

## THE OÖGENESIS OF *BUFO LENTIGINOSUS*.

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The present paper records the results of an investigation of the oogenesis of the American toad, *Bufo lentiginosus*, which was undertaken, primarily, in order to trace the history of the chromatin from the oögonia to the maturation period of the oöcytes and thus to complete my study of the chromatin behavior in the germ-cells of this amphibian. The work has necessarily involved a detailed study of the nucleoli, since these structures are closely associated with the chromatin at certain periods of development; and it has been extended to include an investigation of the yolk formation, as the material seemed especially favorable for this purpose.

This study was begun several years ago at Bryn Mawr College, but was laid aside for various reasons until this past year, when it was completed at the Biological Laboratory of the University of Pennsylvania, where I was holding a University Fellowship for Research in Zoölogy. I take this opportunity to express my obligations to Professor E. G. Conklin for many valuable suggestions during the course of my investigations.

### I. MATERIAL AND METHODS.

*Bufo lentiginosus* is found very abundantly in the vicinity of Philadelphia; and, as the tadpoles are easily reared in the laboratory, several different series of preparations have been obtained consisting of larvæ killed at frequent intervals from the time of hatching until metamorphosis. These series give all stages in the development of the germ-cells up to the early growth period of the oöcyte. For the study of the later development of the ova, young toads with a body length of 1.5-5.5 cm. were collected at various times from June until

September. In order to compare the development of the ova in the young toad with that of the ova in the adult, portions of the ovaries of mature females were preserved at different times during the summer months. As the eggs appear to develop along similar lines in all toads, ovaries of young females were used principally for these investigations, since in them the ova are more nearly uniform in size than they are in the adult, and in a single section it is possible to find a large number of eggs in practically the same stage of development.

The very conflicting results that have been obtained by the investigators who have studied the development of the germ-cells in amphibians can doubtless be attributed, in part at least, to the great diversity of ways in which the material has been preserved. Carnoy and Lebrun, who have studied the germinal vesicle in the eggs of many different species of amphibians, unhesitatingly recommend Gilson's fluid as the best fixative for the amphibian egg. I have not found that this liquid gives a satisfactory fixation of the egg of *Bufo*, as it usually causes a decided shrinkage of the nucleus and, at certain stages, a distortion of the nuclear contents. A number of different fixing fluids have been tried during the course of these investigations, among which may be mentioned Zenker's fluid, corrosive-acetic (5 per cent acetic acid), corrosive-formalin (Bouin's method), picro-acetic, Flemming's solution, chromic-acetic, and Hermann's fluid. Flemming's solution (strong formula) is the best fixative for the oögonia and the early growth stages of the oöcytes, although Zenker's fluid and Hermann's fluid give very good results. After the yolk has formed Flemming's solution does not penetrate the egg sufficiently well to give a satisfactory fixation. For this later period I have found that the chromic-acetic solution recommended in a previous paper (King, 49) gives the best preparations. Corrosive-acetic acid is also a good fixative for the egg at this period of its development, but it is especially valuable for the maturation stages. Corrosive-formalin and picro-acetic do not give a satisfactory fixation of the egg of *Bufo* at any stage of its development.

In *Bufo*, as in the turtle and in the frog according to the investigations of Allen (1, 2), the germ-cells arise in connection with the endoderm. Allen's recent account of the origin of the sex-cells in *Rana pipiens* agrees essentially with what I have found in *Bufo*. I cannot be sure, however, that in *Bufo* the ridge of germ-cells is separated from the endoderm "by the approximation of the lateral plates of mesoderm," although many sections give this impression. During this early period of development, when so many organs are rapidly being differentiated from embryonic tissue, it is impossible to tell exactly what forces or combination of forces are at work shifting the materials from one place to another. It is possible, as Allen suggests, that the germ-cells themselves take an active part in the processes which separate them from the endoderm, since it is apparently only through their own activity that they reach their final position in the embryo. Since Hertwig (41), Boveri (12), and others have traced the germ-cells back to segmentation stages, and Conklin (21, 22) has found various organ-forming substances in definite areas in the unsegmented egg, it seems meaningless to speak of organs as arising from any definite "germ-layer," although the convenience of such a starting point for the study of the development of any structure is obvious. Owing to the character of the embryonic cells it is seemingly impossible to trace any organ in *Bufo* back to early cleavage stages, and the sex-cells are not clearly defined until the tadpole is about five days old. At this stage of development the germ-cells still retain their earlier embryonic character, and they are in contact with and closely resemble the endodermal cells. Instead of asserting that the germ-cells in *Bufo* are endodermal in origin, it seems to me more in keeping with the results of the investigations on other more favorable forms to assume that these cells in *Bufo* are of like generation with the primitive endodermal cells and that both kinds of cells arise from neighboring regions of the unsegmented egg. It may sometimes be possible to determine the organ-forming regions in the unsegmented egg of *Bufo* as Conklin has done in the egg of *Cynthia*.

When the genital ridge is first clearly marked off from the endoderm it occupies a median position between the cardinal veins and beneath the aorta (Fig. 2), as Bouin and Allen have stated is the case in *Rana*. If a section of the ridge in this stage of development is examined under high power one finds that it is composed of two distinct types of cells, one many times larger than the other (Fig. 3). The large cells, which are filled with yolk spherules and have vaguely defined boundaries, are the primordial germ-cells. The nuclei of these cells have the "mulberry" shape which La Valette St. George (78) discovered to be a characteristic of the nuclei in the spermatogonia of *Salamandra*, and they are usually crowded by the yolk spherules into one corner of the cell. The chromatin in these nuclei is in the form of minute, faintly staining granules which are distributed on linin threads or along the nuclear membrane. Each nucleus contains several rounded, deeply staining nucleoli of various sizes. Judging from their staining reactions most of these nucleoli are plasmosomes, and only one or two of the smaller ones are karyosomes. Scattered among these germ-cells, and frequently flattened against them, are numerous small cells which resemble in all respects the cells of the peritoneal epithelium from which they doubtless have been derived. These cells are very much smaller than the germ-cells; they contain no yolk and they have an elongated, deeply staining nucleus which is very large in proportion to the size of the cell. Doubtless these cells migrate into the genital ridge after the formation of the mesentery, since there are no cells of this type in the genital ridge at the stage of Fig. 1, and I have seen nothing that would indicate that they are derived from the germ-cells.

Bouin has stated that he finds in *Rana temporaria* transitional stages between peritoneal cells and primordial germ-cells, and he believes that before the metamorphosis of the tadpole new germ-cells are constantly arising from peritoneal cells. These observations have not been confirmed by Allen (2) in his study of the origin of the germ-cells in *Rana pipiens*, and in *Bufo* I can find no evidence that the germ-cells are

derived from peritoneal cells at any stage of development. The peritoneal cells in the genital ridge vary considerably in size and some may be nearly twice as large as others. In all cases, however, the cell contains comparatively little protoplasm and no yolk; while the nucleus maintains a characteristic appearance and stains very deeply, thus standing out in sharp contrast to the larger, more irregular, and more faintly staining nuclei of the germ-cells.

The development of the genital ridge proceeds from before backward. In a section of the anterior part of the ridge there are usually from 5-8 large germ-cells (Fig. 3), while in a more posterior section there are rarely more than three of these cells. In older tadpoles the difference in the rate of development of the different parts of the genital ridge is even more strongly marked, since the anterior portion of the ridge may have taken on its definite character as an ovary or a testis while the posterior portion remains in an apparently indifferent state.

When a tadpole is ten or eleven days old, the yolk spherules begin to disappear from the cells of the genital ridge and the structure of the germ-cells can then be more clearly seen (Fig. 4). At this time the germ-cells are more rounded than they were at an earlier period and, as they contain fewer and smaller yolk spherules, the polymorphic nucleus is usually found in the centre of the cell. With the exception of the large plasmosomes, the nuclear contents still show little capacity for staining either with plasma or with chromatin stains. By this time many peritoneal cells have become flattened against the germ-cells and have thus assumed the rôle of follicle cells. The boundaries of these follicle cells become very indistinct, and in many cases the cytoplasm seems to disappear entirely leaving the deeply staining nuclei in contact with the germ-cell.

In early stages of development the germ-cells are not always confined to the genital ridge. At the right, in Fig. 4, is a cell (Y) which lies considerably outside of the germinal area and directly under the Wolffian tubule; in Fig. 5, at the

left of the aorta are two germ-cells which lie above the level of the genital ridge. Such germ-cells must eventually come into the germinal area or degenerate, since cells of this character are never found outside of the genital ridge in later stages of development. I have never found cells with the characteristics of germ-cells in the mesoderm or in the ectoderm.

In a tadpole twelve to fourteen days old there is usually found the beginning of a separation of the median genital ridge into two ridges symmetrically placed one on each side of the middle line (Fig. 5). This division of the genital ridge is evidently brought about through the activity of the germ-cells, although I have never been able to find any evidence of amoeboid movement in these cells. A longitudinal section through a tadpole thirteen days old (Fig. 6) shows that, at the time the genital ridge is dividing, the germinal area extends from about the level of the liver nearly to the posterior end of the body-cavity. When the division is completed the anterior portion of each genital ridge contains from two to five germ-cells (Fig. 7), while the middle and posterior portions rarely contain more than one or two germ-cells (Fig. 8). Sections through the posterior region of a genital ridge frequently contain only the peritoneal cells (Fig. 9) which seem to be crowding into the germinal area in increasing numbers at this time.

The primordial germ-cells in the sex-gland of a tadpole about to undergo metamorphosis are similar to those found in the genital ridge at the stage of Fig. 4, except that they contain only a small amount of yolk. After the greater part of the yolk has been absorbed there is found in the cytoplasm of these cells a small, round, deeply staining, apparently homogeneous body which is sometimes, though not invariably, surrounded by a clear area (Fig. 8, V). This body, which I shall call the vitelline body, divides previous to the cell mitosis (Fig. 7, V), and one of these bodies is to be found subsequently in each of the daughter cells.

In addition to the vitelline body, there is found in the

cytoplasm of the germ-cells, usually close to the nucleus, a small centrosome which is surrounded by a rounded, granular attraction-sphere (Fig. 8, C). This centrosome divides very early in preparation for the cell mitosis, and, as shown in Fig. 7, it is sometimes possible to find a section of a cell which contains two centrosomes as well as two vitelline bodies. Such a section shows conclusively that the vitelline body is not derived from the centrosome and that there is no relation between these bodies. I have, as yet, no clue to the origin of the vitelline body which, as will be shown later, is undoubtedly concerned in the formation of yolk nuclei. A structure similar to the vitelline body is found in the cytoplasm of the spermatogonia of *Bufo*, and it can be traced directly to the spermatids where it gives rise to the acrosome of the mature spermatozoon. Since Meves (69), McGregor (63), and Brozman (13) have found that the acrosome of the amphibian spermatozoon is derived from the idiozome, I suggested in a previous paper (King, 52) that the body in *Bufo* which forms the acrosome might possibly be derived "from a condensation of a portion of the attraction-sphere at an early period in the history of the primary spermatogonia." My study of the primordial germ-cells has not given any support to this hypothesis since, although this body is usually found near the attraction-sphere (Fig. 7), the two structures are clearly distinct at all times and there is not the slightest evidence that the former is derived from the latter. In its size and general appearance the vitelline body closely resembles the small nucleoli in the nuclei of the primordial germ-cells, but I have seen nothing that would indicate that it is of nucleolar origin. The later history of this structure in the ova strongly suggests that it is a secretion product of the cytoplasm formed, possibly under the influence of the nucleus, but not from nuclear material.

According to the investigations of Bouin, the increase in the number of germ-cells in *Rana* is brought about through a continuous process of transformation of peritoneal and mesenchyme cells into sex-cells, not by mitosis nor by direct division

of the germ-cells already present in the germinal area. In *Bufo* I have found that the multiplication of the germ-cells is solely through mitotic division of the primordial cells evolved from embryonic issue. Although mitotic figures are comparatively rare during the early stages of development they are found very abundantly when the tadpole approaches metamorphosis, and in a single section of the ovary of a toad killed at this time one may find several cells that are preparing to divide (Fig. 17, P). Stages in the division of the primordial germ-cells are shown in Figs. 10-14. In the early prophase of mitosis the chromatin forms a thick spireme which is so much convoluted that it is impossible to determine whether it is continuous or not. This spireme is subsequently broken into segments of various lengths (Fig. 10). There are 24 of these segments, this being the number that is characteristic of the somatic cells of the species. Usually all of the nucleoli have disappeared before the segments are formed, but sometimes, as shown in Fig. 10, Nu., a nucleolus will persist until a much later period. This would seem to indicate that the nucleoli are not used in the formation of the chromosomes. The chromatin segments shorten gradually and form broad, V-shaped loops which can readily be arranged in pairs according to their lengths (Figs. 11, 12). In the metaphase the chromosomes are arranged in a circle with the angle of the V turned towards the centre of the spindle (Figs. 11, 13, 15); and, as they subsequently undergo a longitudinal division, much narrower V-shaped chromosomes are found at the spindle poles in the late anaphase (Fig. 14).

In sections of the ovary of a tadpole killed at the time of metamorphosis germ-cells are frequently found which appear to contain two or more separate nuclei (Figs. 15, 16, X). Judging from these figures alone one might feel justified in concluding that the germ-cells divide amitotically as well as by mitosis. I have never found a division of the cytoplasm in any of the cases in which sections of the germ-cells contain two or three nuclei, and in every instance the following or preceding sections invariably show a connection between the various nuclei in the cell. It is evident therefore, that the

apparently multinucleated cells are not preparing to divide by amitosis. Their appearance is doubtless due to the fact that in sectioning the cells the polymorphic nuclei were cut in such a way as to completely separate two or more lobes. In my study of the germ-cells of *Bufo* I have never found a single instance where I could be sure that a cell was dividing amitotically; and I am convinced that this mode of division does not normally occur in any of the germ-cells of the ovary or of the testis.

By the time that a tadpole is sixteen to eighteen days old the anterior portion of each genital ridge has developed into a small rounded body, the so-called "Bidder's organ." The structure and development of this organ will form the subject of a separate paper and therefore no further mention of it will be made here, as it has seemingly nothing to do with the development of the ova.

Although sex is doubtless determined at a very early stage of development, the germ-cells of *Bufo* remain in an apparently indifferent condition for a long period, and it is not until the tadpole is about to undergo metamorphosis that its sex can be ascertained with any degree of certainty. Several investigators of amphibian oögenesis have stated that the presence of a central cavity in the genital ridge is the first characteristic by which the young ovary can be identified. In *Bufo* it is possible to distinguish the sexes at a somewhat earlier period of development by means of the arrangement of the cells in the more anterior portion of the sex-gland. In the young male the germ-cells are scattered evenly throughout the testis, each being surrounded by a number of follicle cells; in the young female the germ-cells have a definite arrangement around the outside of the ovary, while the centre is filled with peritoneal cells (Fig. 15). There is no central cavity in any part of the genital ridge at this time.

When the genital ridge has taken on the definite character of an ovary, some of the oögonia still contain a few small yolk spherules (Fig. 15), although all traces of yolk have long since disappeared from the other cells of the body. There is no ovarian wall at this time and the oögonia are surrounded

by follicle cells as in an earlier period. At a slightly later stage of development (Fig. 16), the central part of the ovary is no longer completely filled with peritoneal cells, but it contains a number of intercellular spaces which later unite to form one large cavity (Fig. 17). The central cavity in the ovary of *Bufo* is not, therefore, a portion of the general body-cavity which is brought into the ovary by a fold of peritoneal epithelium as Hoffman has claimed is the case in *Triton* and in various other amphibians, but it is the result of a fusion of the many intercellular spaces which are produced by the rapid increase in the size of the ovary. In Fig. 16 is shown the beginning of the formation of the outer ovarian wall. At the upper part of the ovary a number of peritoneal cells are found with their nuclei flattened against the outer surface of the germ-cells. The outlines of these cells become obliterated and their cytoplasm forms a continuous layer over the oögonia. At a slightly later stage (Fig. 17), many of the peritoneal cells in the interior of the ovary become arranged along the inner side of the oögonia to form the inner wall of the ovary. In the young female as well as in the adult, the ova develop between the two ovarian walls.

The small cells with deeply staining nuclei which are so conspicuous in the ovary at the stages of Figs. 15-17 have been called by various observers mesenchyme cells, peritoneal cells, and follicle cells; while Bouin considers them to be "petites cellules germinatives." In *Bufo* these cells are found with the primordial germ-cells when the latter are first separated from the endoderm at the stage of Fig. 3, and from their general characteristics they are doubtless to be classed as mesodermal cells. Occasionally these cells are found dividing mitotically (Fig. 15, R); but division figures in them are rare as compared with those that are found in the germ-cells. The number of these cells increases enormously as the ovary enlarges; and, since there is no evidence that they divide amitotically, it is probable that there is a continuous migration of cells from the mesentery through the ovary pedicle into the ovary. Many of these cells later become the follicle cells which are found around the egg as long as it remains in the

ovary; the others, as far as I can determine, are actively concerned in the formation of the ovarian walls, the cyst membranes and the zona pellucida.

