the yolk."

The behaviour of that part of the nucleolus which remains within the nucleus until the end of oögenesis varies in different animals. In many cases it seems to persist as a definite body until the chromosomes reappear, and then it disintegrates with the breaking down of the nuclear membrane preparatory to the maturation divisions. Kuhn (10), however, describes in *Daphnia* a large central nucleolus which breaks up to form a number of smaller granules which become spread over the linin network. At the late diakinetik stage when the nuclear membrane breaks down, the remaining nuclear mass disintegrates. Jörgensen (9) working on a species of *Patella* describes, in the youngest oöcytes, a nucleolus

EXPLANATION OF PLATES SHOWING THE BEHAVIOUR OF THE NUCLEOLUS IN PATELLA.

Lettering.—BB., basophil body or basophil staining nucleolus; BP., part of nucleolus which will form the basophil body; DB., disintegrating basophil nucleolus; FB., fragment of basophil substance derived from the basophil nucleolus; FO., fragment of oxyphil substance derived from the oxyphil nucleolus; GA., Golgi apparatus; LN., linin network of nucleus; NC., nurse cell; NE., oxyphil substance extruded from nucleolus; NL., nucleolus; OB., oxyphil body or oxyphil nucleolus; OC., oöcyte; OP., part of nucleolus which will form the oxyphil body; OS., oxyphil substance remaining attached to basophil nucleolus; T., trabecula wall; YS., yolk spherule.

The oxyphil nucleolus and the substance extruded from it into the cytoplasm are shown in red; the basophil nucleolus in black. The chromophility of the cytoplasm is not represented in colour owing to the difficulty of reproducing the gradations in colour between primary oxyphilia and basophilia, and basophilia and secondary oxyphilia.

All the figures are drawn from portions of the ovary fixed in either Bouin with acetic or corrosive acetic and stained with Mann's methyl blue eosin.

PLATE III.

Fig. 1.—Connective tissue cells, showing the oxyphil nucleolus (NL).

Fig. 2.—Portion of trabecula, showing young oöcyte (OC) with oxyphil nucleolus (NL), surrounded by yolk cells.

Fig. 3.—Older oöcyte with a large pear-shaped oxyphil nucleolus (NL) and extruded oxyphil substance in the cytoplasm.

Fig. 4.—Oöcyte at a later stage with nucleolus (NL) rounded off and extruded nucleolar material (NE) in the cytoplasm.

Fig. 5.—An early stage in the differentiation of the nucleolus into two parts. BP will become the basophil body, OP the oxyphil one.

Fig. 6.—Oöcyte during the process of differentiation, showing the extrusion of oxyphil material from the nucleus (NE) and the beginning of basophilia at BP.

Fig. 7.—A slightly later stage than Fig. 6. The basophilia of BP is more pronounced, and the nucleolar extrusion is still proceeding.

Fig. 8.—Oöcyte with distinct oxyphil (OB) and basophil (BB) nucleolar bodies joined together.

apparently amphophil, from which are budded off into the karyolymph two kinds of bodies—namely, amphophile bodies which develop a basophil cap, and oxyphil granules. During growth these become scattered throughout the nucleus and eventually arrange themselves peripherally as “Randnukleolen.” The behaviour of the nucleolus in this species is very different from what is described below in another member of the same genus, a difference which emphasizes the striking cytological dis-similarities which occur in closely related organisms.

TECHNIQUE.

The material used in the present investigation was fixed either in corrosive acetic or Bouin's modified solution, picro-formol acetic acid. The best staining results were obtained by using Mann's methyl blue eosin, which stained the nucleoli red, blue or purple according to the stages of development of the oöcyte. The same histo-chemical reactions, however, resulted from the use of Unna's methylene blue and eosin, lichtgrün and safranin, or hæmatoxylin and eosin used according to Scott's method. Nucleoli at similar developmental stages which stained blue with methyl blue took up the green stain when lichtgrün and safranin were used, or coloured purple when hæmatoxylin and eosin were employed. Such nucleoli are therefore referred to in this paper as the basophil nucleoli, while those nucleoli which colour with eosin or safranin are referred to as the oxyphil nucleoli.

