CYTOPLASMIC INCLUSIONS IN THE EGG OF ECHINARACHNIUS PARMA

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ONE TEXT FIGURE AND FOUR PLATES (TWENTY-FOUR FIGURES)

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INTRODUCTION

The cytoplasm of the egg has attracted a great deal of attention among cytologists during the last few years, in contrast to the almost universal attention paid to the nucleus before that time. A great many observations on cytoplasmic inclusions have been made, but there is a distinct lack of coördination of the results of such work. Cowdry, in his valuable contribution to the literature on mitochondria, has summed up and correlated the observations and conclusions of various authors regarding these structures. Numerous other bodies occurring in the cytoplasm have been reported, but usually investigators have given merely a description of the morphology and staining reactions of these bodies. There have been, however, attempts to consider cytoplasmic inclusions in the light of the physiology of the

cell and to trace the interrelations of distinct bodies through different stages. The possible transformation of mitochondria into secretion granules, pigment, yolk, etc. (Cowdry gives a list of eighty such things into which mitochondria have been reported to change), and the cycle described by Schreiner ('15), in which fragments of the nucleolus wander into the cytoplasm, unite into vegetative threads, and break up into secondary granules which are ultimately transformed into fat drops, are instances of these attempts to consider visible structures as steps in the physiological processes of the cell.

The processes of metabolism in the cell necessarily include activities of both nucleus and cytoplasm. The part played by the nucleus is not fully understood. Some investigators have described particles of material passing out of the nucleus into the cytoplasm. These particles have been regarded as chromatin (Schaxel, '11; Danchakoff, '16), or as fragments of the nucleolus (Schreiner, '15; Nakahara, '17; Walker and Tozer, '09; Hargitt, '19, etc.). According to other investigators, the nucleus acts on the cytoplasmic substrate by liberating enzymes, which diffuse through the nuclear membrane and permeate the cytoplasm. Tennent ('20) has found in Arbacia eggs fertilized by Moira sperm, precipitates in the cytoplasm which are interpreted as the result of enzymes from the nucleus brought in by the foreign sperm.

The present work was undertaken in the hope of demonstrating more exactly the relation between the nucleus and the cytoplasm by comparing the cytoplasmic contents of an egg fertilized by sperm of its own species with that of an egg fertilized by sperm of another species. It was thought possible that the cytoplasm when acted on by two different types of nuclear enzymes might show visible differences. In the study of the particular cross made, Echinarachnius × Arbacia, no such visible differences between the self-fertilized and the cross-fertilized eggs have been found. This does not invalidate the conclusion that the nucleus gives out enzymes into the cytoplasm. It probably indicates that in the particular cross used here the enzymes of the foreign sperm were so much like those of the species sperm that no

visible differences in effect occurred. It should be remembered, however, that minor chemical variations may easily occur without giving rise to visible differences.

Attention was then directed to the cytoplasmic contents of the egg before fertilizaton and the changes which occur after fertilization and during the early stages of development. The study of the cytoplasm of the egg has certain peculiar advantages over the study of the cytoplasm of tissue cells. To be sure, one does not find secretion granules or other structures associated with specialized function, but there are instead those substances necessary for processes of development and differentiation. By the use of various methods, several types of inclusions have been demonstrated and certain conclusions regarding their part in the general metabolism of the cell have been reached.

The work was undertaken at the suggestion of Dr. David Hilt Tennent and pursued under his direction. It is a great pleasure to express my appreciation of his constant and stimulating supervision throughout the course of the investigation.

PREPARATION OF MATERIAL

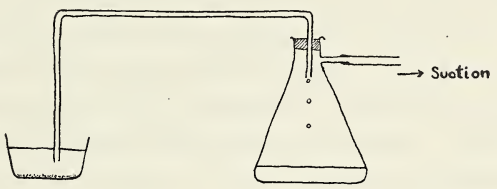
The material for this work was collected, fixed, and imbedded at Woods Hole during July and the early part of August, 1920. The particular eggs used were those of the sand-dollar, Echinarachnius parma. They were fertilized by Echinarachnius sperm or Arbacia sperm, and in all cases parallel series were kept of the self-fertilized and cross-fertilized eggs. Just ('19) has shown that when the eggs are normally shed into sea-water they may be cross-fertilized without special treatment. However, it is very rarely that Echinarachnius females can be obtained at Woods Hole which will shed their eggs. To stimulate them to shed, the test is clipped around the circumference with scissors and the animal placed aboral face down on a watch-glass. Although this was done with individuals from practically every lot of sand-dollars brought in from June 28th to August 4th, only one animal was obtained during that time which shed eggs. Therefore, it was necessary to open the test and shake out the ovaries in sea-

water. The eggs of this form are mature when shed or when they easily shake out of the ovary, and may be fertilized by species sperm as soon as they are clean, but eggs need further treatment before cross-fertilization. To clean from bits of ovarian tissue and coelomic fluid, the water in the finger-bowl in which the ovaries have been shaken is stirred, then allowed to settle for a brief period and the supernatant water poured off. The finger-bowl is then refilled with fresh sea-water and the process repeated several times. In this way the heavier eggs which settle are kept, while the lighter debris is poured off. Since these eggs were not normally fertilizable by Arbacia sperm, it was necessary to resort to some artificial means of breaking down the cortical resistance. As in other eggs, there are three methods of breaking down this resistance, namely, 1) by staling the eggs, 2) by over-insemination, and, 3) by the use of alkali. For the purpose of this work the third method was employed. To determine the optimum concentration of alkali, two experiments were performed. Varying amounts of n/10 NaOH in one case were added to sea-water, and n/10 KOH in the other. After fertilization by Arbacia sperm, the percentage of development was recorded. Very little difference was found between the NaOH and the KOH. The standard strength of twenty drops of n/10 NaOH per 150 cc. of sea-water was adopted as the proportion of alkali yielding the best results. The alkaline sea-water was added to the cleaned eggs from which most of the water had been poured, and as soon as possible the sperm suspension was added and the contents of the dish well mixed. As soon as the eggs had settled, the supernatant liquid containing excess sperm and alkali was drawn off by means of a suction flask connected as figured.