According to the observations of Bouin there are fewer primordial germ-cells in a tadpole of *Rana temporaria* that is 33 mm. long than in one 20 mm. long. As the difference in numbers is considered too great to be attributed to individual variation, Bouin believes that a reduction in the number of primordial germ-cells is brought about at this stage of development through an expulsion of a large number of these cells from the ovary into the body-cavity. He calls this process "ponte d'ovules primordiaux," and he considers that it is analogous to that which occurs in the adult frog when the ripe eggs are expelled from the ovary. Bouin suggests that this process may take place so that "la glande, qui évolue dans le sens mâle, élimine les éléments qui seraient inutiles à son développement ultérieur." None of the other investigators who have worked on the development of the sex-glands in amphibians have described such a reduction in the number of primordial germ-cells and there is nothing similar to it to be found in *Bufo*. The expulsion of primordial germ-cells from the ovary is, therefore, either a process that is peculiar to *Rana temporaria*, or it is one which takes place so quickly in other species that it has escaped the attention of the investigators working in this field.

As the tadpole approaches metamorphosis, the ovary increases in size very rapidly and it usually appears lobed when examined in toto under a low power of the microscope. This lobed appearance of the young ovary furnishes a means by which it can be distinguished from the testis without making use of sections.

### III. THE SECONDARY OÖGONIA.

Soon after metamorphosis the primary oögonia give rise to a new generation of cells, the secondary oögonia, which are aggregated into cysts or "cell nests" that are arranged much

as are the primary oögonia shown in Fig. 17. The cells of a cyst are all descendants of one primary oögonium, and the cyst wall is formed evidently by the follicle cells which had previously surrounded the parent cell. The secondary oögonia are somewhat smaller than the primary oögonia, but they closely resemble them otherwise. They have a polymorphic nucleus containing a faintly staining reticulum and several plasmosomes. In the cytoplasm is a vitelline body (Fig. 18, V) and also a minute centrosome surrounded by a granular attraction-sphere (Fig. 18, C).

The cells of a cyst do not always divide simultaneously, and resting cells as well as cells in all stages of division may be found in the same cyst (Fig. 19). In the early prophase of mitosis a thick spireme is formed, as in the primary oögonia. This spireme breaks into segments (Fig. 19, S), presumably twenty-four, which condense into V-shaped chromosomes in the metaphase (Fig. 19, O). The spindle is the same shape as that found in the earlier generations of cells, and there are distinct centrosomes at the spindle poles which are devoid of any radiation (Fig. 19, O, R).

#### IV. THE DEVELOPMENT OF THE OÖCYTES TO THE SYNIZESIS STAGE.

Considerable controversy has arisen among investigators regarding the origin of the oöcytes in the amphibian ovary since, at the period of the transformation of oögonia into oöcytes, cell and nuclear boundaries are frequently obscured and the cyst contents appear as a syncytium.

In his classic work on *Bombinator igneus*, Goette (35) states that in the young ovary the protoplasmic bodies of the central cells of a cyst fuse into a single mass which contains at first several separate nuclei; later the nuclei also fuse to form the mulberry shaped germinal vesicle of the egg. This view has been slightly modified by Bataillon (6), who concludes, from his observations on *Rana* and on *Bufo*, that after the fusion of the cytoplasmic bodies of the cells of a cyst one

of the nuclei wins the upper hand and subsequently absorbs all of the others.

Gemmil's (34) observations seem to indicate that "in der Regel geht aus einem Zellnest nur ein Ei hervor, und zwar durch direkte Entwicklung aus einem der Elemente des Zellnestes. Von den übrigen Elementen bilden sich einige wieder zurück und beteiligen sich an der Bildung der Granulosa, der Rest aber geht zu Grunde." According to Gemmil, there appears to be a struggle among the cells as to which shall form the ovum; space being the chief factor which decides the contest. The cell which lies in the centre of a cyst has seemingly the most room for development and this is the one which usually wins. The fate of the other cells depends upon how far they have differentiated before the one cell becomes the ovum and so governs the rest. The cells which are least differentiated assume the rôle of granulosa cells. Those further developed cannot go backwards; they either have to become eggs or disintegrate. If the cyst happens to be larger than usual, as many as four of the cells may have room to develop into functional eggs. Since extra space is rarely obtained by the cyst, all of the cells which have passed a certain stage of development before the egg has formed are, as a rule, forced to disintegrate, and traces of the débris from these cells are to be found for some time in the protoplasm of the developing egg. Hoffmann's opinion regarding the origin of the egg in the Anura is similar to that of Gemmil, since he believes that one cell of a nest outstrips the others in development and forms the ovum while the others degenerate and become granulosa cells. Semon's observations lead to a similar conclusion.

Nussbaum and also Knappe (54) find a mulberry shaped nucleus in the primordial germ-cells, and they assert that this nucleus divides by amitosis into several small nuclei. One of these nuclei increases rapidly in size and becomes surrounded by the greater part of the cytoplasm of the cell, thus forming the egg; the other nuclei become arranged around the periphery of the egg to form the follicle epithelium. Ac-

cording to Eismond (27) an ovum may arise either from one of the cells of a nest which has outstripped the others in development, or from a fusion of all of the cells of a cyst. He also considers that "la formation des nids n'était pas un anneau indispensable dans le cycle de l'oögenèse, c'est-a-dire qu'en même temps que la formation des nids du sens strict, se faisait aussi la différenciation progressive des oöcytes directement aux dépens des produits de la dernière division des oögonies, comme cellules indépendantes." The conclusion that ova may arise directly from oögonia accords with the view advanced in 1870 by Waldeyer (91) and supported later by the researches of Balfour (4) on elasmobranchs.

Bouin has investigated the formation of the ova in much greater detail than have any of the other workers on amphibian oögenesis. He finds, as do other investigators, that secondary oögonia are enclosed in cysts, and he states that all of the oögonia in a cyst divide simultaneously. After several divisions, the number of which he does not determine, the character of the cells changes considerably and "oögonia of transition" are formed. The latter are clearly defined cells with rounded nuclei in which there are several chromatin nucleoli, but no traces of a chromatin reticulum. This stage is succeeded by one in which the nuclear membrane disappears and the karyoplasm is separated from the cytoplasm only by clear area. At a later stage of development granular threads appear in the nucleus which are formed, doubtless, of the minute chromatin granules scattered in the karyoplasm. These threads increase in number very rapidly and form a distinct chromatin reticulum, while a new nuclear membrane encloses the nuclear contents. All the cells of a cyst develop up to this stage, but later, owing to some unknown causes, only a part of the cells continue their development as oöcytes; the others degenerate and are either dissolved gradually or devoured by the phagocytes. Degenerating cells never form follicle cells but probably serve as nutriment for the victorious oöcytes. The results of Bouin's investigations agree essentially with those reached by Balfour in his study of

elasmobranchs. The latter investigator states that "some of the nuclei of each nest are converted into the nuclei of the permanent ova, others break down and are used as the pabulum at the expense of which the protoplasm of the young ovum grows."

Judging from the number of cells in a fully formed cyst, there are at most four or five generations of secondary oögonia in the ovary of *Bufo*. After the last oögonial division resting nuclei are formed, and the cyst is filled with small cells which appear much like that shown in Fig. 20. At this time cell and nuclear boundaries are very much more indistinct than in earlier stages, yet they can readily be made out in preparations fixed in Flemming's solution and stained with iron hæmatoxylin. If the material is properly preserved the cells never form a syncytium; nor is there any fusion of the nuclei, or any absorption by one nucleus of its less fortunate neighbors. Each cell in a cyst develops into an oöcyte, and, although I have examined a large number of cysts in this stage of development taken from many different individuals, I have yet to find a single instance in which there is a degeneration of any of the germ-cells in a cyst or any change of germ-cells into follicle cells. It seems very probable that the cells which several investigators have considered to be degenerating young oöcytes, were, in reality, cells in which the nuclei were in the condition shown in Fig. 25. This contracted state of the nuclear contents, to which McClung (62) has applied the term synizesis, is a definite constructive stage in the development of the young oöcyte of *Bufo*, and it is not due in any way to a degeneration of the nucleus or of the cell.

Owing to the crowded condition of the cells in a cyst the young oöcyte is more or less polygonal in outline. The nucleus is very large in proportion to the size of the cell, and it is invariably oval or slightly irregular, never possessing the polymorphic form characteristic of the nuclei in the earlier generations of cells. At this period the chromatin shows little capacity for staining and, as in the resting oögonia, it is in the form of minute granules which are either scattered along

the nuclear membrane or distributed on the linin fibres which form an irregular reticulum. The nucleus contains several deeply staining nucleoli of various sizes which are suspended in the meshwork of the reticulum or held against the nuclear membrane. In preparations stained with safranin and gentian violet the larger nucleoli invariably take the safranin while the rest of the nucleus is stained blue with the gentian violet, and these bodies must, therefore, be considered as plasmosomes; the very small nucleoli, which are found chiefly at the points of intersection of the linin threads, are karyosomes since they take the chromatin stain. In the cytoplasm, which stains very faintly and appears somewhat reticular, there is a vitelline body (Fig. 20, V); but I have not been able to find any traces of a centrosome or of an attraction-sphere in this or in any later period in the development of the oöcyte. As there are no centrosomes at the poles of the maturation spindle (King, 51), it seems probable that the egg centrosome disappears after the last oögonal division and that the attraction-spheres found at the poles of the segmentation spindle are formed in conjunction with the sperm-nucleus, probably under the influence of the centrosome imbedded in the substance of the sperm-head.

As the oöcyte enlarges its outline becomes more regular and much more distinct. The nucleus, which measures about 0.01 mm. in diameter at this time, soon assumes the rounded form which it retains up to the maturation period (Fig. 21), and its reticulum appears continuous and much more sharply defined than at an earlier period (Fig. 22). The number of nucleoli is not appreciably increased during the early growth period of the oöcyte.

#### V. SYNIZESIS AND POST-SYNIZESIS STAGES.

Although the stage in the development of the oöcyte shown in Fig. 22 is practically at the beginning of the growth period it corresponds, apparently, to the stage at the end of the growth period of the spermatocyte (King, 52; Fig. 15). In

both cases the nucleus contains a granular reticulum which appears to be continuous; and in both oöcyte and spermatocyte this stage is followed immediately by one in which there is a gradual condensation of the nuclear contents leading to synizesis (Fig. 25). The beginning of the process of condensation in the oöcyte is shown in Fig. 23, where the greater part of the chromatin reticulum is seen to be collected in the centre of the nucleus. In the following stage the contraction of the nuclear reticulum becomes more marked (Fig. 24), and eventually all of the nuclear contents forms a more or less rounded mass in the centre or at one side of the nucleus (Fig. 25). In favorable preparations the contraction figure is found to be composed of a tangled mass of exceedingly fine filaments in the meshes of which there are several round, apparently homogeneous bodies which are doubtless the plasmosomes: a number of the filaments run out from the central body and connect this structure to the nuclear membrane. At this stage it is impossible to follow in detail the changes that are taking place in the nucleus or to determine what relation the fine filaments bear to the nuclear reticulum of the earlier stage. The condensation of the nuclear contents in synizesis is not carried quite as far in the oöcytes of *Bufo* as it is in the spermatocytes where the contraction figure frequently appears as a rounded, apparently homogeneous mass connected by a few fine filaments to the nuclear wall (King, 52; Figs. 20-22).

In toads killed at the time of metamorphosis the ovaries contain large numbers of secondary oögonia and young oöcytes, although only a few of the latter have reached the synizesis stage at this time. Contraction figures are frequently met with in the ovaries of young toads killed about four weeks after their metamorphosis, and they are found very abundantly afterwards until the toad has attained a body length of about 1.5 cm. As I have already pointed out in the case of the spermatocytes of *Bufo*, I do not think it possible that the contraction figures are due to a bad preservation of the material as Janssens (47) has asserted is the case in *Batracoseps* atten-

uatus, since oöcytes with their nuclei in synizesis are found in all parts of the ovary and frequently lie adjacent to oöcytes in which the chromatin is in the form of a clearly defined continuous spireme (Fig. 22). Any method of fixation that would cause such a decided distortion of the nuclear contents in the one cell must of necessity have some effect on a neighboring cell which is in but a slightly different stage of development. In *Bufo* synizesis is not due to the degeneration of certain cells as Kingsbury (53) has claimed is the case in *Desmognathus fusca*, since only in very rare instances are degenerating eggs to be found in the ovaries of young toads. Degenerating eggs, whether they are found in the ovaries of young toads or in those of adults, are usually deeply pigmented and they are invariably filled with phagocytes; they never resemble in any way the oöcytes shown in Figs. 24-25.

Synizesis, which is a well recognized stage in the development of the oöcytes and spermatocytes of many forms, has, for the most part, been ignored by the investigators who have worked on the germ-cells of amphibians, or its presence has been considered as evidence of a degeneration of the cell. Gemmil describes a stage in *Pelobates fuscus* in which the nucleus of the young oöcyte contains a star-shaped mass of chromatin which lies in the middle of a clear area and sends out processes to the nuclear membrane. It is evident, from the figures which Gemmil gives, that synizesis is the normal stage in the development of the ova of this amphibian. Nussbaum figures condensation stages in the young germ-cells of *Rana fusca* when they are enclosed in a cyst membrane. He has, however, mistaken the order of sequence in the development of the cells, as he considers that the contraction stage preceded one in which the cell contains a mulberry-shaped nucleus. Bataillon, Leydig, and Hoffman also mention the appearance of contracting figures in the course of the normal development of amphibian ova, although they venture no opinion as to the significance of these bodies.

Bouin has entirely overlooked in *Rana temporaria* the young oöcytes shown in my Figs. 20-22, and the earliest stage that he

figures as an oöcyte (Plate XII; Fig. 6), is about like that of my Fig. 39. He does not believe that synizesis is a normal stage in the development of the oöcytes of *Rana*, although he figures contraction stages of the nuclei in cells which he considers as oögonia that are not able to develop into oöcytes. His description of the nucleus of one of these "degenerating" cells is as follows: "On constate que les microsomes constitutifs du réticulum chromatique se gonfluent, se soudent les uns aux autres, forment des amas irréguliers qui se colorent comme les chromosomes des noyaux en mitose. Ces amas peuvent rester isolés dans l'aire nucléaire ou s'amalgamer en un bloc chromatique de faibles dimensions." The one figure which Bouin gives of such nucleus (Plate XI; Fig. 15), shows the synizesis stage in *Rana* which corresponds closely to that in *Bufo* shown in Fig. 25; and many of his other figures show post-synizesis stages comparable to those in *Bufo* (Plate XI; Figs. 10, 11: Plate XII; Figs. 2-5).

In a recent paper Lams (57) has given a description of the stages in the early development of the oöcytes of *Rana temporaria* which were overlooked by Bouin. According to this investigator the nuclear membrane does not disappear at any time during the transition of the oögonia into the oöcytes. In the young oöcytes the chromatin filaments gradually condense at one pole of the nucleus until they form a rounded, deeply staining mass which appears much like that shown in my Fig. 25. In post-synizesis stages this contracted mass resolves into a system of filaments which subsequently divide longitudinally and scatter throughout the nucleus. This work of Lams, with that of Bataillon and Leydig, furnishes conclusive evidence that synizesis is a normal stage in the development of the oöcytes of *Rana*.

It is unfortunate that the contracted condition of the nuclear contents during synizesis prevents a detailed study of the changes taking place in the chromatin at this time. It is evident that during synizesis the nuclear reticulum is no longer continuous, and that it becomes broken up into a large number of exceedingly fine filaments. Some of these filaments appear

to be composed of a series of minute granules; others of delicate linin threads. As the plasmosomes can still be found during synizesis it is probable that they play no part in the changes taking place in the chromatin.

From the contraction figure shown in Fig. 25 there is evolved a long, apparently continuous, much convoluted spireme which is made up of a series of deeply staining chromatin granules distributed on a linin thread (Fig. 26). In the meshes of this spireme there are several nucleoli of various sizes, and there are also from one to five irregularly shaped, apparently homogeneous nuclear masses which are distributed along the nuclear membrane. These masses all stain intensely black with iron haematoxylin as does also the spireme. If, however, preparations have been satisfactorily stained with safranin and gentian violet the spireme is deep blue, the very small nucleoli appear red, while the large nucleoli and the masses against the nuclear membrane are purple, thus indicating that they are composite structures although they usually appear homogeneous at this time.

From the stage shown in Fig. 21 to that of Fig. 26 the oöcytes do not grow to any appreciable extent and the nuclei measure from 0.011-0.013 mm. in diameter. After synizesis there is a rapid increase in the amount of cytoplasm and in the size of the nucleus (Fig. 27). The chromatin spireme becomes more evenly distributed throughout the nuclear space, and it is noticeably thicker than at the stage of Fig. 26. In the succeeding stage the spireme begins to split longitudinally (Fig. 28). As the sister portions of the spireme are only about one-half of the thickness of the spireme at the stage of Fig. 27 it is evident that there is a true longitudinal division of the spireme at this time and not a folding together of chromatin filaments similar to that which occurs in the young oöcytes of the rabbit according to the investigations of von Winiwarter (93). At the stage of Fig. 29 the greater part of the spireme has divided and many of the sister threads have separated a considerable distance. When the splitting of the spireme has been completed the sister threads lie parallel, for the most

part, although they are not connected in any way. The threads do not present the clear cut, granular appearance of the spireme shown in Fig. 26, as they have a jagged outline and send out fine projections on either side.

