IV. *The Oogenesis of the Fowl* (Gallus bankiva).*

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[Plates 15–20.]

Contents.

1. Introduction.

In modern times few branches of cytology have received closer attention than has been devoted, by many observers, to the problems of oogenesis in animals. The time and labour expended in this field have furnished a rich harvest, contributing much to the better understanding of the structure and function of the various parts of the living

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cell, and supplying the solid foundations on which is built our knowledge of embryology. Almost every group of animals has been studied from this point of view, the vertebrates, however, receiving the most attention. The literature, on the oogenesis of birds alone, is considerable and in many ways unique, being crowned by the monumental work of Dr. Modeste Van Durme (25), entitled 'Nouvelles Recherches sur la Vitellogenèse des Œufs d'Oiseaux aux Stades d'Accroissement, de Maturation, de Fécondation et du Début de la Segmentation,' published in 1914.

It is remarkable, however, that none of the many workers on this subject, not even Van Durme herself, has described the Golgi apparatus in the avian oocyte. I started work on the oogenesis of the fowl (*Gallus bankiva*) in the hope of being able to rectify this deficiency. I succeeded in demonstrating the Golgi apparatus, and at once perceived that its structure and behaviour in the bird studied were exceedingly complex, and in many ways unique. In April, 1924, I published a short preliminary account (2). In the present paper I am attempting to furnish a full account of my work on the Golgi apparatus in the oocytes of the fowl, and to treat of such other points of interest in connection with them as have arisen in the course of the investigations.

Part of the work described in this paper was carried out in the Department of Zoology, Trinity College, Dublin, under Prof. J. Brontë Gatenby, to whom my thanks are due. I am also indebted to my friend J. H. Woodger, Esq., of the Middlesex Hospital Medical School, and I wish to take this opportunity of thanking him and Prof. J. P. Hill, in whose department this work was completed.

2. Summary of Previous Work.

Swift (22) has shown that the primordial germ-cells in the chick arise anterior and antero-lateral to the embryo, in a specialised region of the germ-wall endoderm, just at the margin of the area pellucida. They are at first in the space between the endoderm and ectoderm, but later wander into the mesoderm and the forming blood vessels. They are carried in the blood stream to all parts of the embryo. He identifies these primordial germ-cells with the "endodermal wander cells" of Dantschakoff (6), which she considered took no part in blood formation, but disappeared from the blood-stream about the 22-somite stage. Swift finds that they collect in the vessels of the splanchnic mesoderm in embryos of 20–22 somites. In embryos of 23–25 somites they are to be found in the splanchnic mesoderm near the angle of the cœlom. From here they pass into the primitive gonad as it forms at a later stage. It is not possible to distinguish the sex of the embryo until about the sixth or seventh day.

Describing the primordial germ-cells, Swift states that they vary in size from 14μ to 20 or 22μ in diameter, but average about 16μ. The nucleus varies from 8μ to 12μ in diameter. He considers that the attraction sphere is characteristic of the primordial germ-cells of the chick. "It is present in all stages, and, from the origin of the germ-cell to its entrance into the indifferent gonad, does not undergo any change. It appears as a condensed or flattened sphere of cytoplasm resting usually on the nuclear membrane,

on that side of the nucleus which is the farthest removed from the cell membrane. It may rest on the nuclear membrane directly, in which case it has a concavity towards the nucleus, or it may be separated from the nucleus by some distance. It has an average diameter of 3 to 4 micra, but in some cases, where it is flattened against the nucleus, its long axis may measure 6 micra. At times it is very discrete and seems to be surrounded by a definite membrane. The cytoplasm immediately around it appears less dense than the rest, so that the attraction sphere seems to be situated in a vacuole. In all stages and with all stains the sphere is prominent and, unless obscured by yolk spheres, is easily seen." He states that the centrosomes can be seen as black dots after trichloracetic acid fixation and iron-hæmatoxylin staining. Yolk is abundant in the younger germ-cells, but gradually decreases in amount during embryonic life. It, however, remains in the primordial germ-cells long after it has disappeared from the tissue cells. The mitochondria are rod-shaped and scattered through the cytoplasm until about the ninth day, when they tend to become grouped around the attraction sphere.

VON BERENBERG-GOSSLER (1) has described what he interprets as Golgi elements in the germ-cells of the 110 hours chick prepared by BENDA'S method. They appear as rod-like bodies around the centrosphere or attraction sphere.