By this method all the liquid save about 8 cc. could be quickly withdrawn, leaving the eggs undisturbed on the bottom of the finger-bowl. Fresh sea-water was then added. After self-fertilization the supernatant water was similarly withdrawn and replaced by fresh sea-water.

In any one series of eggs preserved the eggs from only one female were used and they were fertilized by sperm from one

male of the same species or from one male Arbacia. In every case three finger-bowls were kept. In one, kept as a control, were placed eggs, 150 cc. of sea-water, and no sperm; in the second, eggs fertilized by species sperm, and in the third, eggs fertilized by Arbacia sperm by the aid of alkali. A sample of unfertilized eggs was preserved, and from the second and third finger-bowls samples were preserved at varying intervals after insemination. For example, series 4 was fixed every fifteen minutes, series 9 every twenty minutes, series 17 every ten minutes, etc. The original bowls were kept until the following day and examined to make sure that no contamination had occurred before actual insemination, as proved by the failure in every case of the eggs in the control to form fertilization membranes or to cleave. In



order to prevent chance fertilization, hands, instruments, and dishes were washed in fresh water before opening each animal. In addition, the animal itself was rinsed in fresh water and then in sterilized sea-water before being opened. Frequently all dishes, pipettes, and instruments were put into a large kettle and boiled.

The fixing fluids used were picro-acetic (saturated aqueous picric acid 95 parts, glacial acetic acid 5 parts), sublimate-acetic (saturated aqueous corrosive sublimate 100 parts, glacial acetic acid 5 parts), Bouin's fluid, Allen's warm modified Bouin (Bouin 50 cc. urea 1 gram, chromic acid 0.75 gram) made up immediately before using, Perenyi's fluid, Meves' fluid (Lee, Vade-Mecum, 7th ed., p. 328), Champy's fluid (3 per cent potassium bichromate 7 parts, 1 per cent chromic acid 7 parts, 2 per cent osmic acid 4 parts), Cajal's fluids, Helly's Zenker-formol, strong Flemming,

and Flemming without acetic acid. In the last-named fluid one series was fixed for one day and another for seven days. The fixation in Perenyi and Cajal was very poor, and therefore the material was discarded. All the material was imbedded at Woods Hole in soft paraffin, then taken to Bryn Mawr, reimbedded, sectioned, and stained. The sections were for the most part 4μ in thickness.

The stains employed were Heidenhain's iron hematoxylin, Auerbach's acid fuchsin-methyl green, lithium carmine and Lyons blue, basic fuchsin and methylene blue, Benda's alizarin and crystal violet, safranin, safranin and gentian violet and orange G, and for special tests, sudan III and Ziehl's carbol-fuchsin. Samples of material fixed in solutions containing osmic acid were also mounted unstained. Of these stains, the iron hematoxylin and the Benda stain proved the most satisfactory and were the most widely employed. In making up the alizarin the directions given in Guyer's Animal Micrology were followed rather than those given by Benda himself or by Cowdry in describing Benda's method. Guyer gives the following formula for Benda's solution of sulphalizarinate of soda: 1 part of saturated aqueous solution of stain to 80–100 parts of water. Benda's own directions are to add 1 part of a saturated alcoholic solution of the stain to 80–100 parts of water. Both methods were tried but Grübler's sulphalizarinate of soda was found to be practically insoluble in alcohol. The stain made from the saturated aqueous solution of the dye gave excellent results.

OBSERVATIONS

As has been mentioned above, there were no visible differences between the self-fertilized and the cross-fertilized eggs. Any given method of fixation followed by the same stains gave identical results in the two cases. In order to compare them the better, sections of self-fertilized eggs and sections of cross-fertilized eggs were mounted side by side on the same slide. This insured exactly the same degree of staining.

A number of structures were found in the cytoplasm following different methods of fixation and staining. All the bodies

found could be demonstrated in the unfertilized egg, but some of them changed or disappeared during subsequent stages of development. Where there were progressive changes in the cleavage stages, a whole series was mounted on one slide. Thus any variation due to differences in technique of staining was eliminated, since all stages of the same series received the same treatment.

Gatenby ('19 b) and others have classified cell inclusions in two main groups: first, inert inclusions like deutoplasm and, second, active or living inclusions like mitochondria. As deutoplasm are classed fat, glycogen, yolk, etc. The egg of Echinarachnius parma contains a considerable amount of fat. The glycogen, if there had been any, would have been dissolved out by the technique employed in preparing this material. The cytoplasm is packed with spherical or plate-like bodies of nutritive material which is identified as yolk. Active inclusions in the form of mitochondria have been demonstrated. There is still another type of structure found in these eggs. It is an inert inclusion and yet is not deutoplasm. It will be considered under the heading 'Precipitations.' The occurrence of these substances will be considered more in detail.

A. Deutoplasmic inclusions

1. Fat. It is known (Partington and Huntingford, '21) that fat droplets reduce osmic acid to osmium dioxide and assume, therefore, a dense black appearance after the use of a fixing fluid containing osmic acid. Accordingly, eggs which had been fixed in Fleming, Flemming without acetic, or Meves' fluid were mounted unstained and examined for fat. No fat was found in the material which had been fixed for seven days, but in the Flemming and in the Flemming without acetic material which had been fixed for eighteen to twenty-four hours there were numerous black bodies. Figure 1 shows an unfertilized egg fixed in Flemming without acetic and mounted unstained. There are in the cytoplasm large blackened masses surrounded by fine droplets of blackened material of uniform size, and in some cases there are clumps of fine droplets without any central larger drop.

This emulsified condition is a characteristic of fats. It seems probable that the original large drop of fat is being split up into smaller parts and that the scattered fine particles of blackened material throughout the cell have been formed by such emulsification of larger masses.