There is absolutely no uniformity in the arrangement of the chromatin threads after the splitting of the spireme. At times the sister threads seem to lie close together throughout their whole extent (Fig. 30); again the sister portions of the spireme lie parallel for a short distance and then become widely scattered throughout the nucleus (Fig. 31); in rare cases, as shown in Fig. 32, the chromatin threads are as evenly distributed throughout the nucleus as they are at the stage shown in Fig. 27, and there is nothing except the size of the nucleus and the character of the threads to indicate that there has been a splitting of the spireme. I am very sure that such a condition of the chromatin as that shown in Fig. 32 could not have been brought about by a gradual lengthening of the spireme, since the great majority of nuclei intermediate in size between that of Fig. 27 and that of Fig. 32 appear similar to those shown in Figs. 28-31.

Soon after the stage of Fig. 30 the spireme breaks transversely, forming, in most cases, long double segments which vary considerably in length (Figs. 33, 34, 36, 37). The sister portions of the segments may lie parallel or they may be intertwined in various ways; they may be united at one or at both ends, forming a figure 8 or an oval ring; in other cases both ends of the segments are free and the threads cross in the form of an X or a Y. The condition of the chromatin threads shown in Figs. 30-34 is found in nuclei having a diameter of 0.015-0.02 mm.

I have tried to reconstruct a nucleus in the stage of development shown in Figs. 33-34, by placing together a series of camera drawings of all of the sections of the nucleus, in the hope that I might be able to determine by this means the total number of chromatin segments. Owing to the fact that the segments are of different lengths and that they are united in a great variety of ways, it has been very difficult to arrive at any

exact conclusion regarding their number. I believe, however, that the nucleus at this stage contains only the somatic number of chromosomes (24) which are usually arranged in twelve pairs. The question at once arises as to the value of the sister segments which form a pair. Is the splitting of the spireme shown in Figs. 28-30 a longitudinal division of chromosomes united end to end in the spireme or is it a separation of univalent chromosomes which had conjugated side by side? This question is very difficult to answer since it is impossible to determine what changes the chromatin undergoes during synizesis. As the nucleus apparently contains but twenty-four chromatin segments which in later stages of development are scattered throughout the nucleus and only occasionally found in pairs, I am inclined to the opinion that each of the sister segments represents an oögonial chromosome. The paired arrangement of the chromosomes at the stage of Figs. 33-34 strongly suggests that in the oöcytes of *Bufo* synapsis is coincident with synizesis as it is apparently in the spermatocytes; yet for various reasons, which will be given later, I am inclined to consider that synapsis does not occur until the beginning of the maturation period.

At the stage of Figs. 20-21 all of the young oöcytes in a cyst are approximately of the same size and in practically the same stage of development. As the synizesis period approaches the oöcyte which lies nearest the cavity of the ovary grows very rapidly and soon becomes several times the size of its neighbors. A section of a cyst with the oöcytes in this condition is shown in Fig. 37. The large cell bordering the cavity of the ovary has a diameter of 0.043 mm., and its nucleus measures 0.023 mm. in diameter. This oöcyte is surrounded by a number of follicle cells and its nucleus contains paired chromatin threads. The other cells in the cyst are very nearly of the same size; each measuring about 0.015 mm. in diameter and containing a nucleus measuring 0.01 mm. in diameter. These smaller oöcytes are in early post-synizesis stages of development, and they are not degenerating, as several investigators who have found a similar condition of the

cells of a cyst have claimed. The development of these cells is slower than that of the one cell simply because the size of the cyst is limited and there is no space for a more rapid growth.

Soon after the stage shown in Fig. 37 the cyst wall is ruptured, owing doubtless to the pressure of the growing oöcytes, and the larger cell becomes separated from the rest of the cyst and surrounded by a membrane which attaches it to the wall of the ovary. Inside of this membrane there are always found a number of elongated follicle cells which are undoubtedly concerned in the formation of the zona pellucida which later develops around the egg (Figs. 36, 39). As the other cells of the cyst enlarge each in turn becomes similarly attached to the ovarian wall. The cysts do not all develop at the same rate. In the ovaries of toads with a body length of 1.5 cm. one may find some cysts containing oögonia, others filled with young oöcytes in various stages of development up to that shown in Fig. 37, while in many cases the cysts have become disorganized and the ova are separately attached to the ovarian wall.

## VI. THE NUCLEOLI AND THE LATER GROWTH STAGES OF THE OÖCYTES.

The irregular shaped masses of nuclear substance found against the nuclear membrane or in the meshes of the chromatin reticulum at the stage of Fig. 26 seem to increase in size as the nucleus grows and one of them usually becomes much larger than any of the others. These bodies appear homogeneous and stain black with iron haematoxylin or purple when the preparation is stained with safranin and gentian violet. When the nucleus has attained a diameter of about 0.025 mm. and the splitting of the spireme has been completed, numerous fine granular fibres are seen to project from these masses which do not stain quite as intensely as before (Fig. 34). At the next stage (Fig. 35) one obtains the first clue to the structure of these bodies. With the use of iron

haematoxylin the masses now appear grayish, and they are found to be composed of a meshwork of exceedingly fine fibres inclosing several darker homogeneous bodies. In preparations stained with safranin and gentian violet a much better differentiation is obtained. The meshwork of fibres invariably takes the blue of the gentian violet, while the rounded bodies in the interior, which are of various sizes, react differently towards the stain; the larger of these bodies, which are usually slightly irregular in outline, stain a reddish purple; the medium-sized ones, which are rounded and have a smooth outline, stain uniformly red, while the very small granules take the gentian violet. From the staining reactions of these masses, therefore, it is evident that they contain two different substances; fine fibres which are doubtless composed of chromatin not used for the chromosomes, and rounded bodies which are nucleoli.

For convenience in description I shall apply the term compound-nucleoli to the complex masses shown in Figs. 26-35 and also to the irregular nucleolar bodies shown in Figs. 39, 40, 43, 45, etc., reserving the term nucleoli for the smaller rounded bodies found in the interior of the larger masses at the stage of Figs. 35-36. The nucleoli which stain uniformly red with safranin will be called plasmosomes, while those that stain like chromatin will be considered karyosomes. In order to distinguish the chromatin of the chromosomes from that of the meshwork which forms part of the compound-nucleoli I shall refer to the former as "basichromatin" and to the latter as "oxychromatin." I am aware that these terms are not being used strictly in the sense in which they were introduced by Heidenhain (37), since both kinds of chromatin show the same color reactions with all methods of staining employed. Their use has been considered advisable here, however, in order to avoid the introduction of new terms.

At the stage in the resolution of a large compound-nucleolus shown in Fig. 36, the oxychromatin meshwork is much more clearly defined than at an earlier period and the threads are thicker and more granular. The number of nucleoli found in

the nucleus at this time greatly exceeds that found at any previous stage in the development of the oöcyte, and it is evident that a new formation of these bodies must take place during or soon after the synizesis stage. The compound-nucleoli in a nucleus do not resolve simultaneously. The larger masses are always the first to break up, and one or two of the smaller bodies may remain unchanged until the nucleus is twice the size of that shown in Fig. 36. Soon after the stage of Fig. 36 the meshwork of fibres becomes very loose and frequently breaks into several parts, while the nucleolar bodies begin to leave the fibres and scatter about the nucleus (Fig. 38). At the stage of Fig. 39 the resolution of the largest compound-nucleolus has been completed and the nucleus contains a number of nucleolar bodies of various sizes as well as several masses of tangled oxychromatin threads which are entirely separated from the nucleoli and easily distinguished from the chromosomes.

In his Fig. 15, Bataillon shows a portion of the nuclear contents of an ovarian egg of *Rana* which is very similar to one of the larger resolving masses shown in my Fig. 38. Bataillon believes that his figure shows the beginning of a connection between the chromatin filaments and the nucleoli, and he states that later the filaments disappear entirely, all of their substance going into the uncleoli. These results do not accord with what I have found in *Bufo*, since in the oöcytes of this amphibian the nucleolar bodies are preparing to leave the chromatin meshwork at the stage of Fig. 38, and chromatin filaments are to be found in all of the later growth stages of the ova.

At the stage of Figs. 33-34, the chromosomes stain somewhat more faintly than at an earlier period, and they are composed of a series of minute granules from which numerous fine fibres extend out a short distance on either side. In later stages these side projections become much more numerous and somewhat longer, and the chromosomes then come to have the feathery appearance shown in Fig. 39. At a later period the chromosomes stain so very faintly that in many cases they are to be found only with the aid of an immersion lens, yet

they retain the characteristic structure shown in Figs. 39, 40, 43, etc., and are therefore always to be distinguished from the oxychromatin threads. The chromosomes are never united with the nucleoli, although sometimes, as shown in Figs. 36, 37 and 39, a nucleolus is in contact with a chromatin thread; neither is there any connection between the basichromatin filaments and the oxychromatin threads. The latter stain much more intensely than the former and always appear to be composed of a series of rounded granules, they never have the feathery structure of the chromosomes. After the stage of Figs. 33-36, the chromosomes become widely distributed throughout the nucleus, and only a very few of them are found paired in later stages of development.

In a preliminary paper on the oögenesis of Triton, Janssens (46) gives a brief account of the changes taking place in the young oöcytes which seems to show that the behavior of the chromatin in the eggs of this amphibian is somewhat different from that I have found in *Bufo*. Janssens finds that synizesis occurs during the early growth period of the oöcyte, but he states that the reduced number of chromatin filaments appears shortly after this stage and that these filaments subsequently split longitudinally, the sister threads always remaining together in later development.

Carnoy and Lebrun (15-18; Lebrun, 58, 59) have written an elaborate series of memoirs dealing with the germinal vesicle and the polar bodies in various species of Batrachians. They have not studied the primordial germ-cells or the early growth stages of the oöcytes, and in every case their investigations begin with the young ovum at a stage about like that of my Fig. 27. Although the details of the developmental processes in the ova differ somewhat in the various species, Carnoy and Lebrun invariably find that, in the earliest stage which they have studied, the nucleus contains a chromatin filament which seems to be continuous. Later this filament disintegrates and gives rise to "primitive nucleoli" which move to the centre of the nucleus and there resolve into chromatin threads of various types. These chromatin threads soon break up into minute

granules from which new nucleoli develop to undergo the same series of changes as their predecessors. When the germinal vesicle disintegrates at the beginning of the maturation period certain of the nucleoli escape dissolution to form the twelve chromosomes which undergo a double longitudinal division in preparation for the maturation mitoses. At certain periods during the development of the ova, therefore, the nucleus contains no chromatin except that found in the nucleoli, and there is no "individuality" of the chromosomes or any reduction in the Weismannian sense during the maturation divisions. For Carnoy and Lebrun (16) the most important structures in the nucleus are the nucleoli. "Les nucléoles sont le chef-d'œuvre du noyau: ils représentent le degré le plus élevé de l'organisation nucléinienne." In another paper (15) the statement is made that "les nucléoles sont des noyaux en miniature. Il renferme toujours un appareil nucléinien filamentueux plongé dans un plasma et logé dans une coque mince."

Carnoy and Lebrun give a large number of figures which are supposed to furnish evidence in support of their conclusions. They have, however, seemingly overlooked the important stages which give the clue to the nature of the "primitive nucleoli" and of their relation to the chromosomes (Figs. 28-39). In many of their figures they show feathery chromosomes similar to those shown in my Figs. 39-41, etc.; yet they consider that these chromosomes are products of the resolution of the nucleoli, as are also the granular threads which correspond to my oxychromatin filaments. The feathery chromosomes are often figured in pairs, the sister threads lying parallel or intertwined in various ways. Carnoy and Lebrun state that these paired filaments are not formed by a longitudinal or by a transverse division of a pre-existent nuclear element, but that they are either produced by a single filament folding back on itself and the parts separating, or they are two filaments which have been resolved from two nucleoli lying close together. Sections of nuclei are given by Carnoy and Lebrun which contain numerous nucleoli and no chromatin threads. Such figures are considered to prove conclusively that there

has been no continuation of the primitive filament. It is not difficult to find sections of the nuclei of the young ova of *Bufo*, particularly at the stages of Figs. 40-44, in which no chromosomes can be found. Such sections are possible because the chromosomes, which stain very faintly, are sometimes collected together at one side of the nucleus and sections passing through the centre of the nucleus show only granular karyoplasm, nucleoli, and possibly some of the oxychromatin threads. After the yolk has formed, many fixing fluids do not seem to penetrate the egg sufficiently well to preserve the delicate structure of the chromosomes. I have examined, under an oil immersion lens, every section of the nucleus of an egg preserved in Gilson's or Flemming's solution without finding the slightest trace of chromosomes; while in the nuclei of eggs from the same ovary that were fixed with chromic acetic or corrosive acetic the feathery chromosomes show very distinctly with a comparatively low magnification. I have never found a nucleus in which it was impossible to find the chromosomes, provided the egg had been properly preserved and stained.

In his earlier work on Axolotl, Fick (28) states that the nucleoli are independent structures which probably represent "eine Art Reservestoffbehälter." In a later paper on the ripening of the egg of *Rana* (29) he confirms the work of Carnoy and Lebrun and states that during the growth period of the oocyte there are several generations of nucleoli which alternate with chromatin figures, consequently the continuity of the chromosomes is not maintained during this time. Fick considers that the nucleoli in the egg of *Rana* represent "eine Ruheform des Nucleins im Gegensatz zu den Chromatin-Figuren und Chromosomen, Formen in denen das Nuclein offenbar eine active Rolle spielt." Carnoy, Lebrun, Fick, and also Bataillon agree, therefore, with the conclusion reached many years ago by Schultze (83) from his study of the ripening of the egg of *Rana*, that the chromosomes "nicht aus einem präformirten Kerngerüst entsteht, sondern sich direkt aus den winzigen Keimkörperchen herausbildet."

The observations of other investigators of amphibian

oogenesis stand in direct contradiction to those cited above. Born (9, 10) states that in the egg of Triton the chromatin skein "sich aus dem Chromatingerüst des Ureies direkt herleitet." Although at one period of development the chromatin threads stain faintly and the chromatin substance can only rarely be distinguished from the granular karyoplasm, Born does not believe that the chromatin disappears or leaves the nucleus at this time, but that "sich dasselbe nur äusserst fein in der umgebenden Kerngrundsubstanz vertheilt habe." Later the chromatin threads are formed again, and they appear in pairs, lying parallel or closely intertwined as in *Bufo*. Born does not find that the nucleoli ever give rise to chromosomes, and while he ventures no conjecture as to the function of these bodies he believes that they "stehen in Beziehung zum individuellen Zelleben nicht zur Fortpflanzung."

According to the observations of Jordan (48) on the newt, the chromatin threads "are distinctly traceable through the whole history of the germinal vesicle," although large chromatin granules break loose from the threads at various times and pass over into true nucleoli. Janssens has also asserted that in the egg of Triton the chromosomes persist throughout the entire growth period; but in this egg the chromosomes are entirely independent of the nucleoli.

Lubosch (61) has recently studied the history of the nucleoli in the ovarian egg of Triton with the avowed purpose of testing the work of Carnoy and Lebrun. His material was preserved and stained in a great variety of ways, and he concludes that many of Carnoy and Lebrun's results are due to the methods of technique which they employed. As Lubosch did not study the very young oöcytes, he ventures no opinion as to the origin of the primitive nucleoli. He states that nucleoli are formed periodically at the nuclear periphery, and that they then wander towards the centre of the nucleus where they undergo one of three modes of dissolution: (1) through vacuolization and subsequent differentiation into karyoplasm; (2) through disintegration into granules; (3) through transition into various sorts of chromatin filaments, some of which

are indistinguishable from the chromosomes at the time that the latter are surrounded by a ring of nucleoli shortly before the maturation period. While Lubosch finds that the primitive chromatin network becomes extraordinarily fine at certain stages of development, he states that it never completely disappears and that it is morphologically present in the ripening egg, although it is in a finely divided form.

From the stage of Fig. 39 until that of Fig. 50, when the nucleus has reached its maximum size and the nucleoli have migrated to the centre preparatory to their final disintegration, the nuclei in the ova of *Bufo* contain an almost endless variety of nucleolar figures. In the nucleus shown in Fig. 39 the nucleolar bodies are of various sizes and they react very differently towards the gentian violet and safranin stain. The very small rounded nucleoli are karyosomes, since they stain like the chromatin; the larger, rounded nucleoli may be considered as plasmosomes, since they stain red and are not connected in any way with the chromatin; the irregular body marked X stains purple, and is a compound-nucleolus which has not yet begun its resolution; while the small, slightly irregular bodies are secondary compound-nucleoli which have been evolved from the resolution of a large mass similar to that shown in Fig. 35. At Y is shown a nucleolar body which is very similar to certain of the resolving nucleoli figured by Carnoy and Lebrun. This body is composed of a large, rounded central plasmosome (staining uniformly red) which seems to be giving off a number of small buds that also take the safranin. The outer surface of this plasmosome appears somewhat irregular and stains purple because a number of oxychromatin granules are attached to it. This structure has been produced, evidently, by the resolution of one of the compound-nucleoli which contained only a comparatively small amount of chromatin. The pinching off of small plasmosomes from a larger mass is a very common phenomenon in the ova of *Bufo*, and it is evidently one of the ways in which the number of these bodies is increased.