WOODGER (28) has been able to confirm SWIFT'S work, on the origin of the primordial germ-cells of the chick, by a study of their Golgi apparatus. He demonstrated this by the silver nitrate methods of CAJAL and DA FANO, but the results he obtained with the osmic methods were unsatisfactory. In primitive streak stages he has been able to find in the ectoderm and germ-wall endoderm cells only scattered argentophil granules of different sizes, the larger ones having a clear central region. In the primordial germ-cells of embryos, from the stage of the closing of the medullary folds onwards, he finds a Golgi apparatus consisting of granules or short rods arranged around the archoplasmic sphere or centrosphere. The apparatus is similar to this in the germ-cells of all subsequent stages of incubation, but may vary considerably in individuals.

D'HOLLANDER (7) describes the oogenesis of the bird from the time of the differentiation of the germinal epithelium until the appearance of the primordial follicles. He calls this period the *extra-follicular growth period* in contradistinction to the succeeding *intra-follicular growth period*. He used chiefly HERMANN'S fluid, but also employed FLEMMING'S fluid and LENHOSSÉK'S fluid of sublimate-alcohol-acetic acid. He states that: " La cellule folliculeuse et l'oogonie ont une souche commune, à savoir la cellule indifférente : l'oogonie après plusieurs divisions engendre l'oocyte de premier ordre."

According to him the period of differentiation of the follicle cells and oogonia is followed by a period of multiplication, which terminates before hatching. The growth period of the oocytes starts in embryos at about the 14th day of incubation, and the primordial follicles are formed about the fourth day after hatching. These various processes of differentiation, multiplication and growth are simultaneously in progress in different parts of the same ovary at a certain stage. D'HOLLANDER then describes

in detail the nuclear changes of this period, which are outside the scope of this paper. He describes a crescentic dark zone in the cytoplasm capping the eccentric nucleus on the side farthest from the periphery of the oocyte. In this zone is a spherical structure containing one or more chromophil granules and surrounded by a narrow clear zone. He considers this structure to be the yolk-body of BALBIANI and the zone, capping the nucleus, in which it is situated, the *couche vitellogène* of VAN DER STRICHT. D'HOLLANDER thinks that this so-called *yolk-body of Balbiani* is the centrosphere of the oocyte, and in support of this he demonstrates the three following points :—

"1°. L'existence du corps vitellin dans les oogonies au repos :

2°. Lors de la division des oogonies, la genèse des sphères attractives de la figure de division au dépens de ce corps.

3°. Après la division des oogonies, la réapparition de cet élément dans les tout jeunes oocytes, dans lesquels il persiste et se développe graduellement durant le début de la période d'accroissement."

On this account and by comparing his figures with those of VON BERENBERG-GOSSLER and of WOODGER, and with my own material, I consider that there can be no doubt whatever that this body is the centrosphere containing the centrosome, and that the zone is formed of the mitochondria surrounding it.

FIRKET (9), working on the chick, attributes a dual origin to the germ-cells, and calls them primary and secondary. His " primary germ-cells " are the " primordial germ-cells " of SWIFT and of WOODGER. FIRKET considers that the primary germ-cells do not give rise to the definitive oocytes, but degenerate in the course of ontogeny, although he admits that it is impossible to determine that some of the primary germ-cells, in the cortex of the ovary, do not reach maturity. He considers that they have the value of oogonia about the eleventh day of incubation, and that they enter upon the growth period, but subsequently degenerate and have all disappeared in the chick 14 days after hatching. The secondary germ-cells, he considers, are derived from the germinal epithelium about the fifteenth day of incubation, and give rise to the majority, if not all, of the oocytes which arrive at maturity. FIRKET suggests that the large number of oocytes which degenerate at different stages are those which have failed in a veritable struggle for existence within the ovary, caused by an over-production, and that the longer an oocyte is in the ovary before it is ovulated the more likely it is to become degenerate. He points out that in this case the primary germ-cells would have little chance of becoming definitive eggs.

SWIFT (22), also working on the chick, contradicts FIRKET and holds that the primordial germ-cells give rise to the definitive oocytes, which never arise from the germinal epithelium. In this he is also in disagreement with D'HOLLANDER as he ascribes an epithelial origin to the follicle cells, but insists on the origin of the definitive ova from the primordial germ-cells.

In the present paper I do not propose to enter into this much disputed question of the origin of the definitive germ-cells, but to content myself with taking up the history of

the germ-cells at the stage of the female chick four days after hatching, at which time the definitive germ-cells have been formed, whatever their origin.