In preparations made by the Mann-Kopsch method (11), stained with acid fuchsin and differentiated in picric acid, similar pictures were obtained as with the fixatives previously mentioned. However, the difference in staining reaction of different parts of the nucleolar content of the nucleus, although noticeable, was not so striking as with the other techniques. Sections fixed by the method of Mann-Kopsch were treated with potassium permanganate solution to rectify the loss of staining properties due to the osmic acid, and then treated with Mann's methyl blue eosin, but although the same essential results were obtained the staining reaction was not satisfactory.

The acetic acid fixatives have the further advantage over chrome-osmic acid when dealing with the nucleolus and nucleolar extrusions, as the chrome-osmic preserves the mitochondria, and these stain with acid fuchsin similarly to the nucleolus and its extrusions, and so render it difficult to distinguish between the two.

CHROMOPHILITY OF THE CYTOPLASM OF PATELLA.

In preparations made by fixation in corrosive acetic or Bouin with acetic, and stained by so-called acidic and basic stains, the

cytoplasm colours differently according to the stage of development of the oocytes. With Mann's methyl blue eosin the nuclei of typical somatic cells stain blue and their cytoplasm purple, while with hæmatoxylin and eosin used according to Scott's method the nuclei are purple and cytoplasm red. The undifferentiated cells of the germinal epithelium appear to stain in the same way. However, when such cells begin to develop into oocytes, a change takes place both in their nuclei and cytoplasm. The latter begins to stain basophil (that is, with methyl blue eosin it stains blue, with hæmatoxylin and eosin purple). During growth of the oocyte, by the time that yolk spheres are sparsely scattered throughout the cytoplasm its chromophility undergoes a further change. The blue or purple colour very gradually gives place to red again. This change in staining properties occurs at about the same time as the scattered mitochondrial granules (*II*) commence active division. In the full-grown oocyte the cytoplasm stains similarly to that of the undifferentiated epithelial cells.

There is therefore, in the process of development of the egg from a cell of the germinal epithelium, a change from primary oxyphilia to basophilia, followed by a state of secondary oxyphilia in the mature oocyte.

THE NUCLEOLAR PHENOMENA DURING THE OÖGENESIS OF PATELLA.

The nucleolar phenomena were studied in material fixed in Bouin (with acetic) or corrosive acetic, and stained with Mann's methyl blue eosin, or hæmatoxylin and eosin, or lichtgrün and safranin.

The nucleolus of a typical somatic cell stains oxyphil, as is shown in Fig. 1, Plate III, which represents connective tissue cells from the digestive gland. The nucleus of the youngest oocyte has also a small oxyphil nucleolus. In Fig. 2 is shown a young oocyte growing up from a trabecula of the ovary amongst three yolk cells. As such an oocyte as this grows the nucleolus increases considerably in size until it comes to occupy a large area within the nucleus. During such early growth phases there is a considerable extrusion of the oxyphil substance into the cytoplasm.

In Figs. 2 and 3 are shown the large oxyphil nucleoli (NL), and in the cytoplasm the oxyphil substance (NE) derived from them. This extruded substance appears to dissolve slowly in the cytoplasm, as different fragments show various intensities of colouration; some are as red as the nucleolus itself, while others are purple in colour, due it would seem to intermingling with the ground cytoplasm, which stains blue.

It will be observed from the figures shown in Plate III. that

the early extrusion of nucleolar material takes place when the nucleus is largest in proportion to the volume of the cytoplasm.

At the end of the preliminary growth stages the nucleolus becomes rounded off, and a distinct separation into two parts is noticeable. This is shown in the cell drawn in Fig. 5. From this stage onwards the two parts of the nucleolus react differently to stains. One part of the nucleolus (OP) still stains oxyphil, while the other gradually changes. At first, as shown at BP in Figs. 6 and 7, it stains a reddish purple, the colour change gradually spreads over the whole of this part of the nucleolus, and it becomes more and more bluish till eventually it appears as a definite basophil body, as is shown at BP in Fig. 8. The nucleolus then appears to be made up of two parts, one the oxyphil body, the other the basophil.

During the process of differentiation into two parts there seems always to be an extrusion of oxyphil substance into the cytoplasm. This is shown specially strikingly in Fig. 7, where the nucleolus is lobulated and oxyphil fragments are breaking away from it and passing out through the nuclear membrane into the cytoplasm. The nuclear membrane does not appear to be damaged by this process, which recalls the passage of leucocytes through the walls of blood capillaries. The oxyphil portion of the nucleolus during this process is usually vacuolated.