Sudan III, a specific stain for fat, was used on this material, but gave no decisive results because the drops had been previously blackened and naturally could not be stained red. A further proof of their fatty character was obtained by soaking the sections for twenty-four hours in oil of turpentine. After this treatment the black droplets were completely dissolved out. Since turpentine is a fat solvent, the material which was removed was probably fat.

The large groups of fat droplets are slightly more numerous in the region of the nucleus than they are in the more distant parts of the cytoplasm. This is of interest in the light of the views of Schreiner, Popoff, and others, that granules of nuclear origin pass through the membrane and give rise to fat droplets. The granules are believed to come from the nucleolus. The evidence given here of the accumulation of fat near the nucleus shows nothing more than the fact that they are associated with some kind of nuclear activity. It is also true that an occasional oocyte has been found on the slides in which there is always a large nucleolus present which is entirely absent in the ripe egg.

The continued splitting of the large drops into minute droplets and the dispersal of the latter through the cytoplasm is illustrated in figures 2 and 3. These show eggs from the same series from which figure 1 was drawn, in stages twenty-five minutes and one hour and forty minutes, respectively, after insemination. As development proceeds, there is a gradual decrease in the amount of blackened material present. As the cell prepares for the first division the fat droplets are much fewer, and in the two-celled stage none are visible. It is highly probable that these cells do not show as much fat as is present in the living egg, because some of it must have been dissolved out in the processes of preparation, but the fact that a definite series of changes can be demonstrated is a true indication of what actually occurs.

2. Glycogen. As was mentioned formerly, no glycogen was demonstrated in these eggs. No material was fixed by any of the methods for the preservation of glycogen.

3. Nutritive plates (yolk). By far the most conspicuous and unusual inclusions are those which are best demonstrated after fixation in Flemming without acetic, Meves' or Champy's fluids, and staining in iron hematoxylin or Benda's alizarin and crystal violet. These bodies are shown in figures 1 to 6, 12, or 19 to 24. In figure 12, drawn from an egg fixed in Champy's fluid, they are distinctly plate-like and much larger than in any other lot of material. The more usual appearance is shown in figures 19 to 24, where they are smaller and less distinctly plates. Their fate indicates that they are nutritive in function. They do not, however, respond to all the usual tests for yolk. At first their staining reaction seems to mark them as mitochondria, for in the series illustrated in figures 19 to 24 the fixation is Flemming without acetic and they are stained a deep violet with the Benda stain. They do not always give this reaction, however. The series described above was fixed for seven days. If it be compared with the series shown in figures 4 to 6, which is also Flemming without acetic, but fixed for eighteen to twenty-four hours only, it will be seen that the large bodies are there in both cases, but they differ in staining capacity. They take the violet stain strongly after seven days' fixation, but are pink after one day's fixation. The behavior of these two series when stained with iron hematoxylin shows great dissimilarity also. In the first case the plates are black and in the second they do not stain. Since they are so striking in appearance in some series of eggs, their apparent absence after other fixatives was unaccountable until it was found that in practically every case the material of which these plates are formed could be shown in the cytoplasm even though not in the form of such distinct separate bodies. For instance, when stained in iron hematoxylin after fixation in modified Bouin, the cytoplasm had a decidedly reticular appearance with minute black granules throughout (fig. 18); in the same material stained in Benda's stain (fig. 15), or in iron hema-

toxylin and basic fuchsin, the cytoplasm had a mottled appearance. A distinct difference between the cytoplasm of the center of the egg in which the division figure lies and the peripheral cytoplasm may be seen. This regional differentiation corresponds to the distribution of the nutritive bodies fixed so distinctly in Flemming without acetic. This shows that the nutritive material is present after modified Bouin fixation. Similar results were found after sublimate-acetic fixation. After strong Flemming followed by safranin, gentian violet, and orange G, this nutritive material takes up the orange stain more strongly than any other cell constituent. Therefore, while certain fixing fluids are decidedly more favorable for the demonstration of these plates, the material is not dissolved completely by the other reagents used.

Yolk is the only substance ordinarily found in the cytoplasm of the egg in great quantities comparable to this material. But these plates do not respond to all the tests for yolk given by Miss Beckwith ('14). For instance, yolk turns black after Flemming fixation and iron-hematoxylin stain. These bodies are not black. Yolk is definitely fixed by picro-acetic and sublimate-acetic. Gatenby ('19 b) states that in some animals yolk discs take a deep violet after Benda's stain. He points out that this staining reaction may be due to protein in the yolk in addition to lecithin. The yolk plates in the egg of Echinarachnius, however, do not invariably stain a deep violet, but do so only after prolonged fixation. Some proof of the nature and function of these bodies may be obtained by tracing them through the early cleavage stages to the blastula. They become gradually fewer in number and in the blastula they are almost gone. Figures 19 to 24 show progressive stages in which they have become fewer. Figure 24 illustrates a stage four hours after insemination in which there are spaces left which the large plates formerly occupied and in which many of the minute granules are stained violet. This may be due to the breaking up of the larger masses by a process of digestion. The disappearance of this material is not confined to those series which have been fixed in Flemming without acetic Meves, and Champy. Figure 11 (picro-acetic) shows a much

more spongy and vacuolated cytoplasm than the earlier stages of the series, which indicates a loss of some substance. Figures 16 and 17 show a similar phenomenon in modified Bouin material. Therefore, these plates are of a nutritive character and are used up by the cell during processes of development.

They contain a certain amount of fat, as is shown by staining with sudan III. Fifteen minutes' staining with alcoholic sudan III gives them a very decided salmon-pink color. After soaking for twenty-four hours in oil of turpentine, the sections stained in sudan III gave no color whatever, even when the stain was allowed to act for thirty minutes. There was evidently some fat there which was removed by the turpentine. After treatment with turpentine the plates retained their characteristic form and appearance except that they lacked the capacity to take up sudan III. The nutritive plates are probably of complex chemical structure.

