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Introduction

It is the purpose of this paper to contribute some information concerning certain cytoplasmic components of the ovum of the goldfish, *Carrassius auratus*. The writer's primary concern has been the study of the yolk nucleus although some data is given relative to golgi bodies, mitochondria, and yolk.

Much already has been written about the "yolk nucleus" of invertebrates and vertebrates but, whereas considerable work has been done on the golgi bodies, mitochondria, and yolk of the eggs of fishes, less has been done on the "yolk nucleus" of these forms. Certainly the early work of Balbiani in 1873 (quoted by Henneguy, 1893) is one of the first to describe this body in fishes. Other definite references to the yolk nucleus of fishes include an excellent article by Henneguy (1893), Hubbard (1894), Cunningham (1897), Wallace (1904), Munson (1912), Wheeler (1924), Parat (1926), Hibbard and Parat (1927), Hylton-Scott (1928), Mendoza (1943), and Chaudhary (1949). Whereas some of the articles (Henneguy, Hubbard, Cunningham, Wallace, and Wheeler) discuss the body at some length, other articles refer only briefly or passingly to the structure.

Because of the great variation that must exist in the nature of the yolk nucleus and other cytoplasmic components in the different animals groups and even within the teleost fishes, the writer is loathe to draw too close a comparison between this material and, for example, that of mammals or molluscs. It is to be expected that the entire cytoplasmic structure in the eggs of fishes should be different, if not more complicated, than that of eggs with little yolk.

The writer is somewhat apologetic about further confusing the issue on the nature of the yolk nucleus of vertebrates but, he is firmly convinced that, at least in fishes, different structures have received the name of yolk-nucleus or one of its many so-called equivalent terms (Balbiani's vesicle, dotterkern, idiozome, etc.). On the basis of this preliminary study, the writer is convinced

that the yolk nucleus of the goldfish is not the idiozome complex, one of the accepted interpretations of the structure. Whether or not the term yolk nucleus is appropriate is at present unimportant; the name will be used to describe a certain body in the cytoplasm; it wlll be used, like Beams and King said (1938) "for convenience" and, furthermore, because the writer thinks there is some validity in the term. At this time, the writer is more interested in describing a structure than in debating about the proper name for it.

The use of the air-driven centrifuge has been introduced into this study because, from the writer's brief experience and the review of the literature, it is clearly evident that staining alone, as shown by the cytoplasmic components, is not decisive nor reliable, not only because the execution of the technique varies from one investigator to the next but, also because the very components themselves must exhibit a wide range of variation from one animal group to another.

Finally, the writer wishes to acknowledge with gratitude the generosity shown by Dr. H. W. Beams of the State University of Iowa in introducing the writer to the use of the air-driven centrifuge and for his many attentions when asked for his opinion or advice.

Materials and Methods

Small goldfish of aquarium size were used exclusively in this study. Whole ovaries were fixed in Bouin's and Zenker's fluids and subsequently stained with Delafield's and iron hematoxylin, standard histological reagents and methods were used in running up the tissues. Special preparations included ovaries fixed according to the Champy method followed either by the triple stain (Champy-Kull) or by iron hematoxylin, the silver impregnation method of Aoyama, and Ludford's osmic acid technique with or without the neutral red counterstain. On the whole, the Aoyama and Ludford techniques gave excellent and consistent results. For various reasons, the Champy-Kull method gave the writer the most trouble although the Champy-iron hematoxylin preparations gave excellent results. Vital stains were not used in this study. On the whole, the ovaries of the goldfish are not simple to work with; small cells are particularly difficult to stain well when in sections with larger eggs, and large eggs have a pecularly brittle yolk that is made even worse by prolonged treatment with heat and osmic acid. Centrifuging was performed at various speeds; whole ovaries were placed in an isotonic solution

in the rotor and were centrifuged for 15 to 30 minutes at speeds ranging from 1500 to 1750 revolutions per second with centrifugal forces of 102,000 and 138,000 times gravity. Maximum centrifugal force used for one sample was approximately 294,000 times gravity for 20 minutes. On the whole, smaller eggs required stronger forces for displacement of cytoplasmic components.

Typical Ovum of a Pre-Yolk Stage

The ova of the goldfish are typical teleost ova in their general structure (fig. 1). They range in size from primordial germ cells to large eggs 1200μ in diameter, heavily laden with yolk. Since these ovaries were taken from aquarium forms, it is assumed that these eggs have not necessarily reached their maximal size. The eggs have a typical, thin, enveloping membrane; with subsequent growth of the egg, this membrane is changed to or supplemented by a thick, striated zona radiata with, apparently, minute canals oriented transversely. In addition to these membranes there is a thin, cellular sheath that evidently serves as a follicle and a vascular theca. No meiotic stages were evident at any time; therefore even the largest cells remain at the gonial or early oocyte stages since growth has started but no evidence of meiosis is present. The nucleus or germinal vesicle is approximately one-half to one-third the diameter of the early cells; it tends to be spherical, vesicular, and contains many nucleoli.

The nucleoli occupy almost exclusively a peripheral position in the nucleus. Nucleolar extrusion into the cytoplasm is very likely a fact in these eggs, regardless of the function, if any, that this may serve. The nucleoli take a basic stain with standard stains (e.g. Delafield's), yellow or red with the method of Champy-Kull, and colorless with the Aoyama and Ludford techniques. Following Bouin-hematoxylin preparations, the cytoplasm of the small eggs appears to be quite homogenoeus, for the greater part, showing rather coarse granulation. Except for the yolk nucleus which will be described shortly, there is little else in the small egg, prior to yolk formation, that can be seen readily with standard fixations and stains. In general, small eggs are difficult to stain satisfactorily; they take the stain much more heavily than do the larger eggs. Also, many typical slides of small eggs show areas in the cytoplasm that do not stain, as if fixation were poor or incomplete. Usually this area forms a ring or zone around the nucleus. The reason for this will be evident later.