By comparing preparations fixed in corrosive acetic with those made by osmium tetroxide fixation (e.g. Mann-Kopsch-Altmann technique), it is seen that the early extrusion of nucleolar substance takes place before the dispersion of the Golgi elements, and the latter process commences about the time that the nucleolus becomes differentiated into oxyphil and basophil portions. Such comparisons lead one to assume that the oxyphil material dissolves in the cytoplasm and plays some part in preparing the cytoplasm for the activities of the Golgi elements, which, it was shown in a previous paper (11), are responsible for the formation of yolk spherules. Whether or not this be the case, the nucleolar extrusion ceases for a time, and is not so pronounced from this point onwards, until the end of the oögenetic process.

After the differentiation of the two parts of the nucleolus, the oxyphil and basophil portions may break up into several smaller bodies, or the two parts may remain joined, or they may separate and form distinct basophil and oxyphil nucleoli. The latter seems to be the commonest occurrence, and in such cases very often there is found a small portion of oxyphil substance remaining attached to the basophil body.

From the differentiation stage onwards, the oxyphil part of the nucleolus becomes gradually smaller in proportion to the basophil portion, and a certain amount of extrusion of oxyphil substance goes on. This is seen by comparing Figs. 9 and 10. The two

nucleoli however remain distinct throughout the process of yolk formation, but by the time that the yolk bodies are fairly evenly scattered through the cytoplasm, both the oxyphil and basophil bodies have become vacuolated, and they commence to disintegrate. The oxyphil nucleolus breaks up into fragments which pass into the cytoplasm, as is seen taking place in Fig. 11, while the basophil nucleolus disintegrates and spreads out over the linin network of the nucleus. Fig. 12 shows this process taking place. When the basophil material is spread out in this manner there appears to be a small quantity of oxyphil substance left over, as is seen in Fig. 12 at FO. This seems eventually to pass out into the cytoplasm.

The basophil substance becomes spread out evenly over the linin network and forms little bodies at the nodes of the reticulum (FB in Fig. 13), resembling similar structures in the nuclei of somatic cells which are usually described as karyosomes.

It has been impossible to ascertain accurately whether a small quantity of oxyphil matter does or does not remain in the mature oöcyte, owing to the present difficulty of obtaining oöcytes at the maturation stages. This problem, together with the subsequent fate of the nucleolus, is still under investigation.

DISCUSSION.

It will be noticed from the above description that the oxyphil nucleolus resembles in its general behaviour that of *Libellula* and *Periplaneta* (8) as described by Hogben, in that there is an early extrusion of oxyphil granules from the nucleolus into the cytoplasm. These granules however "pass to the periphery of the oöcyte, where they break up into smaller spheres which become the first vitellus." A somewhat similar process has been described by Gatenby in the

PLATE IV.

Fig. 9.—Older oöcyte with the two parts of the nucleolus separated. Extrusion is taking place of oxyphil substance which had been left attached to basophil nucleolus.

Fig. 10.—Oöcyte at a later stage with a large vacuolated basophil nucleolus (BB), and a small vacuolated oxyphil nucleolus (OB). Yolk spherules (YS) are fairly evenly scattered throughout the cytoplasm.

Fig. 11.—Older oöcyte showing the breaking up and extrusion (FO) of the oxyphil nucleolus and the beginning of the spreading out of the basophil substance (F and FB).

Fig. 12.—Nucleus showing the disintegration of the basophil nucleolus (DB), and the dispersal of its substance (FB) over the linin network (LN). Oxyphil substance produced as the result of this process is seen at FO.

Fig. 13.—Nucleus with basophil substance (FB) fairly regularly arranged upon the linin network. At NE is seen some of the remaining oxyphil substance being extruded into the cytoplasm.

case of *Saccocirrus* (6), where there is an amphinucleolus with basophil preponderance in the youngest oöcytes. From this nucleolus portions break away and pass through the nuclear membrane, remaining attached for a time to its outer surface. A basophil cloud at this stage appears round the nuclear membrane and conceals somewhat the extrusions. This corresponds with a change in the chromophility of the cytoplasm from primary oxyphilia to basophilia. The basophil cloud disperses and the nucleolar granules become arranged spherically around the nucleus. Later they become scattered irregularly in the cytoplasm, and Gatenby shows that by fission they give rise to smaller granules which grow into yolk spherules. In the oöcyte of *Saccocirrus* there are also present small yolk bodies derived from the Golgi apparatus.