While the nutritive plates do not in all cases behave like yolk with regard to staining reactions, yet their obvious function and their shape and distribution point to the conclusion that they must be yolk.

From their method of origin these yolk plates may be linked up with the other cell inclusions. This point will be considered after the discussion of mitochondria.

B. Living inclusions

Mitochondria. In addition to the above deutoplasmic inclusions, there are also active, living constituents which are distinct from the ground cytoplasm. These are the mitochondria. The stain which differentiates them most successfully from other cell inclusions is Benda's alizarin and crystal violet. They show up a deep violet against a background of neutral pink. Iron hematoxylin blackens them, but it also blackens other cell granules. With basic fuchsin and methylene blue they are red, with safranin they are red. It is possible to demonstrate mitochondria after fixation in Flemming, Flemming without acetic, Meves, Champy, and modified Bouin. They are not found

after ordinary Bouin fixation. The shape and distribution of mitochondria in the eggs of Echinarachnius are shown in figures 13 to 17 which were drawn from a series of eggs fixed in modified Bouin and stained in Benda's alizarin and crystal violet, and in figures 4, 5, and 6 which were drawn from a series fixed in Flemming without acetic followed by the Benda stain.

The study of this material indicates that the mitochondria bear a definite relation to the fine uniform fat droplets described above. In figure 4 there is shown an unfertilized egg fixed in Flemming without acetic and stained according to Benda's method in which the mitochondria were deep violet. They are shown as black granules in the figure. The egg also contains granules which appear the same in size, shape, and distribution but which take the violet stain with varying degrees of intensity or which may be quite pink like the ground cytoplasm. There is also a continuous variation in color from the small fat droplets which are brown from osmic-acid impregnation, through similar granules which are less and less brown, to pink granules. It seems probable, therefore, that the small fat droplets which are formed by emulsification of larger fat drops change gradually, as indicated by differential staining, into the bodies which take a deep violet stain after Benda's method and are identified as mitochondria.

In the modified Bouin material, the mitochondria are stained a deep violet with the Benda stain. They are the granules illustrated in figures 13 to 17. These same eggs when stained with iron hematoxylin show many more black granules than can be identified as mitochondria. Figure 18 shows such an egg thirty minutes after insemination which contains a larger number of black granules than the number of mitochondria shown in figures 14 and 15. No granules stain with sudan III after this fixation.

The relation between the mitochondria and the nutritive plates may be considered here. The great mass of plates is already formed by the time the egg is ripe, but there is some indication of how they may be formed. In the eggs from which figures 4, 5, and 6 were drawn the mitochondria were stained violet and the nutritive plates pink. In figure 4 and more especially in figures 5 and 6 some of the mitochondria show pale centers. In fact,

sometimes the inside distinctly took the pink alizarin stain. This is interpreted as showing the formation of the nutritive plates, or yolk, from mitochondria. The nutritive material accumulates at the center and increases until it is nearly as large as one of the numerous yolk plates. When this occurs the mitochondrial remnant is found as a delicate violet rim around the surface of the plate, or there may be in addition a larger bit of the violet-stained material clinging to one side.

There have thus been followed the fat drops which are emulsified into minute fat droplets and are distributed through the cytoplasm where they are probably transformed by some kind of synthesis into mitochondria. These mitochondria in turn build up within themselves the large nutritive plates which furnish energy for the cleavage processes. The line which is drawn between active and inactive inclusions is, in the case of the egg of Echinarachnius parma, purely arbitrary. All transitional stages between deutoplasmic granules like fat, and mitochondria have been demonstrated.

C. Precipitations

After the use of picro-acetic and sublimate-acetic fixatives, striking bodies which stain strongly in iron hematoxylin are found in the cytoplasm. These are the large black masses surrounded by clear areas shown in figure 7. They are invariably at the centers of open spaces, which leads to the conclusion that they are condensed or precipitated from material once occupying the entire space. After fixation in sublimate-acetic they tend to assume a slightly more elongated form than after picro-acetic as figured. Such irregular precipitations are found in all unfertilized eggs fixed either in picro-acetic or sublimate-acetic. Proof that eggs containing them are normal is found in the subsequent history of some series where practically 100 per cent development followed fertilization. It may be argued that these precipitations are the result of the action of the fixing fluids, since, as has been pointed out by Mathews (Physiological Chemistry, p. 120 and p. 1086), the salts of metals and picric acid have the power to precipitate proteins. The following facts prove that this cannot

be the entire explanation of the matter. These masses are actual precipitations of material in the normal cell, for in later stages these fixing fluids do not form such precipitations. Figures 7 to 11 show that the bodies have become fewer as development has proceeded. By the time the first division is well under way all the large masses have disappeared and there is no indication of the larger spaces in which they lay. They do not again reappear after the first division.

Similar precipitated masses may be found following other methods of fixation, but the precipitations do not actively take up any of the stains employed; they are always like the diffusely stained ground cytoplasm. Figures 13, 14, and 15 illustrate their appearance following modified Bouin fixation. In these eggs, stained in alizarin and crystal violet, they were of the pale pink color of the great mass of cytoplasm, and therefore not so conspicuous as when stained in iron hematoxylin after picro-acetic or sublimate-acetic fixation. In some eggs they looked almost like bacteria, but they failed to stain with Ziehl's carbol-fuchsin. Also their behavior in later stages could not be accounted for if they were bacteria.

As to the nature of these bodies, nothing is known beyond the fact that they are precipitations of colloidal material in the cytoplasm. It is probable that these bodies are of the same character as the rods observed by Tennent ('20) in the egg of Arbacia fertilized by Moira sperm, after fixation in sublimate-acetic.