Several small nucleoli are shown in Fig. 39 which are composed of an outer ring of substance, evidently chromatin, since

it stains deep blue, surrounding a central portion which either stains very faintly or appears colorless; similar bodies are shown in Figs. 40, 41, 43, etc. At a later period the central portion of such nucleoli disappears, leaving only the chromatin ring. Subsequently the ring breaks at some point, thus becoming a crescent (Fig. 41), and it then disintegrates into small granules. Nucleoli of this character are probably derived from the oxychromatin of the larger compound-nucleoli, since they seem to be found most abundantly at the stages of Figs. 39-43. Similar nucleoli are figured by Carnoy and Lebrun and also by Lubosch.

The oxychromatin threads produced by the resolution of the large compound nucleoli are massed together at the stage of Fig. 39; but they soon become scattered throughout the nucleus and are bent and twisted in a great variety of ways (Figs. 40-43). Occasionally, as shown in Figs. 40 and 43, two of these filaments lie parallel or cross each other in the form of an X. Such an arrangement is purely accidental, since the filaments never have any definite arrangement in the nucleus. In some cases oxychromatin threads seem to be united with nucleoli (Fig. 43); but as the nucleoli stain differently from the filaments, it is readily seen that there is no true connection between them.

Many of the figures given by Carnoy and Lebrun show granular chromatin filaments strikingly like those shown in my Figs. 40-43. These investigators consider that such filaments are derived from the substance of the nucleoli, and the contact of a nucleolus with a chromatin thread, as shown in my Fig. 43, is considered to be proof that the chromatin thread is being formed at the expense of the nucleolus. The feathery chromosomes are considered by Carnoy and Lebrun to be merely a special form of the filaments and in no way different from the others in origin or in fate. My observations do not admit of such an interpretation, since in *Bufo* the feathery chromosomes can be traced back to the continuous filament formed after synizesis (Fig. 26), while the oxychromatin threads are undoubtedly derived from the chromatin

which did not go into the spireme and they are always produced by the resolution of compound-nucleoli. Oxychromatin filaments similar to those shown in Figs. 40-43 are figured in Bouin's work on the oögenesis of *Rana*. Bouin considers that these filaments are a part of the general chromatin of the egg, and he does not distinguish them from the true chromosomes.

By means of a series of camera drawings of all of the sections of nuclei in about the stage of development shown in Fig. 43, I have endeavored to ascertain the number of oxychromatin filaments and of chromosomes at this time. While the chromosomes appear to be twenty-four in every case, the number of oxychromatin threads seems to vary from 20-50 in different nuclei. This difference in the number of oxychromatin threads in various cases can doubtless be attributed to the fact that the compound-nucleoli from which the filaments are derived vary in number and in size in different nuclei and that these bodies do not all resolve at the same time.

Carnoy and Lebrun distinguish three distinct stages in the development of amphibian oöcytes, and they state that there are many generations of nucleoli which alternate with various kinds of chromatin figures; the nucleus frequently containing one kind of structure exclusive of the other. In *Bufo* I have never found an oöcyte in a stage of development between that shown in Fig. 38 and that of Fig. 50 in which the nucleus did not contain nucleoli, chromosomes, and oxychromatin filaments provided the egg had been satisfactorily preserved and stained. As the ova grow the number of nucleoli increases; but the number of chromosomes remains constant, and the maximum number of oxychromatin filaments is found at the stage of Figs. 40-43. After this time the oxychromatin threads stain more faintly; the granules of which they are composed gradually draw apart (Fig. 48), and finally become scattered throughout the nucleus. Many of these minute chromatin granules can still be found in the nucleus at the beginning of the maturation period.

Although in *Bufo* there is no periodic resolution of nucleoli into chromatin threads followed by the development of a

new generation of nucleoli from chromatin granules, there is a constant formation of new nucleoli and a gradual dissolution of the old ones during the growth stages of the ova. The disintegration of small nucleoli composed of a ring of chromatin enclosing a plasmosome body (Figs. 39-43) has already been described. The beginning of a dissolution of some of the larger nucleoli is shown in Fig. 42. In this nucleus many of the nucleoli are stained black with iron haematoxylin; others appear grayish, since they seem to have lost their capacity for staining intensely. The latter nucleoli are gradually dissolved in the karyoplasm; they are never resolved into chromatin threads. Although the process of dissolution usually involves the whole nucleolus, sometimes only a portion of it disappears leaving one or several small, rounded bodies (Fig. 40, X). It is possible that the small groups of nucleoli shown in Figs. 40 and 43 may have been formed in this manner.

As a rule the majority of the nucleolar masses which lie in the meshes of the chromatin spireme or against the nuclear membrane at the stage of Figs. 26-34, resolve into plasmosomes and oxychromatin filaments at about the time that the larger compound-nucleoli undergo their resolution (Figs. 35; 39, Y). These masses differ from the larger ones in that they contain a relatively greater quantity of plasmosome material and a much smaller amount of chromatin. One or two of these nucleolar bodies, rarely more, escape dissolution at the stage of Figs. 35-38 and appear in later stages as slightly irregular, round or oval structures which stain as uniformly, though in many cases not as intensely, as in an earlier period (Fig. 39, X). These bodies increase rapidly in size after the stage of Fig. 39, and they usually undergo a somewhat different mode of resolution from that of the other compound-nucleoli. At an early period in the resolution of these bodies the oxychromatin, which has been attached to the outer surface of the plasmosome substance (Fig. 40), breaks away and becomes scattered throughout the nucleus, being indistinguishable from the oxychromatin produced by the earlier resolutions of nucleolar masses. Sometimes all of the plasmosome

substance in these bodies forms one large rounded mass which contains either one large vacuole or a varying number of small ones (Fig. 40). Such a structure greatly resembles the large vacuolated nucleoli found by Carnoy and Lebrun and also by Leydig in the ova of various amphibians, and it is also very similar to the "principal nucleolus" described by Maréchal (67) in the selachian egg. In many cases the plasmosome substance in these bodies is divided, the greater part of it forming a large, rounded central mass which usually stains rather faintly and appears either homogeneous (Fig. 41, R) or vacuolated (Fig. 41, S), the remaining portion being broken up into a varying number of small, round, deeply staining bodies which are attached, for a time, to the outer surface of the larger mass and later separate from it to form small plasmosomes.

The large plasmosome bodies shown in Figs. 40, 41 and 51, disintegrate in various ways and at different times. In some cases they persist as rounded, vacuolated bodies until the germinal vesicle disintegrates at the beginning of the maturation period when they are slowly absorbed by the cytoplasm; in other cases they break open during the later growth stages of the ova (Fig. 51, b), and subsequently divide into several rounded portions which are gradually dissolved in the karyoplasm (Fig. 43). It is not uncommon to find these large plasmosome bodies budding off portions of their substance (Fig. 51, c, d); and it is probable that many of the nucleoli found at the stage of Figs. 48-50 have been formed in this way.

The central vacuole of these large plasmosomes frequently contains a number of nucleolini which may be separated or so joined together that they simulate a granular chromatin thread (Fig. 51, C, D). These nucleolini always stain like the plasmosome, and they are evidently granules which have broken away from the inner surface of the ground substance in a manner similar to that by which the small plasmosomes are budded off from the outer surface. It seems probable that Carnoy and Lebrun have in mind linear aggregations of

nucleolini when they state that chromatin filaments are often found in the interior of large nucleoli. As the result of an investigation of the structure of the nucleoli in many kinds of cells Montgomery (70) states: "I am forced to conclude that in all probability there are no skeins of chromatin lying in any metazoan nucleolus, since I have never found any evidence of chromatin in it in any metazoan cell." This statement may well be extended to include the nucleoli in the egg of *Bufo*, since in no case have I ever found chromatin filaments in the interior of rounded nucleoli, although they are often found wrapped around the exterior of a nucleolus (Figs. 38, 40, 43):

In preparations stained with safranin and gentian violet nucleolar bodies are sometimes found which are similar to the compound-nucleoli described above in size and general outline, although they have a very different structure as they are composed of a number of rounded plasmosomes imbedded in a mass of chromatin granules (Fig. 51, a). These bodies are compound-nucleoli containing a large amount of chromatin, which for some unknown reason did not undergo a resolution at the stage of Figs. 35-38.

Nuclei having a diameter of 0.04-0.08 mm. usually appear as in Figs. 39-43. They contain one or two large unresolved compound-nucleoli, a varying number of round plasmosomes and small karyosomes, together with numerous small compound-nucleoli which were set free from the large nucleolar masses at the stage of Figs. 35-38. These small compound-nucleoli, which I shall call secondary compound-nucleoli to distinguish them from the larger bodies, appear homogeneous and only slightly irregular at the stage of Figs. 39-43, and the greater number of them are masses at the side of the nucleus where the largest of the primary compound-nucleoli underwent a resolution at the stage of Figs. 35-36. In a slightly older egg metabolic processes occur which lead ultimately to the formation of yolk. These processes are accompanied by, if indeed they do not produce, a marked change in the appearance and in the behavior of the nucleolar bodies. At this stage of development (Fig. 44) there is a mass of very

irregular nucleolar bodies at one side of the nucleus which greatly resemble the structures figured by Carnoy and Lebrun as nucleoli resolving into their chromatin constituents. As the preparation from which Fig. 44 was drawn was stained with iron haematoxylin the nucleolar bodies appear homogeneous and stain very intensely. Their true character is shown only when preparations are stained with safranin and gentian violet. In such cases these fantastically shaped bodies are found to be composed of a number of rounded plasmosomes imbedded in a meshwork of oxychromatin granules (Fig. 45). The plasmosomes always appear homogeneous and they invariably take the safranin stain; the chromatin stains blue and it is always in the form of fine granules which may or may not be strung together in a filament. There is nothing to indicate that the chromatin in these structures is derived from the plasmosomes or vice versa. These peculiar bodies, which are found very abundantly in the oöcytes of toads with a body length of 4-5.5 cm., are unquestionably secondary compound-nucleoli which have increased considerably in size during the stages of Figs. 39-43 and are now resolving into their constituent parts, oxychromatin granules and plasmosomes. Soon after the stage shown in Fig. 44 these irregular masses break up, and for a short time the nucleus contains a number of plasmosomes surrounded by chromatin granules (Fig. 46). At a later period the oxychromatin granules separate from the plasmosomes and scatter throughout the karyoplasm, and for the first time since before the synizesis stage the nucleus has all of its chromatin separated from the plasmosome substance.

As I was unable to obtain any young toads in the fall with a body length of over 5.5 cm., I have not been able to follow the later changes in the oöcytes in the ovaries of young females, and I have had to make use of the oöcytes developing in the ovaries of adults to complete my study of the oögenesis of *Bufo*. If adult females are killed soon after the breeding season in April or in May the ovaries are found to be filled with young ova, many of which contain nucleolar bodies simi-

lar to those shown in Fig. 44. A section of the nucleus of an egg taken from the ovary of an adult toad killed the latter part of April is shown in Fig. 48. The nucleus has nearly attained its maximum size, and it is slightly oval, measuring 0.19 mm. by 0.22 mm. All of the irregularly shaped nucleolar bodies found at the stage of Figs. 44-46 have disappeared and the nucleus contains a large number of round or oval nucleoli of various sizes which are entirely distinct from the chromatin threads. The smallest of the nucleoli, which stain like chromatin, have evidently been formed by a fusion of a number of the chromatin granules set free by the disintegration of the oxychromatin threads. Most of the larger nucleoli stain very intensely at this time and only a few of them show, by their lessened capacity for staining, that they have begun to dissolve. Since the majority of the nucleoli are derived from the resolution of the secondary compound-nucleoli the greater number of these bodies are massed together in one part of the nucleus. A few oxychromatin filaments in the process of dissolution are still to be found in the nucleus at this time.

During early growth stages the nucleus occupies the centre of the ovum, but at or soon after the stage of Fig. 44 it begins to move towards the future animal pole of the egg. As at this time the nuclear membrane is usually somewhat irregular in outline, several investigators have maintained that the change in the position of the nucleus is brought about through amoeboid movement. This explanation does not seem to me entirely satisfactory since in some cases the nuclear outline is perfectly regular when the nucleus is moving towards the upper hemisphere, and when irregularities in the nuclear outline are found they are invariably distributed uniformly around the membrane, no matter by what means the egg has been preserved.

At the time that the nucleus is changing its position the greater number of the nucleoli are massed together in one part of the nucleus (Figs. 44-48). I have examined many eggs in this stage of development and I have always found

that the greater number of the nucleoli lie in the part of the nucleus that is nearest the periphery of the egg. It hardly seems probable that this arrangement would be found so constantly if it had no significance. It seems to me a possibility, at least, that the accumulation of most of the nucleoli in one part of the nucleus may have something to do with the movement of the nucleus towards the periphery of the egg. This arrangement of the nucleoli strongly suggests also that the polarity of the egg is determined at or soon after synizesis, since the location of the largest of the compound-nucleoli, from which the greater number of secondary compound-nucleoli are derived, indicates the part of the nucleus which will be nearest the animal pole in the later growth stages of the oöcytes.

After the nucleus has reached its final position at the periphery of the egg there is a rearrangement of the nuclear contents so that the nucleoli become distributed fairly evenly around the nuclear periphery, while the chromosomes and the remains of the oxychromatin filaments are found in the centre of the nucleus (Fig. 49). Whether this arrangement is due to the activity of the nucleoli themselves, I have not been able to determine. These bodies always appear round and they never show processes similar to those found by Leydig and also by Eimer (26) and considered by these investigators to be the means through which the nucleoli change their position in the nucleus. It is at this stage of development shown in Fig. 49 that the nuclear membrane is most irregular in outline, and it is very probable that this irregularity is due to the close proximity of the nucleoli. As a rule all of the oxychromatin filaments have disintegrated at this time; the chromosomes can always be found in favorable preparations, although they stain very faintly.

Several investigators, among whom may be mentioned Will (92), Fick, and Leydig, maintain that at or before the stage of Fig. 49 nucleoli pass out of the nucleus into the cytoplasm where they either dissolve or take part in the formation of the yolk. Although I have examined a large number of eggs in

which there were many hundreds of nucleoli lying close to the nuclear membrane I have never found a single case in which a nucleolus was passing through the membrane into the cytoplasm. At certain stages in the development of the ova there are a number of rounded bodies in the cytoplasm which greatly resemble nucleoli, and it is doubtless this similarity in appearance that has led to the assumption that the cytoplasmic bodies are of nucleolar origin.

Soon after the stage of Fig. 49 the nucleoli leave their peripheral position and move towards the centre of the nucleus. Their arrangement at first is somewhat irregular (Fig. 50); but later, as shown in a previous paper (King, 49; Fig. 3), they form a closed ring which surrounds the chromosomes. This arrangement of the nucleoli in the ovarian egg previous to maturation seems to be characteristic of all amphibian eggs, as it has been noted by all of the observers who have studied this period in the development of the ova. All of the nucleoli have begun to disintegrate by the time that the germinal vesicle breaks down at the beginning of the maturation period. They first lose their capacity for staining and many of them become vacuolated. Later they break into small fragments which are absorbed by the cytoplasm.

Although this study of the ovarian egg of *Bufo* has shown that investigators of amphibian oögenesis have classed together, under the general name of nucleoli, several different kinds of structures, it has not, unfortunately, disclosed the manner in which these bodies are formed or their function in the nucleus.

From the resting stage of the primary oögonium to the synizesis period in the oöcyte the nucleus of the germ-cells contains several rounded nucleoli which stain differently from the chromatin and there is not the slightest evidence that there is any genetic relation between them. During synizesis the nucleoli can still be distinguished from the chromatin (Fig. 25); but in early post-synizesis stages (Figs. 26-34) these bodies are contained in the amorphous masses of nuclear substance (compound-nucleoli) left over after the formation of

the spireme, and they cannot be followed since the large masses stain very intensely and uniformly at this time. When the large compound-nucleoli resolve (Figs. 35-38) they liberate, with the secondary compound-nucleoli, many more of the rounded nucleoli, which I have called plasmosomes, than were found in the nucleus previous to synizesis. It is evident, therefore, that plasmosomes are being formed in the nucleus during early post-synizesis stages (Figs. 26-34). Since these nucleoli are formed only in the midst of oxychromatin granules it seems probable that the oxychromatin is concerned in some way with their formation; but the number and size of these bodies and the fact that they invariably stain differently from the chromatin seems to preclude the possibility that they are derived from chromatin substance as Flemming (30), van Bambeke (5), Macallum (65), Hertwig (43), Obst (7), Schockaert (79), Carnoy and Lebrun, Fick (29), and many others have maintained. The part played by the oxychromatin in the formation of the plasmosomes is obscured. There is no apparent decrease in the amount of this substance associated with an increase in the number and size of the plasmosomes during the early development of the oöcyte, and at the time that the oxychromatin filaments disintegrate the nucleus apparently contains its maximum number of plasmosomes (Fig. 48).