Cellular Components

In describing the cellular components of the egg, the writer has thought it advisable to describe each one in turn. Since it is necessary to identify these structures, they will be given a name arbitrarily; at a later point in the paper, the validity for the terminology will be discussed. The principal structures to be discussed are: (1) a golgi network, (2) the yolk nucleus, (3) golgi granules, (4) mitochondria, (5) idiozome complex, and (6) yolk.

Golgi network

In very small eggs up to approximately 75μ in diameter, there is a most conspicuous network which is distributed throughout the entire cell (figs. 2, 3, 4). Normally, in Aoyama preparations, this network consists of filaments and rods well interlaced together but may, in some preparations, take a granular form, or still in others may appear as dark brown blotches ar patches. In addition, there is usually a heavy concentration of this same material in a zone or ring around the nucleus and around the periphery of the egg. This very conspicuous network is present in all material fixed according to the method of Aoyama. As such, it appears in no other preparation. The perinuclear band or zone is, without doubt, the reason for the imperfect staining of the cytoplasm of the small cells fixed in standard reagents. It is interesting to note that this very same ring has been identified before by Wheeler (1924) for the Dab; he refers to the ring in the silver preparations as a "positive" picture and the absence of the ring, the failure to stain, in standard preparations, as a "negative" picture. With growth of the egg to the point where the yolk begins to appear, there is a progressive breaking up of this network into separate filaments and granules, with a resulting loss of the network structure. (figs. 5, 6). With the appearance of the yolk spheres, these same granules and filaments take a position between the yolk spheres and stay in that position until they disappear from view with the accumulation of large amounts of yolk (fig. 14). This conspicuous network shows a marked affinity for silver impregnation. It normally appears black or very dark brown. When small eggs are centrifuged at very high speed, it is thrown easily to the extreme centripetal pole, along with the nucleus (fig. 9). As the egg grows and this network is lost as such, the granules which replace it are stratified in a more complex manner upon rotation at high speeds. For the present, suffice it to say that in the larger eggs this material is concentrated into the cen-

tripetal half of the egg. It is impossible to give the origin of this network because it is already present in the smallest eggs. Its fate is dubious but there are two possibilities. These granules may be concerned with the origin of yolk and also with a reticulum of argyrophylic material that is found between yolk spheres at a later period. In view of the reaction of this network to osmic acid and silver nitrate preparations, and in view of its displacement when centrifuged, the writer judges this material to be a golgi network.

In other preparations, this network is not visible (fig. 1). In standard fixations (Bouin, Zenker), the cytoplasm appears differentiated in some manner but details can not be made out. In the Ludford material, small cells show only scattered granules and filaments of irregular shapes and sizes; these stain heavily with osmic acid (figs. 7, 10). In the Champy-Kull preparations, red masses comparable to those of the Ludford material can be seen easily. These similar materials or bodies, found in both the Ludford and Champy material, are thrown to the centripetal pole on centrifuging. In some of the best Champy preparations, there is evidence of a delicate red network in the cytoplasm but it has been difficult to demonstrate this consistently. Admittedly, failure to demonstrate this red network in more Champy preparations may be merely a matter of faulty technique. Despite the contradiction of the red stain in the Champy material, it is likely that the structures that show in these two fixatives are golgi bodies. This identification is made on the basis of their displacement on centrifuging which the writer considers more reliable than the staining reaction. It is likely that these bodies are either different expressions or forms of the same net-like material that shows in the silver preparations or that they are bodies that react only to the osmic acid but are still related to the golgi granules seen before.

Yolk nucleus

Regardless of the correctness of the identification or the appropriateness of the name, the body described in this paper is one and the same; it appears consistently in all preparations. An evaluation will be made later of this structure and others identified by the same name in other animals. Even in material fixed with Bouin's and stained with Delafield's hematoxylin, this cytoplasmic body appears consistently. In cells of a pre-yolk stage, it is spherical or ovoidal in shape, measures approximately 15μ and occupies a variable position within the cell. In the smallest

cells, it takes a flattened form against the nucleus; with the growth of the egg, it moves progressively toward the cell periphery where it takes its final position. The body seems to be formed of a dense amorphous matrix; discrete granules are difficult to identify always. In cells stained with Delafield's or iron hematoxylin, the body normally stains darker than the surrounding cytoplasm. The mere fact that this body is visible in routine preparations stamps it as different from some of the others so identified. With the use of special stains, the nature of this structure becomes better defined. Eggs fixed and stained according to the Champy-Kull method show the yolk nucleus to have a red matrix with little or no granulation. If the same material is stained with iron hematoxylin rather than with the customary stains (neutral red, aurantia, etc.), the yolk nucleus stains gray or black, but no granulations shows. Aoyama material shows a background matrix of yellow-brown but with very prominent black granules embedded on it and within it (figs. 5, 8). Depending on the degree of silver impregnation, the yolk nucleus may appear as a yellow-brown mass with or without the black granular deposit or as a dense black mass in which all detail is lost. The former occurs when the silver impregnation has been very light and the latter when the over-all impregnation has been excessive. In the smallest eggs, the yolk nucleus appears directly related to the perinuclear zone of golgi material; furthermore, it always appears to take a position between the golgi zone and the nucleus (fig. 3). Lastly, when the cells are fixed with osmic acid (Ludford), the homogeneous background matrix appears light gray and the granules stain an intense black (fig. 7). The granulation in the Ludford and Aoyama material is identical in appearance. The granules appear to extend throughout the yolk nucleus; they are most definitely not superficial incrustations alone. From the staining reactions above, the writer concludes that the yolk nucleus is formed of a mitochondria-like material with golgi bodies embedded in it. The writer has been incapable of finding centrioles within this complex structure. In early stages, it tends to resemble, at first glance, the idiozome complex of cells as described in the cytological literature yet, in later stages, it seems difficult to compare the two. The yolk nucleus as such does not appear to be affected by rotational forces of high magnitude (over 100,000 g.) It may be found at either pole when centrifuged but more often takes a position at the periphery of the cell and either midway between the two poles or at the centripetal pole, wedged in between the nucleus and the cell membrane. Even at