The chief difference between these cases and that of *Patella* is that in the latter the nucleolar extrusions appear to dissolve in the cytoplasm, and it seems most probable that this dissolved nucleolar substance either becomes converted into yolk under the influence of the Golgi elements, or else acts as an enzyme during yolk formation. This is borne out by the fact that there is always considerable nucleolar extrusion before the Golgi elements spread out in the cytoplasm to form the yolk granules.

In the oöcytes of Ascidians, according to Hirschler (?), there are present densely staining granules which give rise both to mitochondria and to a yolk nucleus. While the mitochondria are breaking away and spreading out in the oöcyte, the densely staining granule is temporarily connected with the nucleus by a stalk along which granules appear to flow out from the nucleus. The yolk granules of Ascidians are complex bodies formed as the result of the fusion of a Golgi element and a swollen mitochondrion, so that it may be that the mitochondria in these cases contain a small quantity of nucleolar substance, and this functions as an enzyme in the formation of yolk under the joint influence of mitochondria and Golgi elements.

The chromophility of the cytoplasm of the Ascidian oöcytes undergoes two changes. At first, during the primary diffused stage of the Golgi apparatus, the cytoplasm stains oxyphil. When the Golgi elements come together to form a compact body the cytoplasm becomes basophil, and the change to secondary oxyphilia is concurrent with the scattering of the Golgi elements, and the formation of yolk from them and the mitochondria. In *Patella* the Golgi apparatus is compact in the youngest oöcytes, and the change from primary oxyphilia to basophilia is unrelated to any observable change in the apparatus. The transition from basophilia to oxyphilia seems to take place at the same time as the fission and spread of the mitochondria, and this occurs after the early nucleolar extrusion has taken place and the nucleolus has been differentiated into oxyphil and basophil portions.

One of the most remarkable cases of nucleolar extrusion is that which has been described in Hymenoptera by Buchner (3), Hegner (see 6), and Gatenby (6). In the oöcyte of certain Hymenoptera, the nucleolus buds off bodies which pass out of the nucleus into the cytoplasm, where they give rise to "secondary nucleoli" with a basophil reticulum and an oxyphil nucleolus. The function of such "secondary nucleoli" is not properly known.

The behaviour of the basophil nucleolus of the *Patella* oöcyte is paralleled by that of *Daphnia pulex* worked out by Kuhn (10), and I have observed exactly the same process occurring with the basophil nucleolus of *Limnæa*. The large central nucleolus in the oöcyte of these organisms towards the end of oögenesis becomes vacuolated and breaks up to form a mass of granules which spread over the linin network, as in *Patella*. Kuhn found that with the reappearance of the chromosomes, preparatory to the maturation divisions, the greater part of the nuclear matter disintegrated. Lack of suitable material has so far prevented the observation of the later stages in *Patella*, but the problem is still being investigated.

Maréchal found a process of disintegration of the nucleolus of a somewhat similar nature in the oöcytes of Selachians (12) towards the end of oögenesis. When the chromosomes were reforming, the nucleolus broke up into a mass of granules which became scattered in the nucleus, and in some cases formed rodlets resembling the chromosomes.

It is very difficult to find any explanation of the function of the nucleolus in such cases as these. In *Patella* and *Limnæa*, the basophil nucleolus is undoubtedly a specially elaborated substance, and it seems to me possible that it stands in some functional relationship to the subsequent condensation of the chromosomes as compact rodlets out of the diffused chromatinic reticulum. Possibly this is effected by producing a shrinkage or contraction of the framework upon which the chromatin is distributed, or may be by a kind of flocculation.

This is borne out by the fact that in preparations of the ovotestis of *Limnæa* which I have studied, there is a marked elaboration of basophil substance in the oöcytes, and towards the end of oögenesis, the basophil body disintegrates and spreads over the linin reticulum, as in *Patella*. In the spermatocytes, however, there is an amphinucleolus with definite oxyphil preponderance, which is present in the cells up to the late synaptic stages of the heterotypic prophase. There is no elaboration of definite basophil substance, nor is there any extrusion of nucleolar material during spermatogenesis.