The plasmosomes seem to be of a plastic, semi-fluid consistency; they appear homogeneous until they begin to disintegrate, and in some instances they seem to be capable of increasing in size and of budding off portions of their substance. Judging from the appearance and behavior of these bodies and from the fact that their formation is associated with the rapid growth of the cell and with the formation of vitelline bodies in the cytoplasm, it seems probable that they are products of nuclear metabolism which are possibly depositors of nutritive substance that are to be used at a later period in the history of the cell. This is substantially the view advocated by Korschelt (56) and by Rhumbler (75). Montgomery (70-71) is one of the few investigators who believes that the nucleoli are of extranuclear origin. He states that in the egg of

nemerteans the nucleoli are first found closely applied to the inner surface of the nuclear membrane. "It would seem that the yolk is at first present in the cytoplasm in the form of a diffused, unstainable fluid; that a portion of it, that remaining in the cell body, later becomes segregated as, or chemically changed into yolk globules; and that another portion of it is taken into the nucleus and, after passing the nuclear membrane, is changed into nucleolar substance." Such an origin for the plasmosomes in the ova of *Bufo* seems unlikely since these bodies are not found close to the nuclear membrane until a late period in the development of the ova.

The large nucleolar masses found in the oöcyte at the stage of Figs. 26-34 correspond evidently to the "primary nucleoli" of Carnoy and Lebrun. I have shown that these bodies are complex structures composed of plasmosome material and of the chromatin which did not go into the formation of the chromosomes and that they later resolve into their constituent parts; they are never formed entirely of chromatin, as Carnoy and Lebrun maintain. The fantastically shaped nucleolar bodies found at the stage of Fig. 44 are similar in structure to the large compound-nucleoli shown in Figs. 26-34, and they too resolve into chromatin threads and plasmosomes. In the egg of *Bufo* there is never any connection between the nucleoli and the chromosomes. Only oxychromatin goes into the formation of the compound-nucleoli, and the oxychromatin filaments which are formed by the resolution of these bodies do not at once disintegrate to form a new generation of nucleoli but they gradually break up into minute granules which seem to be absorbed by the achromatic substance of the nucleus. It is impossible to determine whether these granules take any part in the formation of the chromosomes which are found on the maturation spindle.

## VII. THE CHROMOSOMES.

At no period in the development of the oöcyte does the basichromatin disappear nor does it become condensed in the form of nucleoli, and the chromosomes can be traced continuously

from the time that they are first formed by the breaking of the spireme (Fig. 33) up to the stage when the germinal vesicle disintegrates in preparation for the maturation mitosis. At the time that the spireme divides longitudinally (Figs. 28-29) the chromatin filaments are found to be composed of a series of rounded granules from which a few fine fibres project on either side. When the spireme breaks into chromosomes the number of fine projections increases, evidently at the expense of the chromatin granules (Figs. 33, 34, 36). By the time that the oöcyte has reached the stage of Fig. 39, the appearance of the chromosomes has changed considerably. The axial portion of the thread is now composed of minute, faintly staining granules, evidently formed by the breaking up of the larger ones, and the fine projections from the sides are longer and more numerous than at an earlier period. The chromosomes, of which there are undoubtedly twenty-four, thus come to have the feathery appearance that characterizes them from this time until the beginning of the maturation period, and they greatly resemble the filamentous chromosomes found by Rückert (76, 77) in the selachian egg and by Born in the egg of Triton. After the stage of Fig. 39 the chromosomes stain very faintly, since the greater part of their substance seems to be in the form of fine fibres as it was during the synizesis period; they can always be found, however, if the egg has been properly preserved and stained. When the germinal vesicle is about to disintegrate the chromosomes lose their filamentous structure and become greatly condensed, appearing as a single series of perfectly round granules (King, 49; Fig. 8).

The arrangement of the chromosomes in the young oöcyte depends, evidently, on the extent to which sister portions of the spireme have separated before the spireme breaks into segments. The transverse division of a spireme like that shown in Figs. 31 and 32 produces chromosomes that are scattered irregularly throughout the nucleus and only occasionally paired; while the division of a spireme similar to that shown in Fig. 30, gives a paired arrangement of all of the chromo-

somes (Fig. 33, 34). In the later growth stages of the oöcytes the chromosomes become widely distributed throughout the nucleus and they seem to have no definite arrangement, although it is not unusual to find two chromosomes paired as in Fig. 43, or two chromosomes crossed as in Fig. 40.

In a previous paper (King, 49) I have shown that, at the time the germinal vesicle is about to break down in preparation for the maturation mitoses, the twenty-four chromosomes come together forming twelve pairs. The chromosomes of a pair are of the same length, but there is considerable difference in the lengths of the chromosomes of the various pairs. At this time "two of the chromosomes may be united in the form of an X or Y, a single or double figure eight, or they may lie parallel for a part of their length and the ends intertwine in various ways." At a slightly later period the ends of each pair of chromosomes unite forming a closed ring. Immediately following this stage the chromosomes apparently break up into granules and, owing to the changes occurring in the nuclear substance preparatory to the formation of the first polar spindle, it is impossible to trace the chromatin for a short period. When the polar spindle forms a large number of rounded chromatin granules are found near it which soon fuse into several irregular clumps from which the reduced number of chromosomes (12) is formed.

It is unfortunate that the chromatin cannot be traced during the period of the formation of the first polar spindle since it thereby becomes impossible to identify the chromosomes of the first polar spindle with the chromosomes that are found in the oöcyte previous to the disintegration of the germinal vesicle. If, however, the chromosomes can maintain their individuality in the resting nuclei of the oögonia and of the young oöcytes when the chromatin is in the form of minute granules which are scattered irregularly along a linin meshwork or distributed on the nuclear membrane, I see no reason why they should be considered to lose that individuality when they break up into granules at the beginning of the maturation period. After all it may be through the linin that the morpho-

logical continuity of the chromosomes is maintained; and it is very probable that there is a linin connection between the chromatin granules during the early maturation stages which I overlooked in my previous work. When opportunity offers I shall collect new material showing the formation of the first polar spindle in the egg of *Bufo*, in the hope that with some new method of fixation or of staining I can follow the history of the chromatin granules and thus trace the chromatin continuously from the early growth stages of the oöcytes through to the maturation spindle.

As far as I am aware, no investigator of amphibian oögenesis has as yet traced the chromosomes from the ovarian egg directly into the maturation spindle. Schultze, Carnoy and Lebrun, and Fick believe that the chromosomes of the polar spindles are derived from chromatin nucleoli which escape dissolution at the time that the germinal vesicle disintegrates. Other investigators have tacitly assumed that the chromosomes of the ovarian egg pass over into the maturation spindle and they have not given any figures of the critical stages.

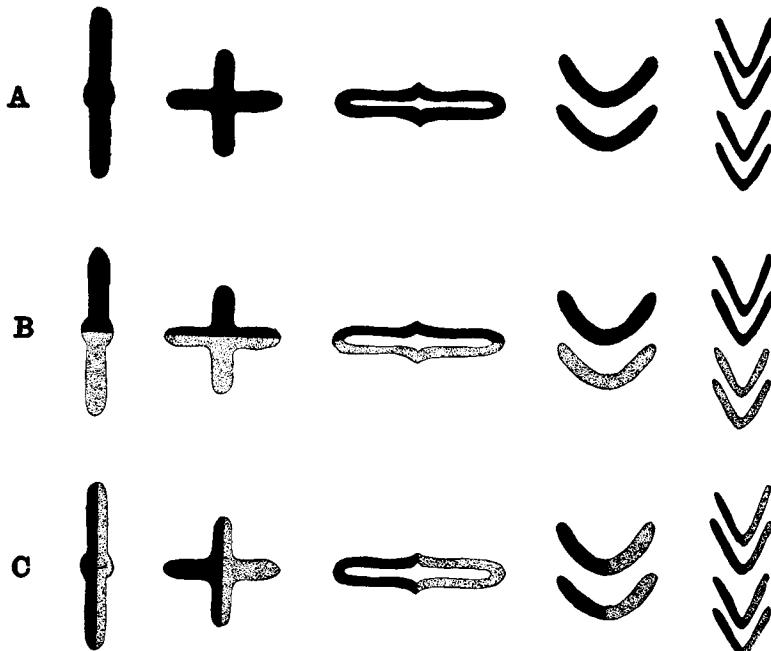
Since there are two periods in the development of the egg of *Bufo* when it is impossible to follow the changes which the chromatin undergoes, it is a difficult matter to decide when and how synapsis occurs. The first period when the history of the chromatin is obscured is during the synizesis stage in the young oöcyte when the nuclear contents become massed together as shown in Fig. 25 and the chromatin appears in the form of exceedingly fine granular threads. This stage is succeeded by one in which the chromatin, which is to form the chromosomes, is in the form of an apparently continuous spireme. Later this spireme splits longitudinally and then divides transversely forming the somatic number of separate segments. Assuming that the chromosomes maintain their individuality during synizesis, it is obviously impossible to determine how they were joined together in the spireme which is formed after the synizesis stage. If the chromosomes were joined end to end, then the splitting of the spireme shown

in Figs. 28-30 is a longitudinal division of univalent chromosomes. On this assumption synapsis is coincident with synizesis and, at the stage of Figs. 33-34, the nucleus contains twelve bivalent chromosomes which are divided longitudinally. This is the interpretation which Janssens has given to the early post-synizesis stages which he finds in the egg of Triton. The fact that in later growth stages the great majority of the chromosomes are not arranged in pairs makes this interpretation improbable for the egg of *Bufo*, since in the eggs of other forms when bivalent chromosomes divide longitudinally the parts remain together or are connected in some way.

If we assume that two chromosomes united side by side in the spireme, then the subsequent longitudinal splitting of the spireme is merely a separation of the chromosomes that were previously paired, and the transverse division of the spireme is the means by which the chromosomes are completely separated from each other. Synapsis, on this assumption, does not necessarily occur during synizesis, since the somatic number of chromosomes is evolved from the spireme. I am inclined to believe that synizesis in the egg of *Bufo* is a process by which the chromatin which bears the hereditary qualities and is to be used for the chromosomes of the maturation spindle is separated from the chromatin which has other uses in the cell. This would seem to bear out Gardiner's (33) contention that "there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter-cells, the other food for the cytoplasm." If this interpretation of synizesis is correct, the chromosomes must have been united in pairs in the spireme that was evolved from the synizesis stage, but synapsis does not take place until the beginning of the maturation period. This interpretation seems the more probable since Jordan failed to find a pairing of the chromosomes in the ovarian egg of the newt at any stage of development.

The second period when it is impossible to trace the history of the chromatin occurs just previous to the formation of the

first polar spindle when the twelve chromatin rings break into granules which cannot be distinguished from the granular achromatic substance of the nucleus. The twelve bivalent chromosomes that are finally formed from the mass of chromatin



- A.—Diagrams showing the changes in the shape of the chromosomes of the first polar spindle in the egg of *Bufo* and the direction of the maturation mitoses. In both mitoses the chromosomes are divided longitudinally.
- B.—Diagrams showing the character of the maturation mitoses if the chromosomes were united end to end in synapsis. The first division separates univalent chromosomes; the second is a longitudinal division.
- C.—Diagrams showing the character of the maturation mitoses if the chromosomes conjugated side by side during synapsis. Both mitoses divide the bivalent chromatin segments longitudinally.

granules that surround the polar spindle vary somewhat in size and in shape. When they have become arranged on the spindle they undergo considerable modification in form, and the longitudinal axis of the chromosome at the time that the

first maturation mitosis occurs is the transverse axis of the chromosome at an earlier period (King, 51). In both maturation mitoses the bivalent chromosomes are divided longitudinally (Text-Figure I, A).

If synapsis occurs when the germinal vesicle disintegrates then the chromosomes must have united end to end, as shown in Text-Figure I, B, otherwise both of the maturation mitoses are equation divisions of bivalent chromatin segments and in neither division are univalent chromosomes separated (Text-Figure I, C). The investigations of Stevens (87, 88) have shown that in *Sagitta* synapsis takes place in the egg by a side by side conjugation of the chromosomes and in the spermatocytes by an end to end union. This is probably the plan that is followed in the germ-cells of *Bufo* if synapsis occurs in the egg during the synizesis period. I am inclined to the opinion that in the egg of *Bufo*, as in the spermatocyte, synapsis occurs shortly before the maturation mitoses and that the chromosomes are united end to end. On this assumption the first maturation division in the egg is a reduction division in the Weismannian sense since it separates univalent chromosomes, and the second division only is an equation division (Text-Figure I, B).

A. and K. E. Schreiner (80-82), who have recently examined the chromatin relations in the germ-cells of many different forms, conclude from their studies that in the germ-cells of *all* animals the chromosomes conjugate in pairs during synapsis, never end to end. This generalization finds an exception in the germ-cells of *Bufo*. In the spermatocytes of this amphibian synapsis occurs during the synizesis stage which immediately precedes the prophase of the first maturation mitosis, and in the prophase and metaphase of division the chromosomes behave in such a way that there seems no possibility of avoiding the conclusion that they were united end to end in synapsis. In the egg, as I have shown, it is not possible to determine when or how synapsis occurs; yet the evidence at my command seems to indicate that in synapsis the chromosomes are united end to end as they are in the spermatocytes.

## VIII. THE FORMATION OF YOLK.

One of the most difficult problems met with in the study of amphibian oogenesis is that concerning the origin of the yolk. This problem includes not only a consideration of the origin and nature of the so-called "yolk-nuclei," but it also involves practically the whole theory of cell action since it cannot be supposed that the yolk formation takes place independently of nuclear activity. The anabolic processes taking place in the cell as a result of the interaction of the nucleus and the cytoplasm are as yet very imperfectly understood, and until we have obtained a clearer insight into the nuclear-cytoplasmic relations it will not be possible to solve the problem of yolk formation in an entirely satisfactory manner.

There is as great a diversity of opinion regarding the nature of the yolk-nucleus and the origin of the yolk in the amphibian egg as there is concerning the origin of the egg itself. The first observation regarding the presence of a yolk-nucleus in the amphibian egg were made by Cramer (23) in 1848. According to this investigator the cell body of the frog's egg contains a small granular ball which later spreads out in the form of a half-moon around the nucleus and gives off granules which develop into yolk spherules. In 1850 Carus (19) investigated the young egg of *Rana temporaria* and failed to find the granular ball described by Cramer. He states that the yolk first appears at the periphery of the egg in the form of single granules as it does in the egg of *Alytes obstetricans* according to the earlier observations of Vogt (90).

A few years later Thompson (89) wrote in regard to the presence of a yolk-nucleus in the frog's egg: "I have in general found it present, and think it more probable that it may be destined to form the external and larger corpuscles of the yolk, while the clearer part immediately surrounding the germinal vesicle may contribute to the production both of these and of the finer substance in which the germinal vesicle is found imbedded."

As Goette failed to find a yolk-nucleus either in the egg of *Bombinator* or of *Bufo*, Hertwig (40) is inclined to attach

but little morphological value to the rounded granular ball which he finds in the cytoplasm of the egg of *Rana*. "Mir scheint er einzig und allein mit der Bildung der Dottersubstanz in Beziehung zu stehen und eine eigenthümliche locale Ansammlung von Nährstoffen darzustellen." He suggests that the name "Dotterconcrement" would be more appropriate for this structure than "Dotterkern." Kolessnikow (55) mentions the presence of granular yolk-nuclei in the eggs of *Rana temporaria*, *Rana esculenta*, and *Bufo variabilis*, but he gives no opinion as to their origin or use.

Henneguy (38) finds a large granular mass, presumably a yolk-nucleus, in the egg of *Rana*, although he fails to find a similar body in the egg of *Bufo vulgaris*, *Triton tæniatus*, and *Triton cristatus*. Henneguy believes that wherever this body is found it is derived from the nucleolar substance of the nucleus and he ventures the interesting conjecture that "c'est un organe ancestral qui, avec les éléments nucléolaires de la vésicule germinative, correspond au macronucléus des Infusoires, le micronucléus étant représenté par le rôseau chromatique, prenant seul part aux phénomènes de fécondation."

Jordan finds a number of granular yolk-nuclei in the egg of the newt which he believes "arise from the cytoplasm and usually disintegrate in the cytoplasm." He is not sure whether these structures are of importance in the formation of yolk or not. Jordan's observations regarding the fate of these yolk-nuclei will be mentioned later.

The observations of several investigators seem to show that nuclear substance is used in the formation of yolk. In 1884, Will brought forward the view that in the ova of amphibians and of insects nucleoli leave the nucleus and migrate to the periphery of the egg where, as yolk-nuclei, they lose their sharp contour and break up into granules which become yolk spherules. Substantially the same view was advanced by Leydig in 1888 to account for the origin of the yolk in the egg of *Triton*. Leydig considers that the nucleoli arise in the nucleus "als Knotenpunkte in dem feinen Netz des Reticulum," and that they move to the periphery of the nucleus

where they "im losgelösten Zustande die Form und Natur kleiner Amöben zeigen." Subsequently they pass through the nuclear membrane into the cytoplasm and move to the periphery of the egg where they form groups of granules which develop into yolk.