maximum forces used (294,000 g.), there was never any tendency for the yolk nucleus to break up into component parts. The granules on the yolk nucleus were just as conspicuous and numerous in the strongly centrifuged material as they were in the normal cells. Whether or not they could be dislodged at higher forces is impossible to say.

It is not possible to state the origin of this body with any degree of assurance since it is present in all but the most minute cells. The fate of the yolk nucleus, however, is at least partially clear. About the time the first yolk spheres make their appearance in the egg (150 μ), the yolk nucleus becomes difficult to identify. This is due to the loss of golgi granules, leaving behind only the matrix proper that stains essentially like the cytoplasm, although somewhat darker, and therefore becomes difficult to follow. Identification of the body becomes particularly difficult, furthermore, because the yolk spheres are increasing in number at this time and taking a peripheral position in the cell. Figure 5 shows a particularly dark yolk nucleus with most of the golgi granules having dropped or dissolved off. Most matrices of yolk nuclei do not stain this dark. Note that no yolk has yet appeared (upper cell). The function of the yolk nucleus cannot be given with certainty. It appears to be related directly to much of the peripheral granulation in the egg and thus, indirectly, it may be related to yolk formation.

Golgi granules

In this category are listed two types of granules somewhat different but, essentially, they seem to belong to the same general category. First, there seems to be a very definite time and place of formation of one type of granule which, for lack of a better name, will simply be referred to as "fine" golgi granules. These make their appearance at the time of formation of the first yolk spheres (150 μ). They appear as fine black granules at one pole of the cell and can be observed only in the Ludford material. Although these granules can be found around the entire periphery of the cell, the invasion of the cell by these bodies proceeds essentially from one pole of the cell (figs. 11-13). They spread throughout the entire cell by the time an egg is 250 μ in diameter. Although the bulk of this granulation consists of very fine, small granules, there is ample evidence of larger masses that also appear at the periphery. This material makes its appearance at the time of yolk formation and naturally leads to the assumption that it is concerned in some way with yolk formation. These granules be-

come scattered throughout the cytoplasm but take a position between the yolk spheres as soon as they start to form. As the eggs grow, the fine granules tend to remain at the periphery of the egg, between yolk spheres, whereas many of the coarser granules take a position toward the center of the cell, to one side of the a-centric nucleus. In the Aoyama material, on the other hand, there is no evident mass influx of granulation such as occurs above. If it occurs here, it does so in such a manner that it is not readily detectable. The same is true for the larger particles; no influx can be detected although much coarse and fine golgi material in the cells is left from the break-up of the golgi network. The coarse granules in the Aoyama material normally stain an intense black. In cells that reach 250 μ, the coarser particles cluster at the center of the cell in a juxtanuclear position whereas the finer particles stay at the periphery between the yolk spheres. Thus, the golgi material here becomes distributed in a manner identical with that of the Ludford preparations (figs. 14, 15). In the centrifuged material, a similar picture is presented regardless of the fixative used (Aoyama or Ludford). Yolk always is thrown to the extreme centrifugal pole. From there to the opposite pole, the cytoplasmic materials stratify in a typical and fairly constant picture. Next to the yolk is a band of very coarse granules that take a very strong osmic acid stain; a similar band of particles takes a heavy silver impregnation (figs. 17, 18). In both fixatives, if the material is well prepared, the granules show a heavy cortical stain and a light or osmophobic center. Without doubt, these are the coarse granules that cluster in a juxtanuclear position in cells of medium size. Next to this zone and towards the centripetal pole, appears a very broad zone or area of fine granulation. These finer granules appear either black (Ludford) or black or brown with silver stains. Between these two zones mentioned, the cytoplasm remains either colorless (Ludford) or pale yellow (Aoyama). There is no doubt in the mind of the writer that this fine granulation is the one that appears at time of yolk formation. The presence of the fine granules in the Aoyama material indicates that the sudden influx of these bodies is not detectable here as it is in the Ludford preparations. Whether these fine granules are black or brown (Aoyama) is unquestionably only a matter of degree of silver impregnation. This fine granulation is very typical of medium-size centrifuged eggs where it virtually fills the centripetal half of the egg. Even in Bouin-fixed material, these two major zones can be identified.