Mlle. LOYEZ (17), in an extremely important paper on the oogenesis of Reptiles, Birds, and Cephalopods, describes in detail the cytoplasmic and nuclear changes observable in the oocytes of the adult ovary of many species of birds, including the fowl. She finds that the differences between the various species of birds observed are comparatively slight. In all the birds a differentiated protoplasmic zone (*couche* or *masse vitellogène*) can be seen in the neighbourhood of the germinal vesicle of young oocytes. A yolk-body (*corps vitellin*) can sometimes be observed in the interior of this zone, which appears to be formed chiefly of the remains of the centrosome and the attraction sphere. No trace of it can be found at the time when yolk-formation commences. Corpuscles are present in the cytoplasm, which she considers are of nucleolar or chromatinic origin and come from the germinal vesicle. The yolk-forming zone (*masse vitellogène*) breaks up and its fragments, with the chromatinic corpuscles, spread out in the protoplasm towards the periphery, where they become incorporated in the ground cytoplasm. The first yolk-spheres appear at some distance from the periphery, which is, at that time, under the influence of substances coming from the follicular epithelium, the yolk-forming zone, and the germinal vesicle. Later a second yolk-centre forms at the periphery. The structure and chemical composition of the yolk-globules varies in the course of development. I will refer again to the work of this authoress on reptiles and birds in the discussion.

In a treatise published in 1914, worthy of being called a masterpiece of cytological investigation, Dr. MODESTE VAN DURME (25) described the oogenesis of the bird from the commencement of the intrafollicular growth period of the oocyte until fertilization. She thus takes up the work at the stage at which D'HOLLANDER left off. In this paper the previous literature is treated extensively and especially the work of Mlle. LOYEZ (17), of which much use is made. VAN DURME describes the nuclear changes, as well as the various cytoplasmic processes concerned in yolk-formation, but the former will be ignored here as they do not affect this work. She describes accurately the yolk-body of BALBIANI, and agrees with D'HOLLANDER and others in identifying it as the " sphère attractive," originating from the centrosome. She says that the yolk-body of the young oocyte persists in the middle of the yolk-forming layer (*palléale*) until the beginning of the intra-follicular growth period, when it may, as in mammals, divide into two, three or four bodies before becoming invisible. The yolk-forming layer, made up of variously shaped mitochondrial bodies, is also soon dispersed, thanks to the gradual invasion of the exoplasm by the mitochondrial structures of the yolk-forming crescent (*croissant vitellogène*). The latter represents the first centre of yolk-formation and is developed around the yolk-body of Balbiani. This process of dispersal results in the appearance of a homogeneous ooplasm with the mitochondria uniformly distributed.

These form, as yolk formation proceeds, four concentric layers in the cytoplasm :— (1) The cortical mitochondrial layer ; (2) the peripheral zone, in which the new

formation of yolk-granules is taking place; (3) the exoplasmic zone with large spheres; (4) the endoplasmic zone with small yolk-spheres. Some fat spheres always persist in the second and third of these layers and sometimes in the fourth, particularly near the germinal vesicle. As the nucleus approaches the surface of the yolk, at the animal pole, the nuclear hood disappears, its constituents giving rise to a special yolk with fine delicate yolk-granules. This represents the plastic yolk, or formative substance, which is destined alone to take part in segmentation. It forms finally a convexo-concave disc enclosing the germinal vesicle.

The peripheral yolk zone persists at the end of the growth stage and contains numerous globules of different sizes. It constitutes the white peripheral yolk. The yellow yolk is produced chiefly by the exoplasmic layer. The spheres of the endoplasmic zone increase and give rise to the white axial yolk of the egg: the latebra of Purkinje bound by the yolk cord to the nucleus of Pander.

The germinal disc rapidly increases in size at maturation. The white yolk, constituting the nucleus of Pander, gradually transforms into the superficial formative yolk and acquires its characteristic smaller, more numerous, heaped up yolk-granules. A multitude of clear yolk vacuoles appear in the middle of the formative disc.

At fertilization the size of the germinal disc still augments and the white yolk of the underlying layer diminishes. The yellow yolk becomes appreciably modified in the vicinity of the latter. A pale less stainable zone with much finer yolk-granules appears around the pronuclei.