In the unfertilized egg there is no uniformity in the orientation of the precipitated masses, but as soon as the spindle for the first division begins to form, they begin to be oriented parallel to the astral radiations extending through the cytoplasm. They appear as if swept about by the currents of more fluid protoplasm flowing in toward the focus of the aster, until they present the least surface in opposition to the direction of flow. Such a flowing in of more fluid protoplasm to the centers of the hyaline areas (such as are shown in figure 10) as the protoplasm goes into a state of gelation at division, was demonstrated by Chambers ('19). In this connection, Bowen's observations on the division

of mitochondria in Hemiptera ('20) may be mentioned. He states that the fact that the mitochondria are oriented with definite relation to the centrosome proves that they are under its directive influence, and therefore there is a mechanism for exact division of mitochondria in mitosis as well as for chromosomes. Since the flowing of material described by Chambers occurs at this time, it is inevitable that any inert masses lying in the cytoplasm should be swept into line, and their orientation by this means need have no connection with any attractive force exerted on them by the centrosome.

DISCUSSION

All of the inclusions found in the egg of Echinarachnius parma have been found in the unfertilized egg and they have decreased during cleavage. This is wholly in accord with the nature of the processes going on in the egg at this time. Its growth period is past and it is about to start on a series of changes involving great energy expenditure. While these changes are occurring there is no opportunity for the aquisition of food material either from maternal tissue or from the medium in which the cells are living. All energy then must come from materials stored up within the egg at the time it is set free from maternal tissues. This does not in any way preclude transformations of some materials in the cytoplasm into others, and it is believed that such transformations have been demonstrated. Here there are reduced to their lowest terms the problems of the biochemist concerning the synthesis of proteins, carbohydrates, and fats, for the large amounts of the substances present in an animal body are merely the sum total of all the minute particles of proteins, carbohydrates, and fats synthesized and transformed in the single cells. The exact steps in the synthesis of the complex chemical compounds which serve as sources of energy in the physiology of the cell are wholly undetermined. There are no reliable microchemical tests for many of these substances and our interpretation of them according to their staining reactions may be quite erroneous.

Regaud has demonstrated that mitochondria are made up of phospho-lipin and albumen. The transformation of mitochondria into fat or of fat into mitochondria has been discussed by a number of investigators, but the intermediate steps are unknown. Dakin states that fatty acids are not directly transformed into amino acids, but the evidence leaves open the possibility of such a transformation through carbohydrates. In that case, is there a carbohydrate stage in the formation of mitochondria or of yolk? Evidence of such a stage would be removed by all ordinary methods of technique in preparing sections. Perez ('03) found in the adipose cells of Formica rufa a transformation of fat globules into albuminous bodies by the digestion of the former.

A number of other authors have touched on this problem of synthesis in the cytoplasm, but their conclusions are not in agreement. According to Popoff ('10), chromidia from the nucleus pass out and change to fat. Saguchi ('20) working on the islet cells in the pancreas, describes lipoid granules being formed from mitochondria. Hollande ('14) observes fat formed from granules near the nucleus and then a transformation of part of the fat into albuminoid bodies. Beckwith ('14) found pseudochromatin granules which developed directly into yolk spheres; Schaxel ('11) found chromatin emitted from the nucleus which formed yolk spheres; Danchakoff ('16) found chromatin emitted from the nucleus which synthesizes more chromatin in the cytoplasm. Ludford ('21) in Patella oogenesis found yolk to be formed by Golgi bodies which were entirely distinct from the mitochondria and the fragments of the nucleolus which he described as being emitted from the nucleus. Nakahara's results ('17) on Pieris, and Perez' work on Formica rufa both point to a transformation of fatty bodies into albuminous bodies. From these many observations it is evident that there are transformations of visibly distinct bodies into one another in the living cell. The great need at present is for some technique which will demonstrate accurately the intermediate steps.

In this paper an attempt has been made to interpret those chemical compounds in the cytoplasm of the egg of Echinarachnius parma which form distinct visible bodies and which can be

stained with ordinary reagents, and to show the part played by them in the processes of development initiated by fertilization.

SUMMARY

1. The cytoplasm of eggs of Echinarachnius parma when fertilized by sperm of the same species shows no visible differences from that of eggs fertilized by Arbacia sperm.

2. Fat drops occur in the unfertilized egg. These drops are emulsified and the fine droplets of fat thus formed gradually become used up or transformed during the early cleavage stages.

3. Small spherical mitochondria are found scattered throughout the cytoplasm and there is evidence to show that they are the direct products of the fine fat droplets.

4. The cytoplasm is packed with plates of nutritive material which have some fatty component in their makeup. They are probably yolk. They are in some cases closely associated with mitochondria, and it is probable that the mitochondria are instrumental in their synthesis. These plates gradually become fewer in number and in the blastula the cytoplasm is quite spongy and full of vacuoles once occupied by the plates.

5. After certain fixatives, picro-acetic and sublimate-acetic, large precipitations of colloidal material in the cytoplasm are stainable with iron hematoxylin. Other fixing fluids preserve them, but do not mordant them so that they actively take up the stain. These precipitations are found in the unfertilized egg when its cytoplasm is in a sol state. They cease being formed as the cytoplasm becomes a gel during its preparation for the first division.

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DESCRIPTION OF PLATES

All drawings, except figure 12, were made at table level by the aid of a camera lucida, using a Zeiss 1.5-mm. oil-immersion objective, and a compensating ocular no. 4. This gave a magnification of 1250 diameters. Figure 12 was made slightly above table level and its magnification was 1170 diameters.

Plates 1 and 3 were then reduced to $\frac{2}{5}$ their size, giving a final magnification of 500 diameters.

Plates 2 and 4 were reduced $\frac{1}{2}$, making a final magnification of 625 diameters, except for figure 12, which is 585 diameters.

PLATE 1

EXPLANATION OF FIGURES

(All eggs illustrated on this plate are from the same series.)