It appears to the writer that some of this fine granulation is

present in the eggs from the beginning, left over from the break-up of the golgi network. It is also evident that there is a mass influx of granules from the egg periphery at the time of yolk formation but is visible only in one preparation (Ludford). It is further evident that there are two major kinds of granules, coarse and fine, that respond to the different techniques as described. Some aspects or phases of this granulation apparently can be better demonstrated with a particular technique. Because of their staining and behavior when centrifuged, these granules are identified as golgi or goli-like material. It is difficult to state what function, if any, they may serve in the egg but the evidence indicates they are related to yolk formation.

Mitochondria

There is very little indication of mitochondria in these eggs. The best evidence is found in the very small cells that have been centrifuged. When the golgi material is thrown to the centripetal pole, it leaves behind a mass of bright yellow-brown material (Aoyama) that is not displaced by forces up to 138,000 g. These granules do not stratify at the centrifugal pole; they simply are not displaced. But because they tend to blend into the yellow cytoplasm of the cell they are difficult to identify in the normal small cells. Nothing comparable was found in other preparations although a red "dust-like" material, tinting the color of the cytoplasm, was found in some of the Champy preparations. At no time was any mass of granules ever thrown to the centrifugal pole in a clearly defined layer in small or large eggs. Nothing ever displaced the yolk spheres from their extreme position at the centrifugal pole. Either there are few or no mitochondria in these eggs or, if they are present, they are not displaced to the centrifugal pole or, simply, faulty technique failed to show them. The great mass of granules in these eggs closely followed the criteria for golgi material.

Idiozome complex

In the smaller cells, it has been virtually impossible to identify anything like a typical idiozome-golgi complex. However, in cells of medium size approximately 200 μ in diameter, a structure appears that may well be an idiozome complex. In these cells, yolk formation is well along; the spheres take a peripheral position and may be one, two, or three rows deep. Between them, in typical Aoyama material, golgi granules can be easily seen since they stain black or deep brown and they lie against a background of yellow. At this time, the nucleus is a-centric in position. In a limited

space of cytoplasm, devoid of yolk, approximately in the center of the cell, and to one side of the nucleus, there is a large mass of bright yellow surrounded very loosely by a large number of the very large golgi bodies. Actually, there large granules fill the yolk-free cytoplasmic area and are only slightly more concentrated around this bright yellow mass which is free of golgi material (figs. 14, 15). This same structure is present in the Ludford material. Here, the large golgi bodies are black and the central part is pale or gray. No centrioles were found in the center of this osmophobic mass, however. A bright yellow mass has been found in smaller eggs (Aoyama material) that resembles this structure but at no time is it as conspicuous as it is in these larger eggs because at no time before do the golgi granules form such a clear, conspicuous mass around it. It appears that this mass does not form or become clearly defined until eggs are of medium size.

Yolk

Two major types of yolk are evidently formed in these eggs; one stains a bright pink with eosin and occupies the bulk of the larger eggs, the other stains gray and is confined to the periphery of the egg. The former consists of small platelets ovoidal in shape and the latter of large spheres. The pink yolk in the center of the egg is characterized by a very conspicuous reticulum that shows marked affinity for silver stains and lies between adjacent platelets of yolk. Neither type of yolk stains with silver but both show a slight affinity for osmic acid hence must have very little fat content if any. The first type of yolk to appear is the gray yolk at about 150 μ. Reference has been made to the fact that at this time, two other major changes occur, one is the disappearance of the yolk nucleus as a definite body and the second is the mass influx of the granular material as evidenced with osmic acid (Ludford). It is difficult to escape the implication that the appearance of the golgi material is related to the yolk origin. Study under an oil immersion lens shows what the writer interprets as evidence of transition from the granular golgi to the gray yolk spheres. Interesting corroboration of this idea is found in the centrifuged material; there, between the centrifugally displaced yolk and the zones of golgi material small transitional forms occur. Thus, it seems inescapable that the golgi material leads to the formation of the gray yolk. These form at the periphery of the cells and retain that position. As they increase in number, they encroach on the central cytoplasm and eventually crowd it out altogether. The second type of yolk appears at about 500 μ and

first shows up in the interstices between the spheres of the gray yolk; this formation occurs primarily in the center of the egg. These new platelets retain an ovoidal shape and eventually constitute 85% or more of the yolk in the egg. Although complete evidence is lacking, it is probable that this yolk also arises from the golgi bodies that lie between spheres of gray yolk. When the largest eggs are centrifuged, there is little displacement; the central yolk retains its central position but the gray peripheral yolk is thrown to the centrifugal pole where it forms a small cap. The nucleus is still displaced to the centripetal pole.

Discussion

It is evident to the person who has just finished reading diverse accounts of the nature of the yolk nucleus that several factors exist that confuse the entire picture. First, we should not necessarily expect to find identical structures in the ova of a spider, guinea pig, or fish. Second, judging from the literature, it is obvious that different men have called different structures by the same name. Third, unfortunately, the very cytoplasmic components that form the yolk nucleus are in themselves so varied in their structure, physiological expression, and affinity for stains that even the same structure appears differently to different investigators. Fourth, it is unfortunate that too much effort has been made to equate bodies in different animal groups.