Bataillon (6) also derives the yolk in the amphibian egg from the substance of the nucleus, but he believes that it is formed from the chromatin. "Des massules chromatiques issues de la vésicule germinative viennent donner dans le plasma ovulaire et à la périphérie d'abord, de véritables éléments cellulaires transitoires dont ils fournissent le noyau, et prendre part à la formation simultané des tablettes vitellines et du pigment."

As a result of a study of the ovarian egg of *Rana* and of *Necturus*, Macallum (64) concludes that the peripheral chromatin nucleoli generate a substance which diffuses gradually through the nucleus into the cytoplasm. "I regard the yolk spherules as formed by the union of a derivative of the nuclear chromatin with a constituent of the cell protoplasm." Support for this view is furnished by the more recent cytological studies of Carnoy and Lebrun (15). These investigators state that the greater number of chromatin granules that are produced by the resolution of nucleoli are not used in the formation of a new generation of nucleoli, but that they are dissolved in the achromatin substance of the nucleus and transformed into nucleinic acid. This acid passes by osmosis through the nuclear membrane and is diffused through the cytoplasm. "Dans les plages formatrices, il rencontre les globulines de réserve imbibées d'eau, et se combine avec elles pour former la paranucléine d'abord, la vitelline en suite. . . . Nous considérons les plaques vitellines comme étant des produits de l'activité du noyau et du cytoplasme: celui-ci fournirait les globulines, le noyau, l'acide paranucléique. Les vitellines sont, en effet, des paranucléo-albumines, c'est-a-dire des combinaisons de paranucléine avec un albumine qui est ici une globuline."

Jordan has observed in the newt appearances which might be interpreted as a migration of very minute granules from

the germinal vesicle into the cell body, and he also is inclined to the opinion that nucleus takes part in the formation of yolk. "One might suppose that granules from the germinal vesicle serve as starting points, centers of attraction or stimulation as it were, while the cytoplasm perhaps through the mediation of the yolk-nuclei, elaborates and supplies the requisite deutoplasmic material out of nutritive elements furnished it by the follicle cells."

Since it is seemingly impossible to harmonize these various observations regarding the yolk-nuclei and the yolk in the egg of amphibians, it can only be supposed, if these observations are correct, that the processes by which yolk is formed differ in various species. The details of these processes must, therefore, be worked out for each species separately, since there is no apparent similarity between them even in closely related forms.

In the egg of *Bufo* it is possible to trace the anlage of the yolk-nuclei back to the primordial germ-cells. As I have already stated, there is present in the cytoplasm of these cells a small, round, apparently homogeneous body which is sometimes, though not invariably, separated from the cytoplasm by a clear area (Fig. 8, V). This body colors very intensely with iron hæmatoxylin, and it always takes the safranin when sections are stained with safranin and gentian violet or with safranin and *Lichtgrün*. In preparations stained with Delafield's hæmatoxylin and orange G. this body is hardly discernible, since it takes the orange stain as does also the cytoplasm. Judging from its staining reactions this body is not chromatin; neither is it a centrosome, since the same section of the cell may show both of these structures (Fig. 7). I have not been able to determine the origin of this body owing to the fact that in very young tadpoles the large yolk plaques in the primordial germ-cells obscure the other cytoplasmic structures, while in older tadpoles, when the yolk is beginning to be absorbed, the small yolk granules show the same staining reactions as this body and therefore cannot be distinguished from it. Not until the tadpole is at least thir-

teen days old can this structure be distinguished with any degree of certainty. I shall apply the term "vitelline body" to this structure and also to other bodies of similar character which appear later in the cytoplasm, reserving the term, "yolk-nucleus" for the granular masses found in the cytoplasm at a much later period of development.

The vitelline body divides previous to each cell mitosis (Fig. 7, V). In sections of the ovaries of young toads this structure is found in the primary oögonia (Figs. 16-17), in the secondary oögonia (Figs. 18-19), in the young oöcytes at the critical period when the cell contents stain very faintly and cell boundaries and nuclear outlines are made out with difficulty (Fig. 20), and also during synizesis and early post-synizesis stages (Figs. 23-31).

In the early stages of development a cell rarely contains more than one vitelline body unless it is preparing to divide. During synizesis the vitelline body enlarges somewhat and at a slightly later period it becomes oval and then constricts through the middle so that it has the appearance of a dumb-bell (Fig. 47, a); subsequently it divides into two rounded parts (Fig. 47, b), which soon separate (Fig. 47, c). The vitelline bodies thus formed divide repeatedly, and by the time that the oöcyte has reached the stage of Figs. 36-39, its cytoplasm contains a considerable number of these bodies which vary greatly in size, although they all appear round and homogeneous. Sometimes at this stage a vitelline body is enclosed in a clear area which marks it off from the cytoplasm, but this is not a constant phenomenon. Occasionally a vitelline body does not divide in the manner described above, but it breaks into three small parts (Fig. 38, Y); in other cases division is unequal and one large and one small body are formed (Fig. 38, X). Since the vitelline bodies vary so greatly in size and are so widely scattered throughout the cytoplasm at the stage of Fig. 39, it seems very probable that some of them are newly formed secretion products of the cytoplasm which appear first as minute granules and gradually increase in size.

The vitelline bodies begin to increase in number before the resolution of the large compound-nucleoli and at a time when the nucleus contains but a very few plasmosomes: they are scattered throughout the cytoplasm, chiefly in the zone midway between the periphery of the egg and the nuclear membrane; and very few of them ever lie close to the nucleus. These facts would seem to preclude the possibility that the vitelline bodies are extruded nucleoli, although in their staining reactions and in their general appearance they are strikingly like these structures.

One of the reasons given by Will for considering the rounded bodies which he finds in the cytoplasm of the egg of *Rana* as extruded nucleoli is that he first finds these bodies in a light area close to the nucleus. Preparations of young ovarian eggs of *Bufo* that have been badly preserved frequently give the impression that nucleoli lie outside of the nucleus in a fluid space marked off from the cytoplasm. If such preparations are examined under an immersion lens, one finds that the light area which apparently surrounds the nucleus is, in reality, a portion of the nucleus itself, since the nuclear membrane is readily found where the clear area comes in contact with the cytoplasm. In such eggs, owing doubtless to the imperfect penetration of the fixing fluid, all of the more fluid portions of the nuclear substance seem to be collected at one side or around the periphery of the nucleus, while the granular achromatin and most of the nucleoli are massed together either in the middle of the nucleus or at one side of it. Projections from this mass sometimes extend across the fluid substance to the nuclear membrane and thus give the appearance of an ameboid nucleus without a nuclear wall. In nuclei of this character nucleoli are sometimes found stranded in the fluid substance and, under low magnification, they appear to lie in the cytoplasm. The clear area which separates the nucleus from the cytoplasm in many eggs is doubtless an artefact produced through the action of reagents: I do not think that it is present in the living egg.

The vitelline bodies are rarely found close to the periphery of the egg at the stages of Figs. 36-39, and I have seen noth-

ing that would indicate that follicle cells or their products enter the egg and produce these structures. As these bodies are not extruded nucleoli it is evident that they must be considered as secretion products of the cytoplasm itself. Since, as Bernard (8), Chittenden (20) and others have maintained, the nucleus is undoubtedly to be considered as an organ of constructive metabolism which "has controlling power over the metabolic processes in the cell, modifying and regulating the nutritive changes" (Chittenden), it is not to be supposed that the formation of the vitelline bodies in the cytoplasm takes place independently of nuclear activity. Although no substance can be seen to leave the nucleus at the time that the number of vitelline bodies is rapidly increasing, it is not improbable that a fluid, possibly an enzyme, passes from the nucleus into the cytoplasm and there causes the formation of these bodies. The same enzyme, acting in the nucleus itself, may be the cause of the formation of the plasmosomes; for these bodies are being produced in considerable numbers in the nucleus at the time that the formation of vitelline bodies is taking place most actively in the cytoplasm. On this assumption it is probable that the vitelline bodies "bear the same relation to the cytoplasm that the nucleoli do to the germinal vesicle," as Jordan has suggested. Whether the substance out of which the vitelline bodies are made is supplied entirely by the cytoplasm, or whether the follicle cells contribute material to the egg for their formation, I have not been able to determine. I have never found follicle cells inside of the egg, although they very frequently enter the cells of Bidder's organ. The function of the follicle cells seems to be to form the egg membranes during the early stages of development and, after the egg has left the ovary, to aid in the absorption of the follicle sacs (King, 50).

Several investigators of amphibian oogenesis, besides Will and Leydig, have found rounded bodies in the cytoplasm of the egg which are doubtless of the same nature as the vitelline bodies in the egg of *Bufo*. Hertwig (42) states that the small bodies which he finds in the cytoplasm of the egg of

Rana are composed of a hyaline substance and appear much like nucleoli, although they cannot be extruded nucleoli since nucleoli never wander into the cytoplasm. Born discovered small oval bodies near the nucleus in the cytoplasm of the egg of Triton which he hesitates to call yolk-nuclei since they never appear granular. Bataillon describes and figures the division of a small body lying in the cytoplasm of the egg of Rana which he considers to be a large nucleolus which has passed out of the germinal vesicle. He states that this body ordinarily disappears when the yolk is formed, and that he once saw it transformed into pigment.

Bodies similar to the vitelline bodies in the egg of *Bufo* have been found in the mammalian egg by von Winiwarter (94), Gurwitsch (36), and von Skrobansky (85). These bodies are present in the cytoplasm in addition to a granular yolk-nucleus. The latter structure, according to the researches of von Winiwarter and Gurwitsch, is homologous to the idio-zome in the sperm-cells. The figures given by von Skrobansky of rounded bodies in the egg of the guinea pig are very similar to those shown in Figs. 36-38. Von Skrobansky states that these bodies increase in number as the egg develops and that they have a tendency to form in groups of two, three, or more which are often surrounded by a clear area. As their appearance is coincident with the disappearance of the yolk-nucleus, he suggests that the substance of the yolk-nucleus becomes differentiated into these rounded bodies, although it is not impossible that they are new differentiation products of the cytoplasm.

Small homogeneous bodies appearing like the vitelline bodies in the egg of *Bufo* have been found in the cytoplasm of the eggs of various arachnoids, myriapods, and vertebrates, and classed with large granular structures as yolk-nuclei. From the researches of Henneguy, Balbiani (3), and others, it is evident that the term yolk-nucleus has been used in a general way to cover a number of different structures in the cytoplasm, as the term nucleolus has been applied to a variety of structures in the nucleus.

When the toad has reached a body length of about 4 cm. and the egg has a diameter of from 0.18-0.2 mm., there appears simultaneously in different parts of the cytoplasm a number of irregular, granular masses which I shall call yolk-nuclei since they are similar in appearance to the structures described under this name by Foot (32), Henneguy, Jordan, Calkins (14), and Munson (72). These yolk-nuclei arise as rather small, irregular patches of granular substance that are not sharply marked off from the surrounding cytoplasm. There is at first no regular arrangement of these bodies; some lie near the nucleus; others are found near the periphery of the egg, while the majority lie in a zone midway between the nuclear membrane and the outer boundary of the egg. When sections are stained with any of the various combination stains previously mentioned, these yolk-nuclei take the plasma stain more deeply than does the cytoplasm and hence are easily seen. It seems strange that such a keen observer as Goette failed to find these bodies in the egg of *Bombinator*.

The manner in which these yolk-nuclei are formed is shown in Fig. 46. Around one of the larger vitelline bodies there appears a clear area, as if the vitelline body had in some way caused a liquefaction of the surrounding cytoplasm (Fig. 46, X). The substance of this area then becomes changed into an irregular mass of minute granules which at first stain but slightly darker than the cytoplasm. In some cases the vitelline body can be found in the centre of the yolk-nucleus (Fig. 46, Y), but as a rule it quickly loses its capacity for staining and then disappears, evidently being used up in the formation of the yolk-nucleus. As shown in Fig. 53, a yolk-nucleus sometimes contains several vitelline bodies of various sizes which stain as intensely as at the stages of Figs. 36-39. In cases of this kind it is impossible to determine whether the vitelline bodies in each yolk-nucleus are produced by the repeated division of the one vitelline body concerned in the formation of the yolk nucleus, or whether several vitelline bodies originally took part in the formation of a single yolk-nucleus. I am inclined to the former view since the vitelline

bodies grow rapidly and divide very readily both in earlier and in later stages of development and they are rarely found in groups of more than three before the formation of the yolk-nuclei.

In eggs with a diameter of 0.25-0.3 mm. the yolk-nuclei are very conspicuous since they stain more intensely than at the stage of Fig. 46, and their number is much less than at an earlier period as several small granular masses fuse to form larger ones. At this stage of development the yolk-nuclei come to have a definite arrangement in the cytoplasm, forming a more or less complete ring midway between the nucleus and the periphery of the egg (Fig. 44). This is not an accidental arrangement found in a few eggs, but it is a constant phenomenon in eggs of a given size taken from different individuals and preserved and stained in different ways. At this time the cytoplasm contains very few large vitelline bodies, most of these bodies having been used up in the formation of yolk-nuclei.

In the egg of the newt Jordan finds granular yolk-nuclei similar in appearance to those found in the egg of *Bufo* at the stage of Fig. 44. Jordan states that these bodies appear about the time that the yolk is beginning to form at the periphery of the egg "from points of independent origin," and that there are never more than nine of these structures. In the very young egg Jordan has found what appears to be "localized condensations of the cytoplasm and a consequent greater avidity for staining fluids," and he finds all gradations between these bodies and the granular yolk-nuclei. From his observations Jordan concludes that "in the newt the yolk-nuclei always arise first as condensations of the cytoplasm and subsequently increase in size and complexity with the growth of the egg." The figures given by Jordan do not show clearly the early development of the yolk-nuclei, although his Figs. 5, 10-12 are sufficiently detailed to indicate that the method by which the yolk-nuclei are formed in the egg of the newt is essentially the same as in the egg of *Bufo*. The subsequent history of these bodies in the egg of the newt is similar to that

of the yolk-nuclei that are first formed in the egg of *Bufo* since, for a time, they lie in a zone half way between the germinal vesicle and the periphery of the egg and later draw near to the germinal vesicle where they gradually disintegrate. The only conjecture Jordan makes as to the probable function of the yolk-nuclei is that "they have a real physiological significance probably related to the construction of yolk."

The granular yolk-nuclei found by Foot in the egg of *Allobophora foetida* bear a striking resemblance to those found in the egg of *Bufo* (Cf. Foot's Figs. 4-5 with my Figs. 44 and 46). According to Foot the yolk-nuclei arise in contact with the nucleus, but, judging from their staining reactions, they are not derived from the chromatin as Calkins (14) maintains is the case in *Lumbricus*. As the yolk-nuclei increase in size they become broken up, and they are either scattered in patches throughout the cytoplasm or aggregated at the egg periphery. Foot concludes that these yolk-nuclei are formed of "archoplasm," and she traces them into the attraction-sphere, the fertilization cone, and the polar rings. Munson finds granular yolk-nuclei in the egg of *Clemmys marmorata* which are similar to the granular masses shown in Figs. 44 and 46. Munson states that these structures are formed of "a kind of metaplasma (or archoplasm) arising in the neighborhood of the germinal vesicle through the combined influence of the nucleus and cytoplasm. From the place of its formation, it diffuses or flows throughout the cytoplasm where it serves as a culture medium of the living substance of the egg; in other words, it serves as food. The true yolk-bodies are a secretion of the living substance of the cytoplasm."

Soon after the yolk-nuclei become arranged in the form of a ring there appears near the periphery of the egg a number of small, round, homogeneous bodies which stain intensely (Fig. 45). These bodies, as their subsequent history shows, are a new generation of vitelline bodies which are directly concerned with the formation of the yolk. From their peripheral position one might, perhaps, be inclined to think that the follicle cells are concerned in some way with the formation

of these bodies. I have never found any evidence that would support such an assumption. Since these vitelline bodies are formed some distance from the yolk-nuclei and are at first nearly uniform in size, it would seem as if they must be new secretion products of the cytoplasm. There is the possibility, however, that they are derived either from the few vitelline bodies that were left over after the formation of the yolk-nuclei or from the granular substance of which the yolk-nuclei are composed. The vitelline bodies increase very rapidly in number and in size, and many of them give rise to small granular yolk-nuclei, similar to those shown in Fig. 46, which always remain at the outer surface of the egg (Fig. 54).

While the new formation of vitelline bodies is taking place at the egg periphery, the ring of yolk-nuclei is gradually moving towards the centre of the egg and at the stage of Fig. 54 it closely encircles the germinal vesicle. In many cases yolk-nuclei seem to come in actual contact with the nuclear membrane. Whether there is a fusion between these bodies and the substance of the germinal vesicle, as Jordan is inclined to believe, I am not able to state. There is no noticeable increase in the size of the nucleus or any unusual change in the nuclear structure as one might expect to be the case were a considerable quantity of substance taken at this time into the germinal vesicle. After reaching the germinal vesicle the circle of yolk-nuclei stains less intensely and gradually disappears (Fig. 56). I am inclined to the opinion that their substance is dissolved in situ to take part later in the formation of the yolk spherules in this region of the egg. This view agrees substantially with that advanced in 1859 by Thompson and since advocated by Jordan.