1 Unfertilized egg. Flemming without acetic, unstained.

2 Twenty-five minutes after insemination. Flemming without acetic, unstained.

3 One hour and forty minutes after insemination. Flemming without acetic, unstained.

4 Unfertilized egg. Flemming without acetic, alizarin and crystal violet.

5 Twenty-five minutes after insemination. Flemming without acetic, alizarin and crystal violet.

6 One hour after insemination. Flemming without acetic, alizarin and crystal violet.

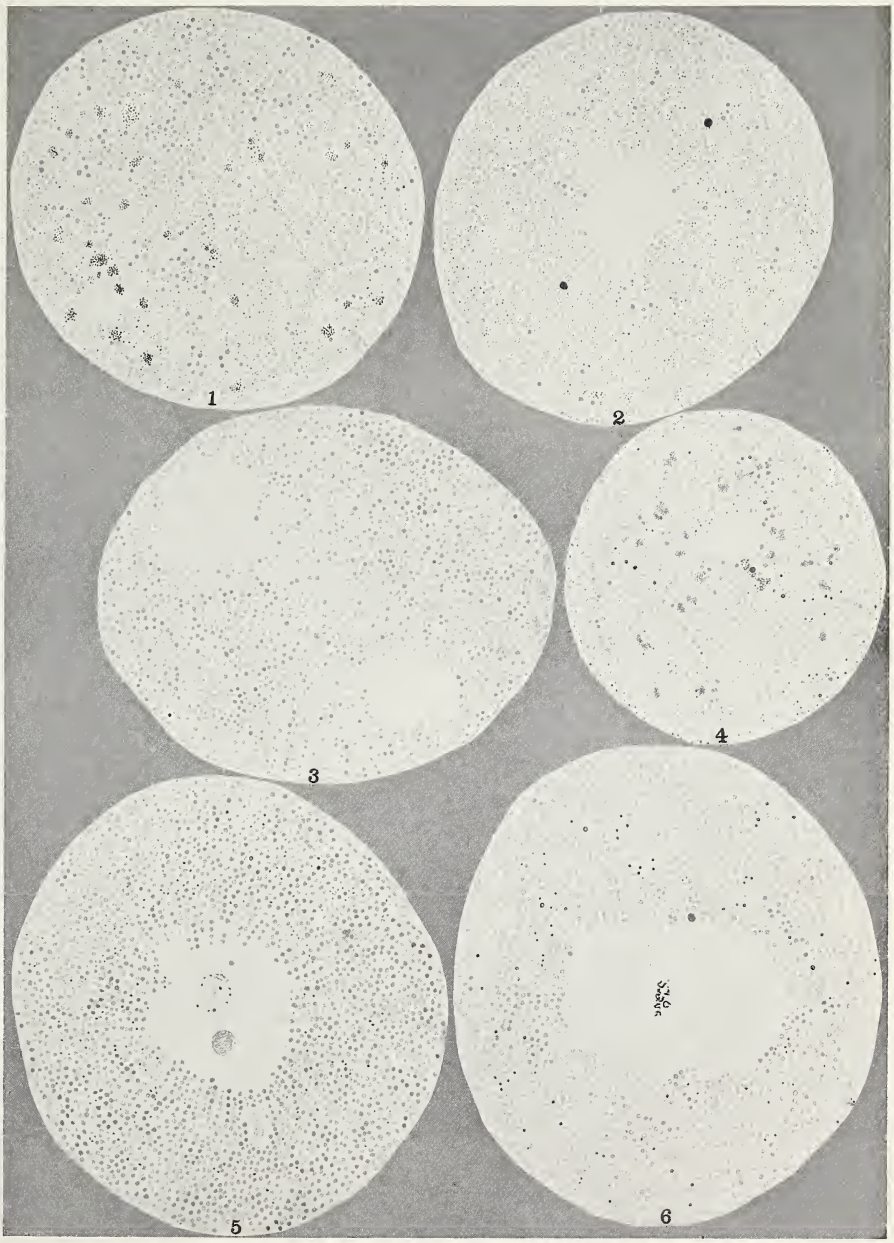
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PLATE 2

EXPLANATION OF FIGURES

(Figures 7 to 11 are from the same series.)

7 Unfertilized egg. Picro-acetic, iron hematoxylin.

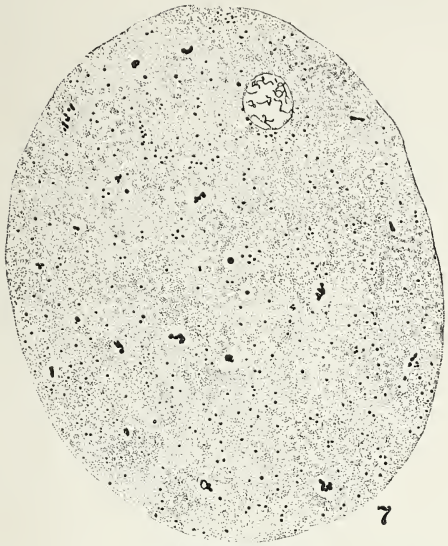
8 Twenty minutes after insemination. Picro-acetic, iron hematoxylin.

9 Fifty minutes after insemination. Picro-acetic, iron hematoxylin.

10 One hour after insemination. Picro-acetic, iron hematoxylin.

11 Three hours and one-half after insemination. Picro-acetic, iron hematoxylin.

12 One hour and ten minutes after insemination. Champy, iron hematoxylin



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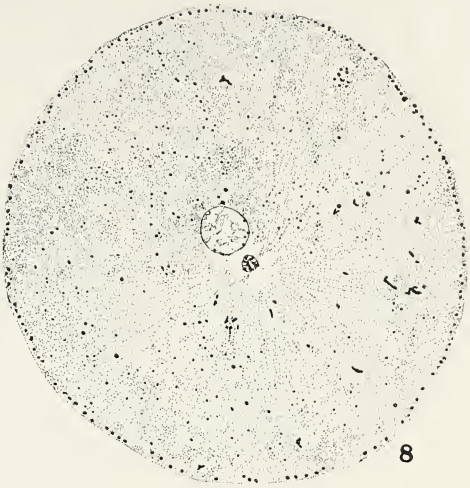