The yolk nucleus of the goldfish was described as made of a semi-granular matrix resembling mitochondrial material, with golgi-like bodies embedded in it. These evaluations were based on staining reactions. There is no question that the matrix and the incrustations are completely different structures. This may or may not correspond to the Balbiani corpuscle in the perch defined as a mass of mitochondrial fibers with contained vacuoles of true golgi (Hibbard and Parat 1927). Certainly, the writer found no central delicate vesicle in a granular mass as described by Balbiani (according to Henneguy, 1893), nor is there the cell-like structure described for the dab (Wheeler, 1924). It further is evident that the true yolk nucleus described by Wallace (1904) for several fishes (Zoarces, etc.) is in reality a pallial layer or growth ring. That is, it has utterly no similarity to anything else in the literature (for fishes) called a yolk nucleus. To a body that does resemble the yolk nucleus (judging from his drawings), Wallace gives the name of centrosphere but does not elaborate on its nature. In the opinion of the writer, the pallial layer (Wallace's "true yolk nucleus") and the yolk nucleus described for

the goldfish are completely different. The writer did not find a distinct pallial layer in the goldfish. However, in the egg of the teleost, Neotoca bilineata, (Mendoza 1943), the writer found both a typical pallial layer and a conspicuous yolk nucleus. As recently as 1949, Chaudhary described for five marine fishes, both a pallial layer or plasmatic zone and a yolk nucleus of Balbiani. The writer doubts that the pallial layer as seen in fishes is an artifact as claimed by Wheeler (1924) for the dab. Certainly there is nothing in goldfish resembling the lamellated structure found in spiders. The writer does not deny that the yolk nucleus could be an idiozome complex but all evidence so far tends to deny this for the goldfish. Needless to say, the possibility that the yolk nucleus of mammals is an idiozome complex is strongly supported by the work of Beams and King (1938) and Beams and Sheehan (1941). Working with the plaice and flounder, Cunningham (1897) doubted the centrosome nature of the yolk nucleus because of its origin, movement within the cell, and its fate. In those forms, the yolk nucleus moves toward the cell periphery, occupies a position at the edge of the yolk and then disappears. He doubts that, if it were a centrosome, it would move out, disappear, and then reappear later at the nuclear surface. The writer believes that the structure of the yolk nucleus in the goldfish, its movement to the cell periphery, its loss of golgi bodies at time of yolk origin, and its failure to be disintegrated at 294,000 g. excludes the possibility of its forming an idiozome complex. Upon centrifuging, the yolk nucleus does not suffer the same fate described by Beams and King for the guinea pig. Furthermore, the finding of an idiozome-like structure in the goldfish further prevents the writer from accepting the idiozome explanation. Thus the writer is in agreement with Cunningham about the idiozome nature of the yolk nucleus.

As to the origin of the yolk nucleus, it is impossible to give a final answer. In another teleost, Neotoca bilineata, the writer (unpublished data) identified the origin of the yolk nucleus as a nucleolar-like body, an opinion reported several times in the literature. In the goldfish there is good evidence of nucleolar extrusion, a phenomenon reported before in fishes (Wheeler 1924; Chaudhary 1949), for snakes (Kaushiva 1949), and for mammals (Zlotnick 1948). Similarly, the origin of the yolk nucleus in a juxtanuclear position in the very earliest cells has been described before by Hubbard (1894), Wallace (1904) and many others. Prior to this time, it is completely impossible to identify

the body. It arises in the midst of or internal to the perinuclear ring of golgi.

So far as the function of the yolk is concerned, the writer feels that it is related to the granulation that is present at time of yolk formation and therefore may be related to yolk formation. It is further interesting that it disappears at the time yolk starts to form. Certainly today we cannot accept some of the older accounts of the function of the yolk nucleus; they are repeated here only for their historical significance. According to Henneguy (1893), Balbiani described the yolk nucleus as being a cell derived from the follicle cells; another early writer expressed the belief that it forms the female pronucleus in the fertilized egg, and, lastly, Henneguy himself expressed the opinion that this body is an ancestral organ comparable to the macronucleus of the infusoria.

The writer is not interested in entering the long-debated argument concerning the nature of the golgi bodies and the mitochondria, in part because this study is by no means an exhaustive analysis of these two types of bodies. However, some information is worth recording at this time. The writer has found two types of bodies which, because of their affinity for osmic acid stains and silver impregnation and because of their very definite centripetal displacement upon centrifuging, have been called golgi or golgi-like bodies. Furthermore, the large granules, if properly impregnated, show the characteristic osmophobic center with an osmophilic cortex. It has been impossible to identify this structure in the finer granules. Because of the lack of complete reliability in distinguishing between mitochondria and golgi on the basis of stains alone (note identification by Hibbard and Parat, 1927, of blackened filaments in Cajal and Da Fano methods as mitochondria), the writer relies strongly on the centripetal displacement of these bodies presumed to be golgi. Needless to say, it is difficult to harmonize the behavior of these bodies upon centrifuging with the recent account of Adamstone (1952) in which he described the golgi as canals in the cytoplasm with mitochondria attached to their surface.

With respect to the typical golgi apparatus in an idiozome complex, nothing has been definitely identified in these eggs. Admittedly, the position of the yolk nucleus in the early egg is typical of that of the idiozome complex but the detailed structure is not typical. The one large body that may be an idiozome in a medium size egg, does show some concentration of granular golgi around an osmophobic center that stains bright yellow with

Aoyama preparation. The golgi consists of granules and not filaments and it is a very loose aggregation, not a tightly woven network. Finally, the writer is not desirous of entering a discussion concerning the reality of the golgi material. For a fairly recent evaluation of the problem, the reader is referred to Hibbard's extensive account (1945) and the Bensley's more recent but pertinent summary (1951). Suffice it to say that recent reports indicate that golgi bodies can be seen in living material (see Bensley's account). If the golgi be artifacts, as some investigators think, they are artifacts that have a consistent origin in the cell, a consistent staining reaction, a definite and consistent density, a definite developmental sequence during growth of the egg, and a definite and consistent fate.