As a rule the yolk-nuclei that are first formed have moved close to the germinal vesicle by the time that new yolk-nuclei are to be found at the periphery of the egg, and there is a cytoplasmic zone between them which is free from vitelline bodies or yolk-nuclei (Fig. 54). In exceptional cases, as shown in Fig. 53, the formation of the peripheral vitelline bodies and yolk-nuclei takes place even before the older yolk-

nuclei have become arranged in the form of a ring, and the only portion of the cytoplasm which does not contain these structures is that surrounding the germinal vesicle. As these cases are found so infrequently I have not been able to determine whether the yolk-nuclei later become arranged as in Fig. 54, or whether all of them remain at the periphery of the egg to take part in the formation of the yolk there.

In *Bufo*, as in other amphibians according to the investigations of Vogt, Goette, Schultze, Born, Iwakawa, Jordan, and Dubnissos, yolk spherules first appear in the outer regions of the cytoplasm and usually simultaneously in different parts of the egg. There are apparently two methods by which the first yolk spherules may be formed in the egg of *Bufo*. Both of these methods sometimes take place in the same egg; whether this is true for all eggs I am unable to say. The yolk develops so rapidly that it is difficult to follow the processes of its formation in any detail.

Soon after the stage of development shown in Fig. 54 a varying number of small, oval bodies appear in the peripheral yolk-nuclei (Fig. 56). These bodies, which stain somewhat less intensely than the vitelline bodies, are yolk spherules which are being formed, evidently, from the substance of the yolk-nuclei. As the yolk spherules increase in number the granular yolk-nuclei fade away and they have completely disappeared by the time that the yolk forms a continuous layer around the periphery of the egg.

In some cases certain of the vitelline bodies at the periphery of the egg grow very large (Fig. 55). The outlines of these bodies become irregular (Fig. 52, a), and subsequently they break into from two to four rounded, homogeneous pieces (Fig. 52, b) which in turn divide into smaller bodies (Fig. 52, c-e). As a result of the repeated division of the one vitelline body there is formed a mass of small oval bodies (Fig. 52, f) which are of the same size and shape as the small yolk spherules, although at first they stain more deeply than the yolk spherules and can therefore readily be distinguished from them. Later these bodies stain less intensely and seem to pass

over into yolk spherules. It is very probable that the aggregations of small yolk spherules shown in Figs. 55-56 have had such an origin, although it is possible that they were derived from the substance of a yolk-nucleus. During the early stages of yolk formation the cytoplasm at the outer boundary of the egg frequently appears vacuolated. This does not seem to be a constant phenomenon, however, and it may be due in part, at least, to the action of reagents.

In the egg of *Bufo* there are two generations of yolk-nuclei, both formed from or under the influence of the vitelline bodies, and both evidently concerned in the formation of yolk. The first yolk-nuclei that are formed appear simultaneously in different regions of the cytoplasm and they later move close to the germininal vesicle where they gradually fade away. One can readily trace every step in their development from the stage of that shown in Fig. 46 to that of Fig. 56. The yolk-nuclei belonging to the second generation are formed at the periphery of the egg and they are transformed directly into yolk spherules. The various stages in the development of these bodies can also easily be followed. With these facts in mind the following statement by Crampton (24) is of interest: "The accounts of the origin of the yolk-spheres from cytoplasmic elements at places removed from the nucleus, or from several centres, or in all parts of the egg at once, fail to take into consideration an earlier stage marked by the origin from the nucleus of a true yolk-matrix which subsequently disintegrates and spreads throughout the whole cell-body as in *Molgula*." It would seem as if enough cytological work had been done to make it clear that one cannot deduce general rules applicable to all eggs from the study of one particular egg, no matter how carefully the work may have been done.

According to my investigations the formation of the yolk in the egg of *Bufo* is closely associated with the vitelline bodies and also with the granular masses produced by them, the yolk-nuclei. I have elsewhere stated that the vitelline bodies are probably secretion products of the cytoplasm formed, possibly, under the influence of an enzyme given off by the nucleus. The

granular yolk-nuclei are undoubtedly composed of nutritive material which is subsequently aggregated into yolk spherules. In some instances the substance of the vitelline bodies seems to be transformed directly into yolk spherules; the intermediate stage, that of the formation of yolk-nuclei, being omitted. This would seem to indicate that the vitelline bodies are themselves but aggregations of nutritive material which is in a semi-fluid condition rather than in the form of granules. It may be that during the early stages in the development of the ova "yolk is present in the cytoplasm in the form of a diffused unstainable fluid," as Montgomery has suggested, and that this fluid is first collected into the rounded vitelline bodies and later changed into yolk spherules.

The part taken by the nucleus in the formation of yolk in the egg of *Bufo* is as yet obscured. I have never seen any nucleoli or any minute granules leave the nucleus which might have an influence on the formation of the yolk. If, as seems probable, the nucleus directs and controls the nutritive processes in the cell, then in the formation of yolk it must act either through a fluid substance which it gives out into the cell-body, or it must exert its influence directly on the deutoplasmic substance of the cytoplasm. In many kinds of eggs, according to the investigations of Conklin, Crampton, Calkins, Foot and Floderus (31), the yolk is formed first around the nucleus and then produced progressively towards the periphery of the egg. In these cases it may be supposed that the cytoplasm surrounding the nucleus is directly stimulated by the nucleus to produce yolk. In amphibians and many other vertebrates the yolk first appears at the periphery of the egg. In these cases the nucleus has a less direct influence on the yolk formation, and this influence is probably exerted through the action of a fluid substance which passes by osmosis through the nuclear membrane into the cell-body. The investigations that have seemed to show that yolk is derived directly from nucleoli, or from chromatin, or from follicle cells, are all open to question, and until they have been confirmed by further research I shall be inclined to believe that yolk formation is

one of the anabolic processes in the cell which, although it is directly or indirectly controlled by the nucleus, does not depend upon the nucleus for its material substance.

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#### EXPLANATION OF PLATES.

All figures were drawn with the aid of a camera lucida. They have been reduced one-third.

Abbreviations used in lettering the figures:

- Al., alimentary tract.  
Ao., aorta.  
C., centrosome.  
C. V., cardinal vein.  
E., endoderm.  
G., germ-cells.  
H., heart.  
L., liver.  
L. M., lateral plates of mesoderm.  
M., mesoderm.  
N., neural tube.  
No., notochord.  
Nu., nucleolus.  
P., early prophase of mitosis.  
T., Wolffian tubule.  
V., vitelline body.

FIG. 1.—Portion of a transverse section through the middle of a tadpole six days old.  $\times 91$ .

FIG. 2.—Portion of a transverse section through the middle of a tadpole nine days old showing the location of the germinal ridge.  $\times 91$ .

FIG. 3.—Section of the germinal ridge at the stage of Fig. 2.  $\times 1,000$ .

FIG. 4.—Section of the germinal ridge in a tadpole eleven days old.  $\times 1,000$ .

FIG. 5.—Transverse section of a divided germinal ridge in a tadpole thirteen days old.  $\times 1,000$ .

FIG. 6.—Longitudinal section showing the extent of the germinal ridge in a tadpole thirteen days old.  $\times 47$ .

FIGS. 7-9.—Sections through the germinal ridge in a tadpole fifteen days old showing the character of the cells at different levels.  $\times 1,334$ .

FIG. 10.—Early prophase of mitosis in a primordial germ-cell.  $\times 1,334$ .

FIGS. 11-12.—Equatorial plate in a primordial germ-cell. All 24 chromosomes are shown.  $\times 1,334$ .

FIG. 13.—Longitudinal section of a spindle during the metaphase. Only 5 of the chromosomes are shown.  $\times 1,334$ .

FIG. 14.—Late anaphase in a primordial germ-cell.  $\times 1,334$ .

FIG. 15.—Section of the ovary at the time when sex is first apparent. Taken from a tadpole with very well-developed hind legs.  $\times 1,000$ .

FIG. 16.—Section of a young ovary showing the beginning of the formation of a central cavity. Taken from a tadpole about to undergo metamorphosis.  $\times 1,000$ .

FIG. 17.—Section of a young ovary containing a central cavity. Taken from a tadpole at the time of metamorphosis.  $\times 1,000$ .

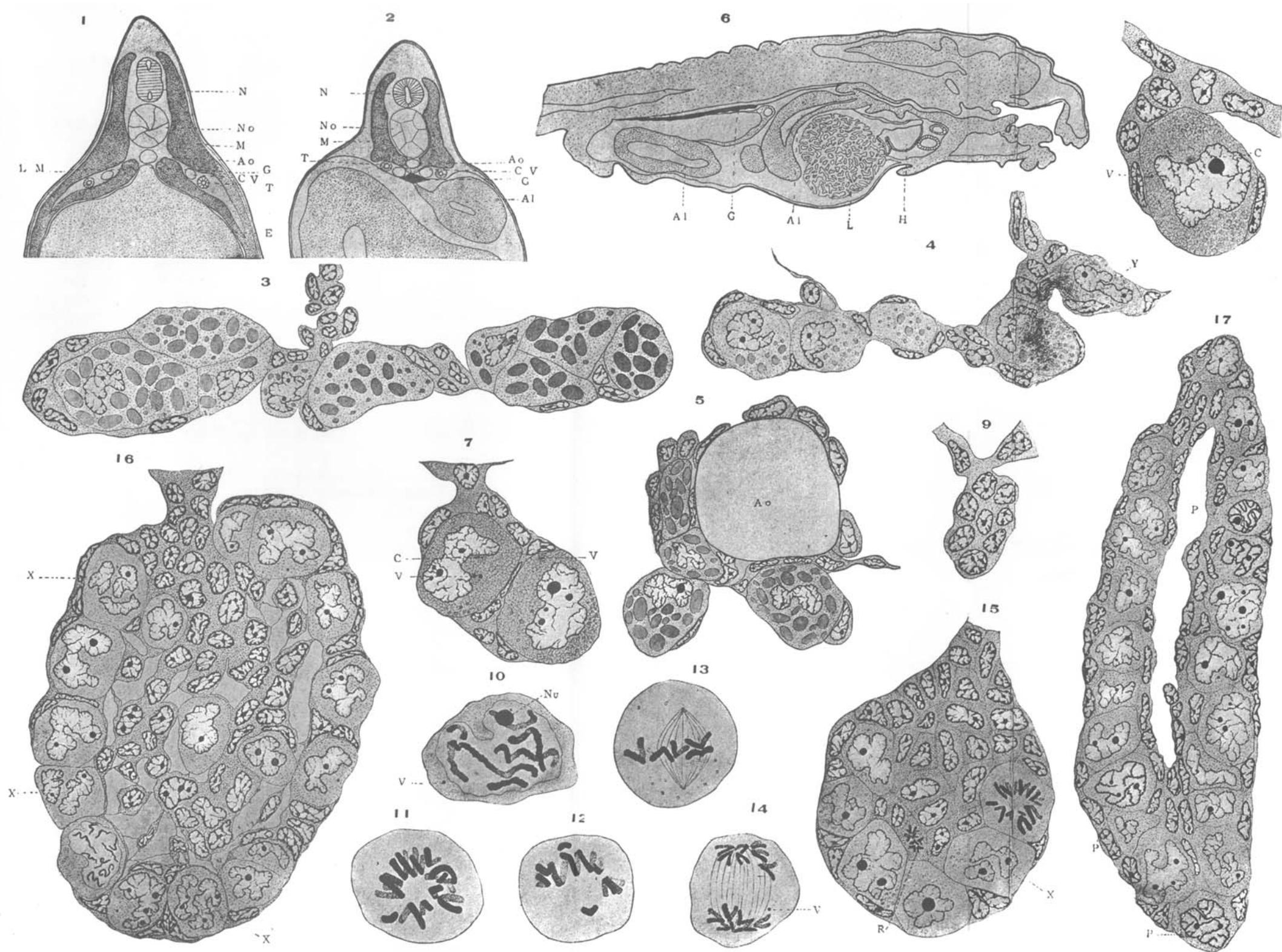


FIG. 18.—Cyst of secondary oögonia in the resting stage.  $\times 1,334$ .

FIG. 19.—Cyst containing secondary oögonia in various stages of mitosis.  $\times 1,334$ .

FIG. 20.—Young oöcyte with oval nucleus.  $\times 1,334$ .

FIG. 21.—A slightly later stage than Fig. 20. The nucleus of the oöcyte has assumed a rounded form.  $\times 1,334$ .

FIG. 22.—Early growth stage of the oöcyte. The nucleus contains a well defined, apparently continuous spireme.  $\times 1,334$ .

Figs. 23-24.—Stages showing the gradual condensation of the nuclear substance previous to synizesis.  $\times 1,334$ .

FIG. 25.—Synizesis stage.  $\times 1,334$ .

Figs. 26-27.—Post-synizesis stages. Part of the chromatin has been evolved in the form of a continuous convoluted spireme: the nucleoli and the rest of the chromatin appear in the form of irregular masses lying against the nuclear wall or in the meshes of the spireme.  $\times 1,334$ .

Figs. 28-29.—Stages showing the longitudinal splitting of the spireme.  $\times 1,334$ .

FIG. 30.—Slightly later stage. The sister portions of the spireme have begun to separate.  $\times 1,334$ .

FIG. 31.—Young oöcyte surrounded by its zona pellucida. In the nucleus the sister portions of the spireme are almost entirely separated.  $\times 1,334$ .

FIG. 32.—Section of an oöcyte in which there is a complete separation of the sister portions of the spireme.  $\times 1,334$ .

Figs. 33-34.—Nuclei of the young oöcytes showing the division of the spireme into double segments.  $\times 1,334$ .

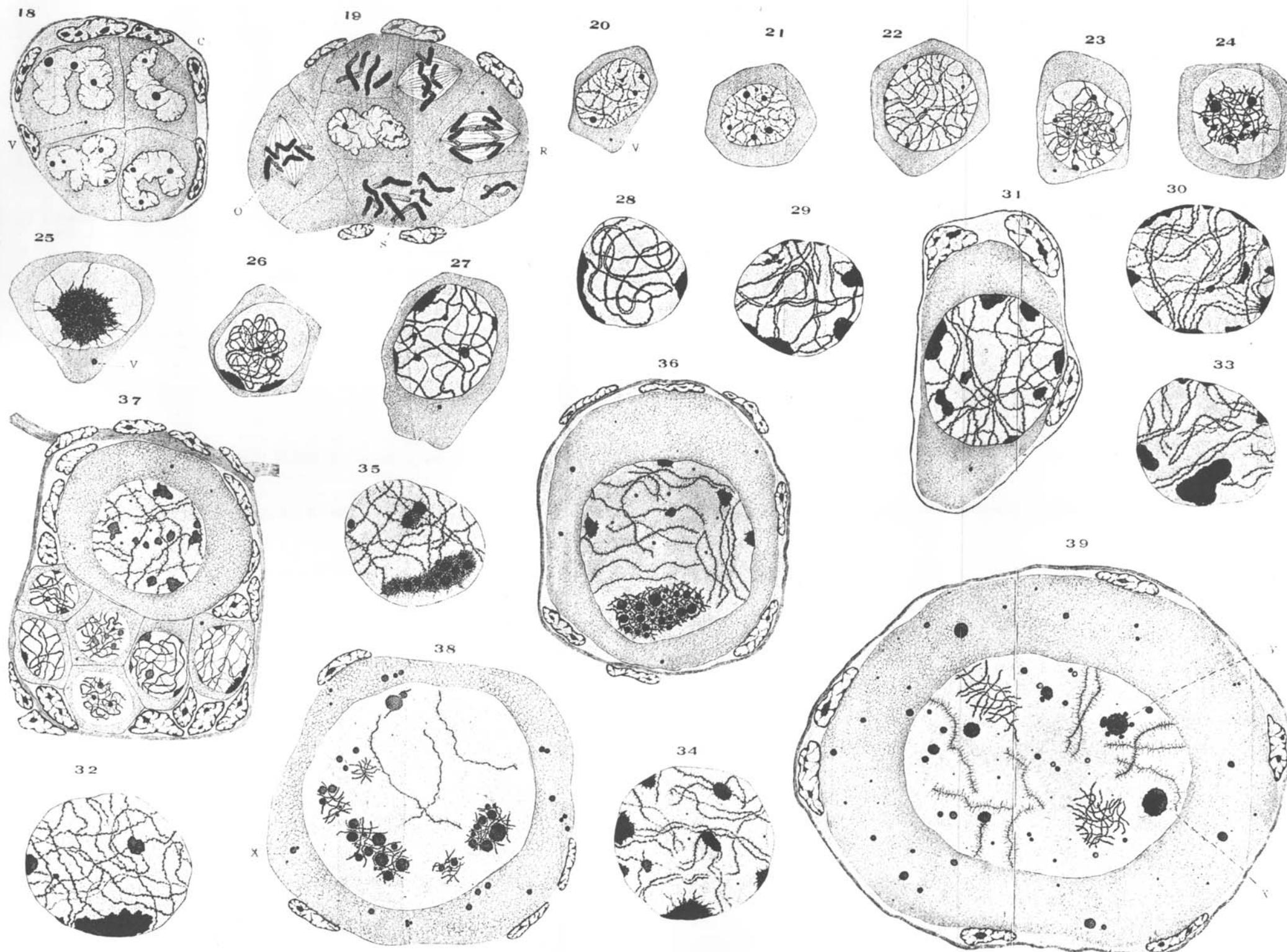
FIG. 35.—Section of the nucleus of a young oöcyte showing the beginning of the resolution of the amorphous masses shown in Figs. 26-34.  $\times 1,334$ .