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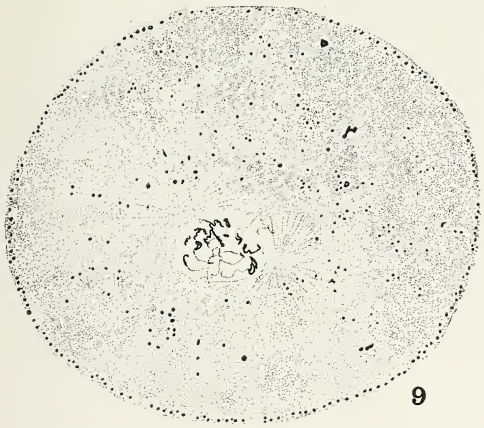

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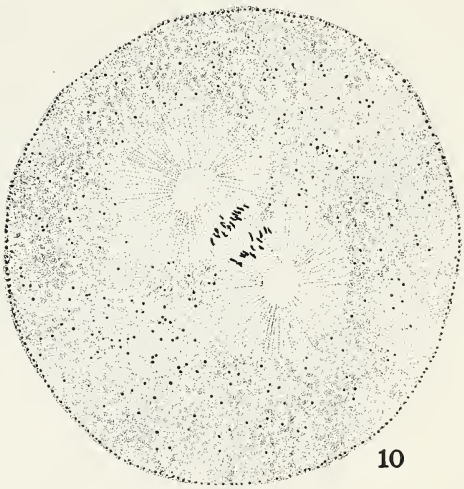

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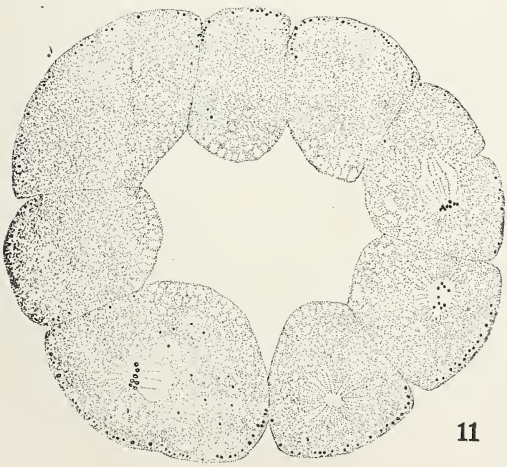

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PLATE 3

EXPLANATION OF FIGURES

(All eggs illustrated on this plate are from the same series.)

13 Unfertilized egg. Modified Bouin, alizarin and crystal violet.

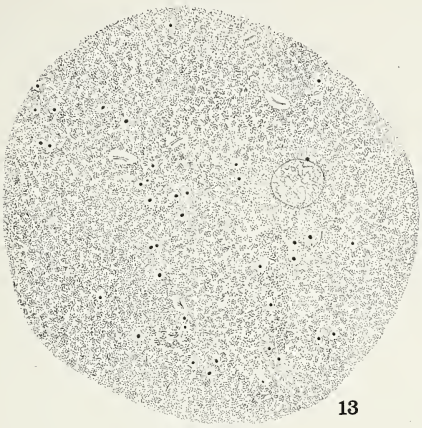
14 Fifteen minutes after insemination. Modified Bouin, alizarin and crystal violet.

15 Forty-five minutes after insemination. Modified Bouin, alizarin and crystal violet.

16 Two hours and forty-five minutes after insemination. Modified Bouin, alizarin and crystal violet.

17 Eight hours and fifteen minutes after insemination. Modified Bouin, alizarin and crystal violet.

18 Thirty minutes after insemination. Modified Bouin, iron hematoxylin.



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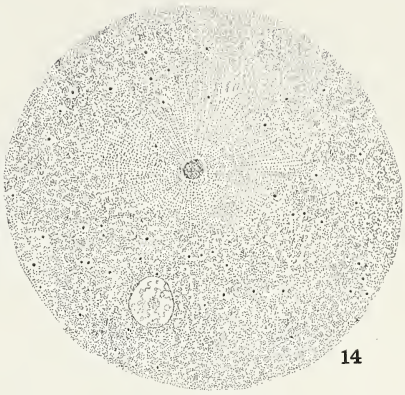

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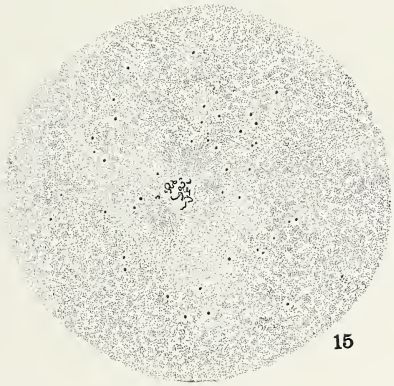

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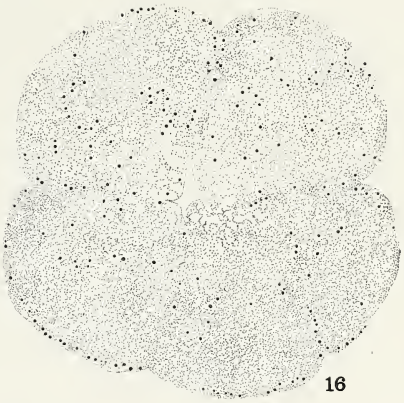

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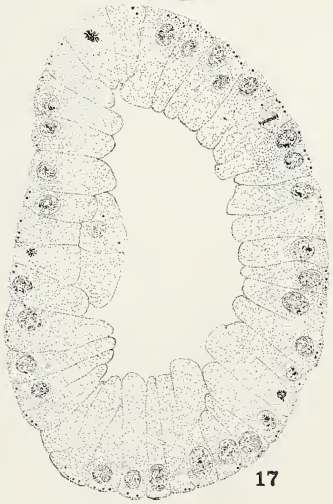

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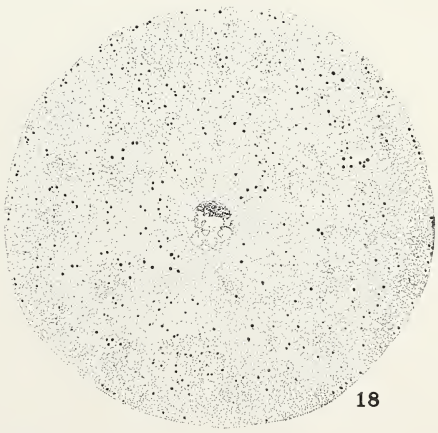

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PLATE 4

EXPLANATION OF FIGURES

(All eggs illustrated on this plate are from the same series.)