There has been little opportunity to identify mitochondria in these eggs; the techniques used were not particularly suited to their identification. In some preparations there was slight evidence of such material but at no time, even with centrifugal forces of more than 100,000 g., was it possible to displace this into a definite stratum. Furthermore, in displacing components in the cytoplasm, yolk spheres always occupied the extreme centrifugal pole; never was there seen a layer of some substance, heavier than the yolk, and displacing it from the extreme polar position. The extreme centrifugal position for mitochondria has been described before by Brambell (1924) for the eggs of Patella, a molluse, by Beams and King for the eggs of the guinea pig (1938), and by Beams and Sheehan for the eggs of the human (1941). However, in one heavily yolked form, a spider, Nath found (1928) that the albuminous yolk took an extreme centrifugal position and was followed by a layer of hyaloplasm with the nucleus and mitochondria, the fatty yolk taking the customary centripetal position. While it is possible that, in the goldfish, the layer of coarse granules next to the yolk may be mitochondria, they are the ones that exhibit particularly well the typical golgi reaction to osmication. Furthermore, these same granules are always thrown to the centripetal pole in smaller cells. It is not impossible furthermore, that there may be a general scarcity of mitochondria in these eggs, a condition that has been reported before for crustacean eggs (Bhatia and Nath, 1931); and for certain Indian snakes (Lal, 1933). Finally, it is quite possible that mitochondria does occur in these eggs but that it has not been displaced at the speeds used and will require forces in excess of 300,000 g.

Little effort has been made to determine the types of yolk that occur in these eggs; however, brief statements of fact can be pre-

sented. It is very likely that no true, fatty yolk occurs in these eggs because osmication of yolk spheres was by no means consistently found, even in slides not bleached with turpentine or hydrogen peroxide. More significant, however, is the fact that no yolk was ever found at the centripetal pole on centrifuging, a position characteristic for fatty yolk (Brambell 1924, Nath 1928, Mendoza (unpublished data on the carp egg). It is possible that both types of yolk are of a proteinaceous nature and contain little or no fat. This possibility of the absence of fatty yolk is, of course, difficult to reconcile with the often-reported fact that golgi, presumably so abundant here, normally gives rise to fatty yolk whereas mitochondria, presumably so scarce here, gives rise to albuminous yolk.

Summary

1. The yolk nucleus is a semispherical body, approximately 15 μ in diameter, at maximum size, and consists of a matrix of finely granular, mitochondria-like material, with golgi-like granules embedded in its structure.

2. The yolk nucleus arises as a semi-flattened mass in a juxtanuclear position and, during the growth of the egg, moves to a peripheral position in the cell as an intact body.

3. The yolk nucleus disappears at the time of yolk origin and presumably is related to the origin of yolk.

4. Upon centrifuging at forces up to 138,000 g., the yolk nucleus was not disintegrated into component parts but was more often left in an intermediate position in the cell or was thrown to the centripetal pole as an intact body.

5. The writer does not consider this yolk nucleus to be an idiozome complex.

6. Bodies identified as golgi or golgi-like material stain strongly with osmic acid and silver stains and are clearly displaced to the centripetal pole upon centrifuging.

7. Some golgi material arises as a very heavy network in the cytoplasm, frequently forming a heavy ring of perinuclear material in the very small eggs, but breaks up into coarse granules and filaments during the growth of the egg.

8. There is, at the time of yolk origin, a polar ingression or infiltration of a very fine granular material also presumed to be golgi in nature. This granulation is believed to form or help form the first type of yolk.

9. It is possible that the golgi materials are related similarly to the origin of the second and most abundant type of yolk.

10. In view of the fact that all yolk is displaced to the centrifugal pole it is likely that both types of yolk are of a proteinaceous nature with little or no fat content.

11. Mitochondria are either scarce or absent from the goldfish egg; no clear stratum of material identifiable as mitochondria was found.

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Illustrations

All illustrations are unretouched photomicrographs. The negatives, 2¼ inches square, were then enlarged about 50% for mounting. With eventual reduction for printing, the ultimate magnification will be essentially that at which the photographs were originally taken. Photographs were made at the following magnifications: 100X, 200X, 440X, and 1,000X.

Figure 1. Typical ova of the goldfish ovary (Champy-iron hematoxylin, 100X) before growth and differentiation have started. Note the number and position of the numerous nucleoli.

Figures 2, 3, 4. These photographs show the golgi network (1,000X) so typical of the small oogonia when prepared according to the method of Aoyama. In figure 3, the dark mass adjacent to the nucleus (light area in the center of the cell) is the yolk nucleus, over-stained in this preparation.

Figures 5, 6. These figures (Aoyama, 1,000X) show the granules that result from the break-up of the golgi network in the smaller eggs. The upper cell in figure 5 shows relatively few granules along the cell periphery, before yolk formation. This figure also shows the yolk nucleus with most golgi granules having been lost; only few very large granules remain. The lower cell in figure 5 and the cell in figure 6 show the golgi granules of both sizes, between spheres of the gray yolk.