FIG. 36.—Section of a young oöcyte showing the differentiation of one of the amorphous masses into a meshwork of chromatin threads and rounded nucleoli.  $\times 1,334$ .

FIG. 37.—Section of a cyst containing oöcytes in different stages of development.  $\times 1,000$ .

FIG. 38.—Stage following that of Fig. 36, showing the relation of the nucleoli, the oxychromatin threads and the chromosomes. In the cytoplasm are numerous vitelline bodies.  $\times 1,334$ .

FIG. 39.—Section of a young oöcyte. The chromosomes are scattered throughout the nucleus and they have assumed the feathery appearance which characterizes them throughout the rest of the growth period. The oxychromatin threads are entirely separated from the nucleoli and have become very granular. In the cytoplasm are numerous vitelline bodies of various sizes.  $\times 1,334$ .



FIGS. 40-41.—Sections of the nuclei in oöcytes of a young toad with a body length of 3.5 cm. A large nucleolar body, oxychromatin threads and feathery chromosomes are shown.  $\times 1,000$ .

FIG. 42.—Section of the nucleus in the oöcyte of a toad with a body length of 3 cm. Some of the nucleoli stain faintly and are evidently in the process of dissolution.  $\times 1,000$ .

FIG. 43.—Section of a nucleus in an oöcyte of a toad with a body length of 4 cm. showing the fragmentation of a large nucleolar body, scattered oxychromatin threads, and a pair of chromosomes.  $\times 1,000$ .

FIG. 44.—Part of a section of an egg taken from a young toad with a body length of 5.5 cm. The yolk-nuclei are collected in a zone lying midway between the nucleus and the periphery of the egg. Diameter of the egg is 0.23 mm.; of the nucleus, 0.11 mm.  $\times 1,000$ .

FIG. 45.—Drawn from an egg taken from the same ovary as that from which Fig. 44 was taken. Differentiation of the compound-nucleoli with the aid of safranin and gentian violet.  $\times 1,000$ .

FIG. 46.—Part of a section of an egg taken from a toad with a body length of 5 cm. The yolk-nuclei are forming at the expense of the vitelline bodies.  $\times 1,000$ .

FIG. 47.—Division stages of a vitelline body.  $\times 1,334$ .

FIG. 48.—Section of the nucleus of an egg taken from the ovary of an adult toad killed the latter part of April. The plasmosomes are separated from the chromatin and most of them are massed at one side of the nucleus. Diameter of the nucleus, 0.2 mm.  $\times 333$ .

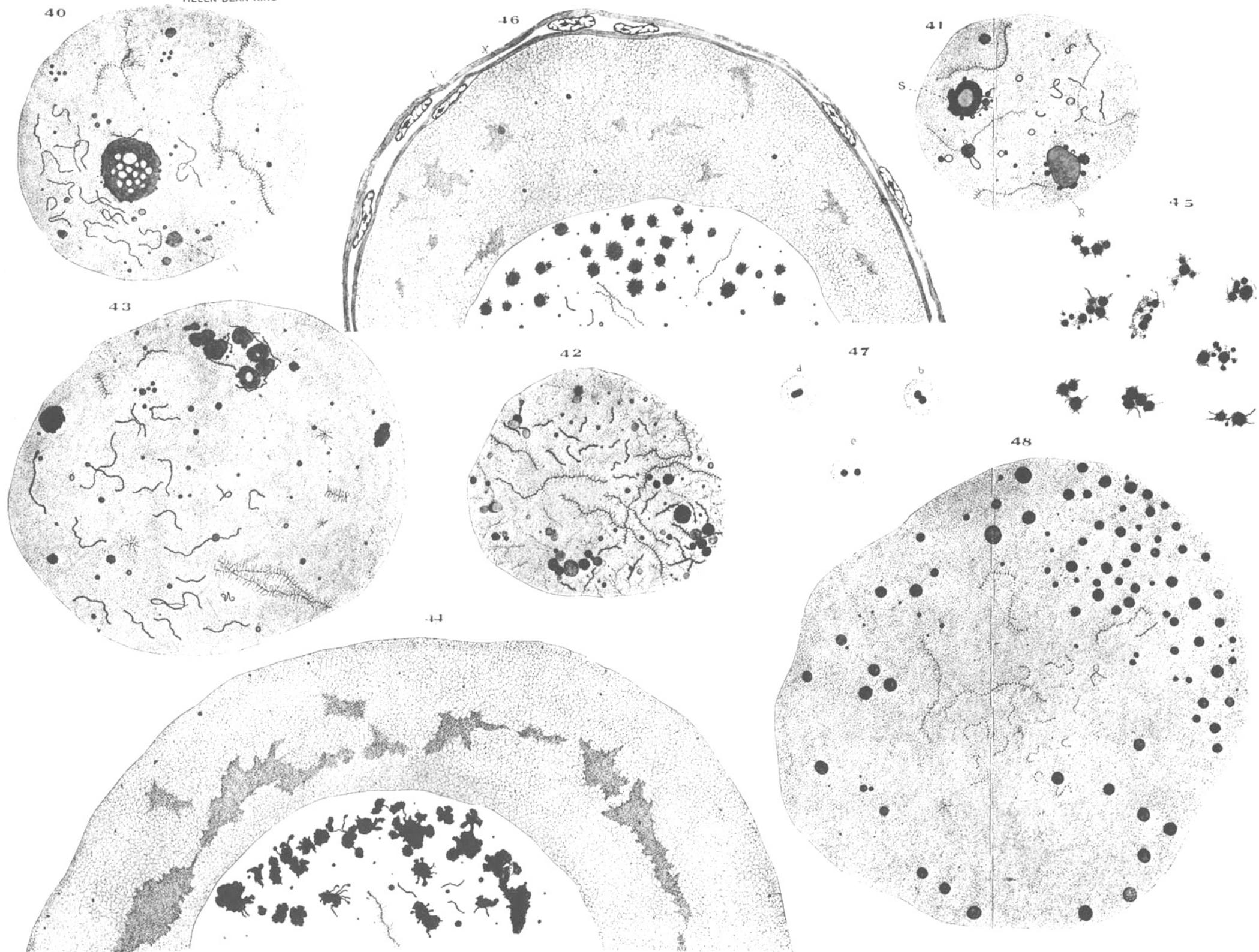


FIG. 49.—Section of the nucleus of an egg taken from an adult toad killed the latter part of April. The chromosomes occupy the centre of the nucleus, while the plasmosomes are very evenly distributed about the nuclear periphery.  $\times 333$ .

FIG. 50.—Section of the nucleus of an egg taken from an adult toad killed early in May. The plasmosomes have migrated to the interior of the nucleus and they enclose the chromosomes.  $\times 333$ .

FIG. 51.—Peculiar types of compound-nucleoli found in many of the nuclei during the later development of the oöcytes.  $\times 1,334$ .

FIG. 52.—Stages showing the formation of yolk spherules from a vitelline body.  $\times 1,334$ .

FIG. 53.—Yolk-nuclei and vitelline bodies in an egg taken from an adult toad killed the latter part of April.  $\times 1,000$ .

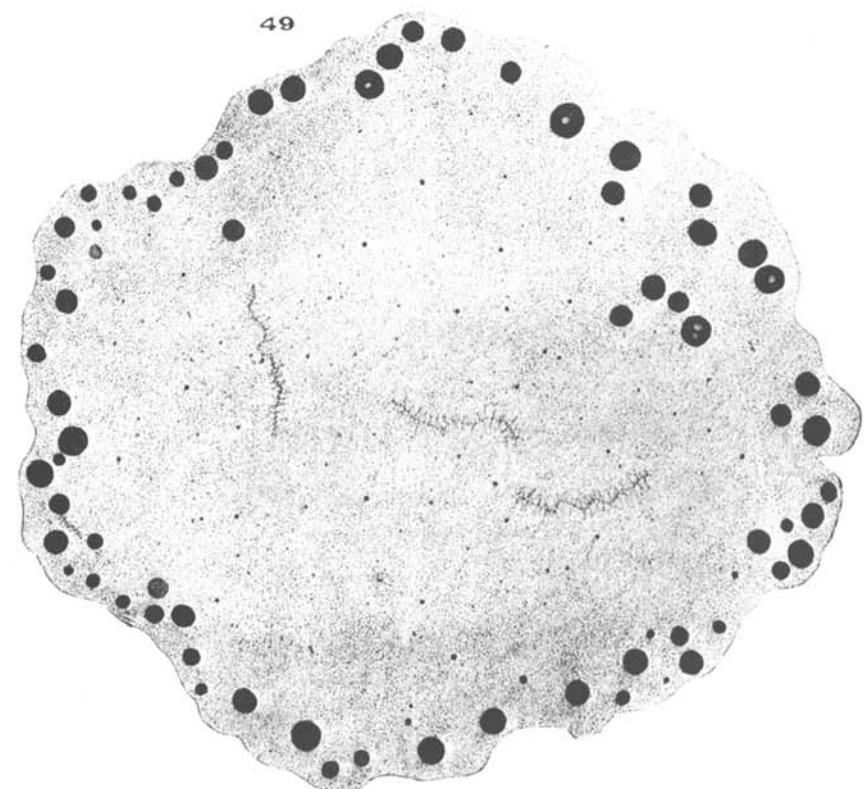
FIG. 54.—Part of a section of an egg taken from a young toad with a body length of 5.5 cm. New yolk-nuclei are forming at the periphery of the egg, and the older ones closely surround the nucleus.  $\times 1,000$ .

FIG. 55.—Formation of yolk spherules at the periphery of an egg taken from an adult toad killed in May.  $\times 1,000$ .

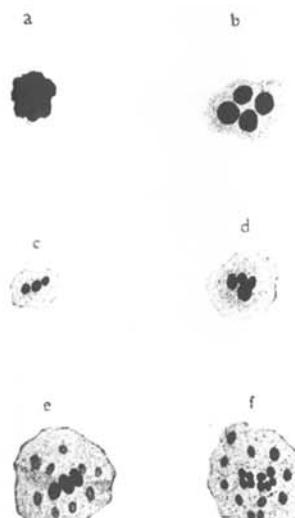
FIG. 56.—Part of the section of an egg taken from an adult toad killed in May. At the periphery of the egg a layer of yolk spherules is forming at the expense of yolk-nuclei and vitelline bodies. The layer of yolk-nuclei around the nucleus is beginning to disappear.  $\times 667$ .

THE OÖGENESIS OF *BUFO LENTIGINOSUS*

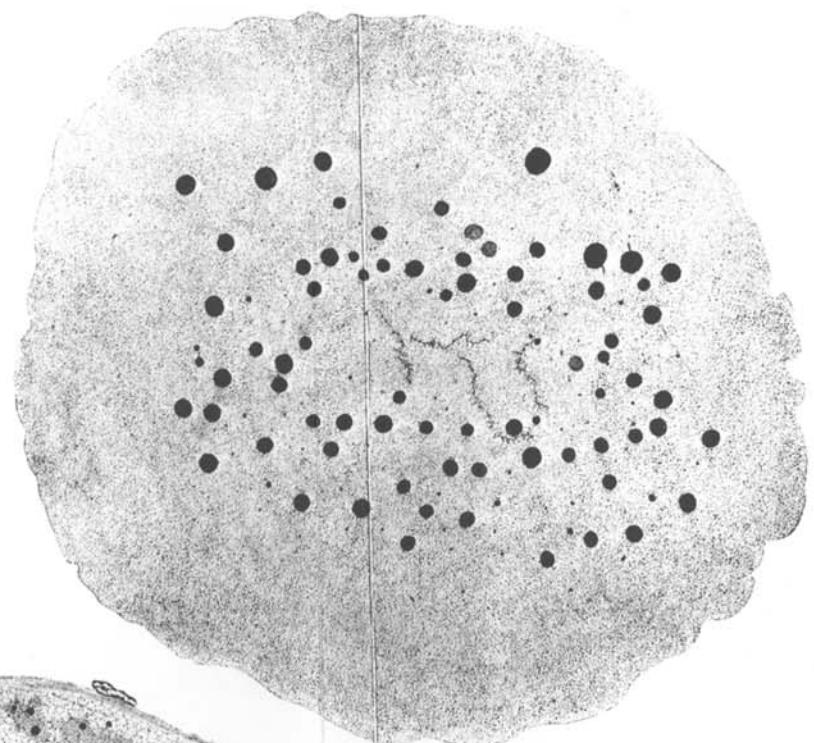
HELEN DEAN KING



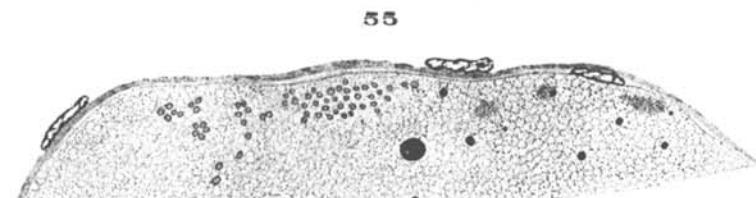
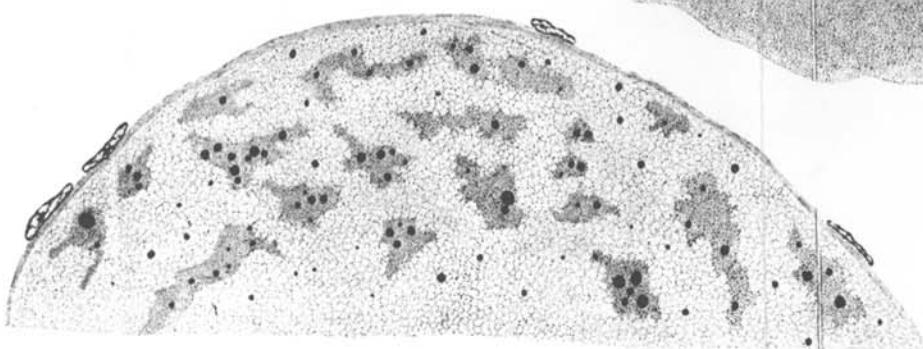
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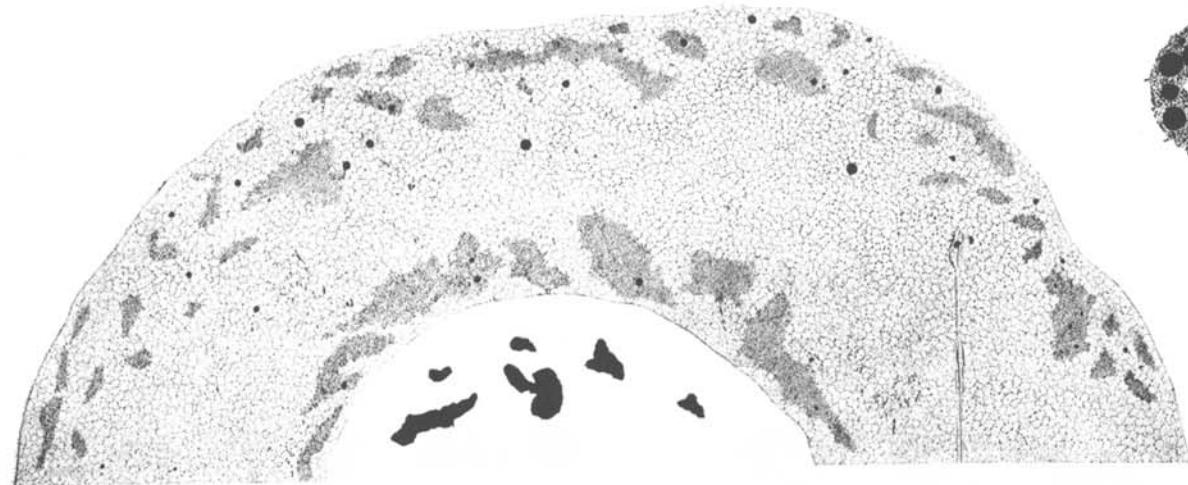
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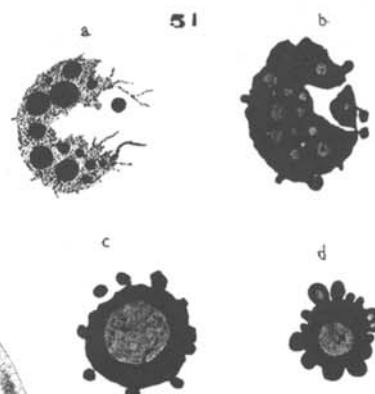
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