19 Unfertilized egg. Flemming without acetic, alizarin and crystal violet.

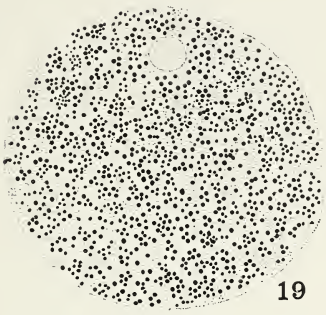
20 One-half hour after insemination. Flemming without acetic, alizarin and crystal violet.

21 One hour after insemination. Flemming without acetic, alizarin and crystal violet.

22 One hour and one-half after insemination. Flemming without acetic, alizarin and crystal violet.

23 Two and one-quarter hours after insemination. Flemming without acetic, alizarin and crystal violet.

24 Four hours after insemination. Flemming without acetic, alizarin and crystal violet.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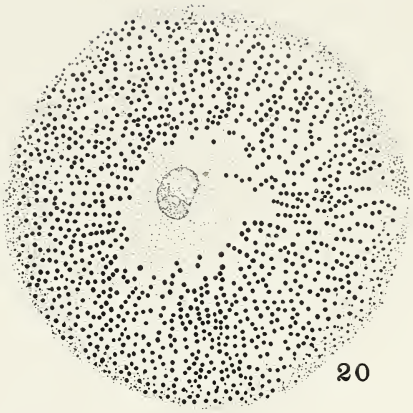
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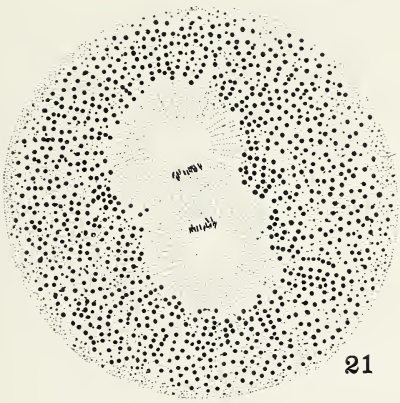
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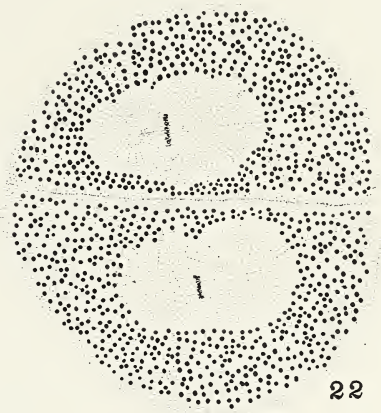
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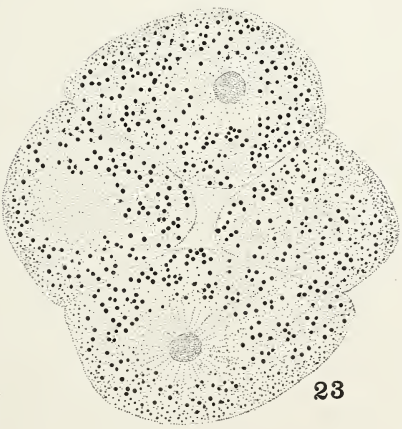
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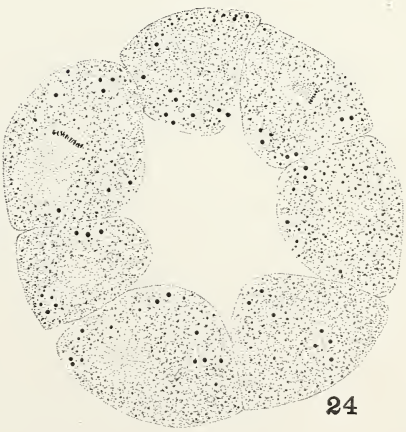
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VITA

I, Hope Hibbard, was born at Altoona, Pa., in 1893. My father is Herbert Wade Hibbard, my mother Mary Scofield Hibbard. I attended the University of Missouri from 1912–18, and received the degree of A.B. in 1916 and of A.M. in 1918. I held the position of Student Assitant in Zoology at the University of Missouri in 1915–16, and of Assistant in Zoology from 1916–18. In 1918–19 I was Honorary Scholar in Biology and Assistant Demonstrator in Biology at Bryn Mawr College, and from 1919–21 I held the Fellowship in Biology at Bryn Mawr College. During the summer of 1917 I attended the Marine Biological Laboratory at Woods Hole, Mass., on a scholarship from the University of Missouri, and during the summer of 1920 on a scholarship from Bryn Mawr College.

My work at the University of Missouri was done under the direction of Dr. George Lefevre, Dr. W. C. Curtis, and Dr. G. S. Dodds. My Major work at Bryn Mawr College in Morphology was done under the direction of Dr. D. H. Tennent; my Associated Minor in Physiology was done under the direction of Dr. S. C. Brooks and Dr. A. B. Yates; my Independent Minor in Organic Chemistry was done under the direction of Dr. R. F. Brunel. My Ph.D. preliminary examinations were completed on January 17, 1921, my final examinations on May 23, 1921.

For particular assistance in the preparation of this dissertation I wish to thank Dr. D. H. Tennent to whom I have already expressed my appreciation in the Introduction.

I have published, in collaboration with Mary J. Guthrie, "Cleavage and Mesenchyme Formation in Toxopneustes variegatus." Biol. Bull., Vol. 37, 1919.