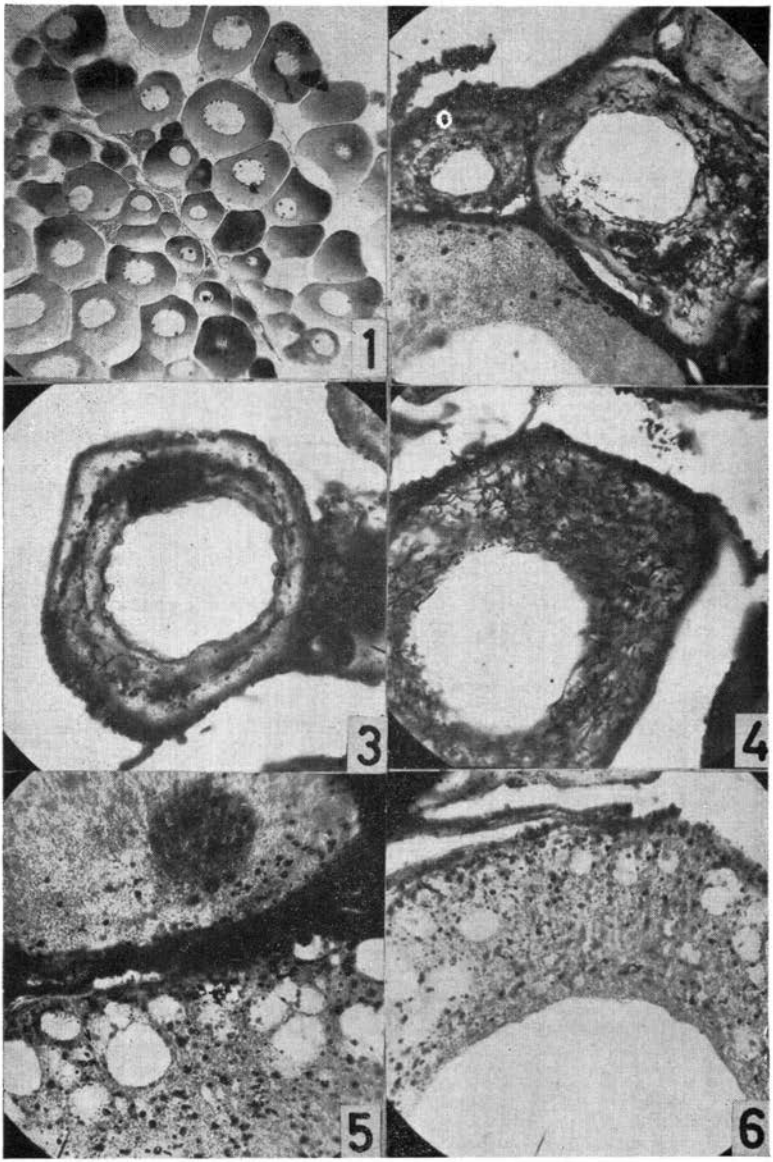
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Figure 7. This cell (Ludford, 1,000X) shows the yolk nucleus with embedded golgi granules; also shown are sparsely scattered osmophilic golgi granules in the cytoplasm (see fig. 10).

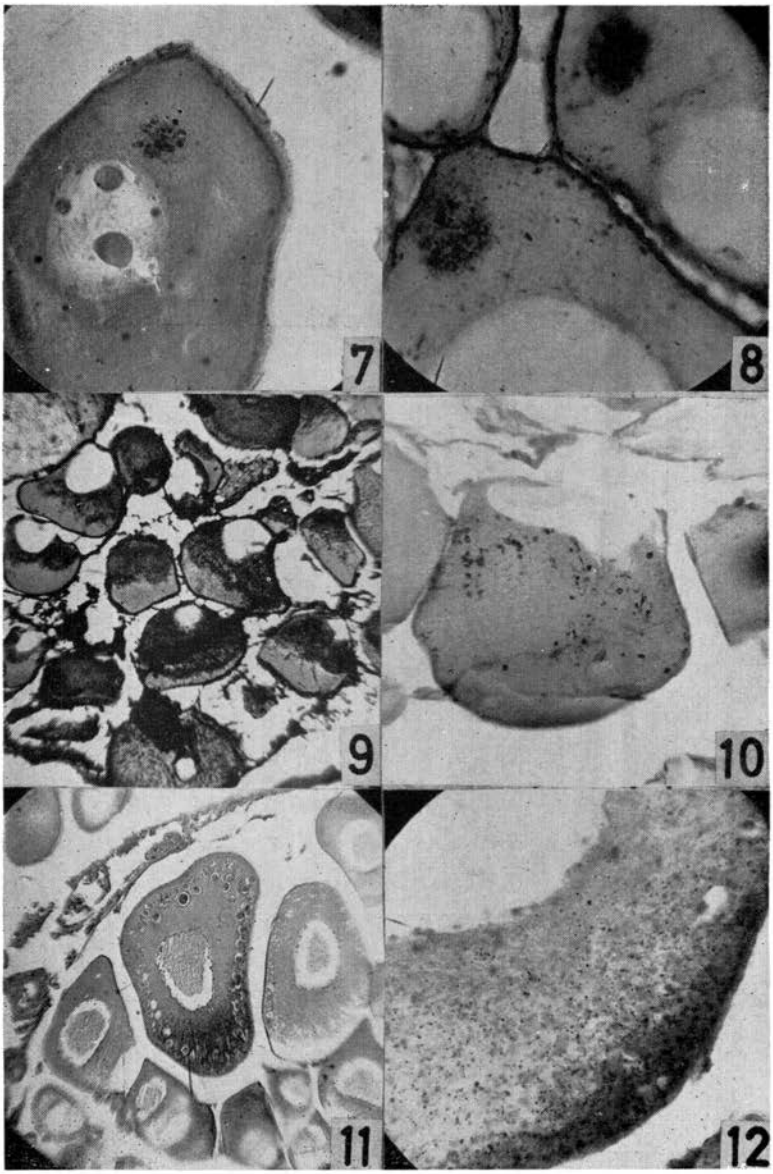
Figure 8. Yolk nuclei in Aoyama preparations (1,000X); the golgi granules stain black; the finely granular matrix stains brown.