Recent work on staining reactions has shown that we have no stain which can be relied upon as a criterion for chromatin. In preparations of the ovotestis of *Limnæa stagnalis* fixed in

corrosive acetic or Bouin (with acetic), and stained with Mann's methyl blue eosin, I find that the chromosomes of the spermatocyte during reduction division appear amphophile with distinct basophil preponderance, and later the same chromatinic substance forming the head of the spermatozoon appears brilliantly red. Gatenby finds a similar thing in *Saccocirrus* (6), after fixation in Petrunkevitch and staining in Ehrlich's or Delafield's hæmatoxylin and eosin or Biebrich scarlet. It is therefore absolutely unjustifiable on staining tests alone to call a basophil staining nucleolus a chromatin body, or to deny that an oxyphil nucleolus may contain chromatin.

It is also known that the fixative used may determine to a great extent the nature of the subsequent staining. Jörgensen, in his work on *Patella*, found the nucleolus amphophile with basophil preponderance in the youngest oöcytes, while in the species of *Patella* described in this paper the nucleolus was in most cases distinctly oxyphil, although there were exceptions where it could be described as amphophile with distinct oxyphil preponderance.

I attribute this difference in a large degree to the fixative used, for in making preparations with *Limnæa* material, I find that after fixation with Bouin's fixative the nucleolus stains oxyphil, while with prolonged fixation in corrosive acetic it stains basophil.

Observations such as these detract from the value of much work on nucleoli and chromatin based upon staining reactions alone. Also we know that differences in staining may be the result of differences in the surface membrane of the objects stained. In the case of *Patella* however, where there is a functional differentiation of the nucleolus into two parts, I consider it is justifiable to assume that the difference in staining reactions of oxyphil and basophil nucleoli corresponds to a difference in chemical constitution. That there is a difference in the chemical composition of the nucleoli of different animals has been shown by Jörgensen (9), who, as the result of digestion experiments with pepsin, divides nucleoli into three classes—namely, those immediately digested, those digestible after prolonged exposure to the ferment, and those indigestible to begin with, but soluble later.

Jörgensen concluded "that we can only say with certainty that the nucleolar substance is a functional organ during egg growth, and is no worthless metabolic product"; and all that we are justified in adding at present, is that it is in several species related to the formation of yolk, while in others it gives rise to "secondary nucleoli," and that it may stand in some functional relationship to the formation of the small condensed chromosomes from the much spread out structural forms the latter assume during the various formative processes in the oöcyte, but such a hypothesis does not imply any interference with the integral continuity of the chromosomes throughout the germ cell cycle.

SUMMARY.

A.—Chromophility of the Cytoplasm of the Oöcyte of Patella.

1. Transition from an undifferentiated cell of the germinal epithelium to an oöcyte is marked by a change in the chromophility of the cytoplasm from oxyphilia to basophilia.

2. During the early stages of oögenesis, the cytoplasm remains basophil. A change towards secondary oxyphilia occurs about the time that the Golgi elements have become scattered and the mitochondria are commencing active division.

3. From this stage onwards, the cytoplasm becomes gradually more oxyphil till definite secondary oxyphilia is attained.

B.—Behaviour of the Nucleolus of the Oöcyte of Patella.

1. The nucleolus of the youngest oöcytes resembles that of certain of the somatic cells in being an oxyphil body.

2. During the early stages of growth before the commencement of yolk formation, the oxyphil body extrudes portions of itself into the cytoplasm. These fragments disintegrate or dissolve.

3. At an early stage the oxyphil nucleolus becomes differentiated into two parts, one of which remains oxyphil, while the other begins to stain basophil.

4. There is an extrusion of oxyphil substance during the differentiation of the nucleolus, especially marked in that part which is becoming basophil.

5. Definite basophil and oxyphil parts of the nucleolus are established, and in most oöcytes they separate into distinct spherical bodies. During the later stages of oögenesis, the oxyphil nucleolus dwindles in size, and ultimately fragments into granules which are extruded into the cytoplasm, while the basophil nucleolus disintegrates and becomes distributed upon the linin network, mostly in the form of little accumulations of basophil material around the nodes of the reticulum.

6. It is suggested that the oxyphil substance extruded from the nucleus is related to yolk formation. The basophil substance is tentatively assumed to stand in functional relationship to the condensation of the spread out chromosomes into discrete chromosomes from the chromatinic network. This does not imply any interference with the integral continuity of the chromosomes throughout the germ cell cycle.

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