Figure 9. Centrifuged cells (Aoyama, 200X) showing the centripetal displacement of the golgi network in small cells (the centripetal pole is toward the top of the page). This is the same network shown in figures 2-4. The light, circular area in many of the cells is the nucleus, similarly displaced to the centripetal pole.

Figure 10. A centrifuged cell (Ludford, 1,000X) showing the displacement of the golgi granules toward the centripetal pole. The flattened, light area at the top is the nucleus. Displacement of granules in this cell evidently was incomplete. Compare this to the normal cell in figure 7, similarly fixed.

Figure 11. The large cell (Ludford, 200X) in the center shows the invasion of the cell cytoplasm at one pole by the fine, black, golgi granules.

Figures 12, 13. These detailed photographs (Ludford, 1,000X) show the nature of the osmophilic granulation indicated in figure 11. In figure 12, yolk formation is just starting; in figure 13, yolk formation is well along.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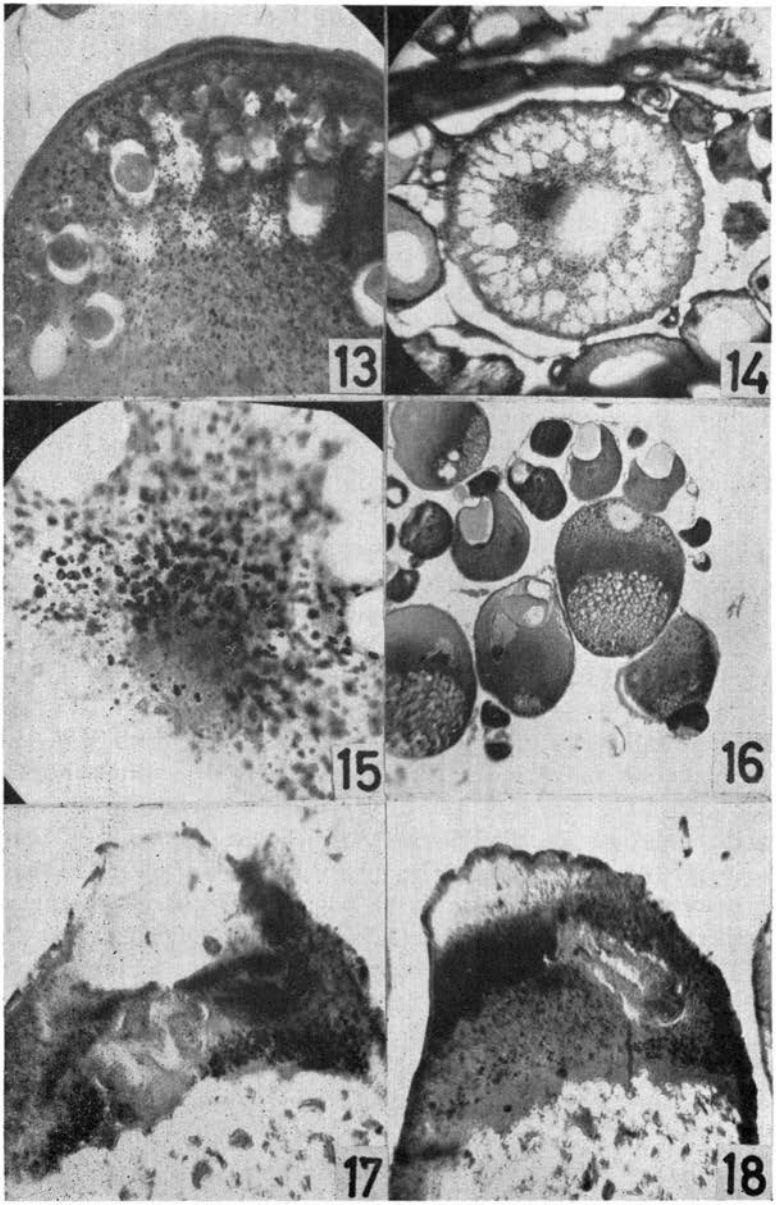
Illustrations

Figure 14. This large cell (Aoyama, 200X) shows a structure that may well be the idiozome of these eggs. The light area in the cell center is the nucleus; the yolk spheres are at the egg periphery. The black granules scattered throughout the cytoplasm, between yolk spheres, are golgi granules. The possible idiozome complex is a mass of loosely scattered golgi granules.

Figure 15. An oil immersion photograph of the same structure shown in figure 14. The nucleus is at the lower left corner.

Figure 16. A Bouin-hematoxylin preparation (100X) of centrifuged cells. The nuclei are the light areas at the top of the cells. The gray yolk spheres are at the centrifugal pole (lower portion of cells). The dark granular zone adjacent to the yolk represents the band of coarse golgi granules; the mottled area adjacent to the nucleus is filled by fine golgi granules. This is the appearance they give in Bouin preparations.

Figures 17, 18. Both cells (Ludford, 1,000X) show the displacement of golgi granules on centrifuging at forces above 100,000 g. The yolk is at the bottom, the nucleus (fig. 17) is at the top. The nucleus has ruptured in figure 18 and can be seen in the midst of the golgi granules. Adjacent to the yolk are the large golgi granules. The extreme centripetal pole of the cell in figure 18 is filled probably by some cytoplasm and nucleoplasm of the ruptured nucleus.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