At the outset of segmentation the germinal disc diminishes in size and a superficial peri-nuclear zone appears, with finer less stainable yolk-granules, distinct from the coarser grains of the peripheral zone. It occurs also around the first segmentation spindle and the nuclei of the first blastomeres. The white yolk of the nucleus of Pander does not take part itself in segmentation but gradually diminishes and disappears, profiting the formative yolk. A sub-germinal cavity, which may appear as early as the growth stage and is always apparent at the outset of segmentation, underlies the germinal disc. Polyspermy occurs and gives rise to accessory sperm nuclei, around which the yolk undergoes a process of superficial and incomplete cleavage.

3. Material and Technique.

The material employed in the course of this work was the ovaries of the domestic fowl (*Gallus bankiva*). The chief method used was Da Fano's cobalt silver nitrate technique. It was found that the best results could be obtained with this method after fixation for 10 or 12 hours, a quick wash in aq. dist., impregnation in the silver nitrate solution for 48 hours, half an hour's wash in several changes of aq. dist., and reduction for 8–12 hours. The following material was prepared successfully by this method:—

Ovaries of two 4 days old chicks.
Ovary of one 6 weeks old chick.
Ovary of one 3 weeks old chick.
Ovary of one 11 weeks old chick.
Ovaries of two adult fowl.

Serial sections were made of some of this material. Much of the material was mounted unstained and untoned, in which condition it was found to be most suitable for general study. Some of each lot of material was toned with gold chloride solution and subsequently fixed with " hypo " solution. It was then mounted, either stained or unstained. The most useful staining after this method was found to be safranin and light green, but MANN'S methyl blue eosin, and hæmatoxylin and BIEBRICH scarlet, both gave good results. Some sections of each lot were stained also with iron-hæmatoxylin. The iron-alum has the effect of removing the silver from the untoned sections and a good iron-hæmatoxylin stain is produced. The removal of the silver is much accelerated by keeping the iron-alum solution warm. If the sections are toned and fixed before the treatment with iron-alum the reduced silver in the section is not removed at all, or only very slightly, when the sections are left in warm iron-alum solution for several hours. This is important as it allows the production of an excellent iron-hæmatoxylin staining without impairing or destroying the visibility of the argentophil portions of the Golgi apparatus.

Material from an adult was also prepared by the NASSONOV method and gave very beautiful results, the impregnation of the Golgi apparatus being finer and better than by the silver methods. It was fixed in CHAMPY'S fluid, but without the addition of the pyrogallic acid solution which NASSONOV recommends, for 24 hours and then washed in running water for 24 hours. It was then placed in 2 per cent. OsO_4 solution in the thermostat at 37° C. for seven days. After washing again in running water for 24 hours it was upgraded and imbedded. Some of the sections were subsequently counterstained with acid fuchsin and aurantia, after treatment with potassium permanganate solution, followed by oxalic acid solution, to restore the staining affinities of the tissues. The Golgi apparatus, as revealed by this method, appeared in every way similar to that observed in the silver preparations, but was clearer and the general fixation was much superior. It is remarkable that previous workers, with the exception of SUBBA RAU and LUDFORD (21), failed to impregnate the Golgi apparatus in ovarian or early embryonic material of the fowl by the osmium methods. I myself failed in many attempts with the KOPSCH, MANN-KOPSCH, and SJÖVALL methods, before employing that of NASSONOV.

Adult ovarian material was fixed also in formol-corrosive-bichromate solution and stained by the CHAMPY-KULL method. It proved very successful for the mitochondria and yolk.

Material from an adult ovary was fixed also in CHAMPY'S fluid and gave excellent results when followed by CHAMPY-KULL'S staining method, or by iron-hæmatoxylin and orange G. Some material from an adult ovary was centrifuged, at approximately 6,000 revolutions per minute, for half an hour in normal saline. The radius of revolution was $5\frac{1}{2}$ inches. It was then fixed in FLEMMING'S fluid without acetic acid. This material gave beautiful results when stained by the CHAMPY-KULL method. Material from two adult ovaries was fixed in BOUIN'S fluid, and gave good results when stained with iron-hæmatoxylin and orange G, or with MANN'S methyl blue eosin.

In all cases the ovary was excised and transferred to the fixative as quickly as possible after the fowl was killed. In killing the fowl the head was severed without the use of an anæsthetic. In no case did more than four or five minutes elapse between the death of the bird and the moment when the ovary was placed in the fixative. The possibility of post-mortem changes in the oocytes was thus reduced to a minimum. All the material was imbedded in paraffin or double imbedded in celloidin and paraffin.

4. Observations.

(1) *Foreword.*

This description of the oocytes is not intended to be a complete account of the oogenesis of the bird, but rather to furnish a detailed account of the Golgi apparatus alone, and, in this respect, to be complementary to that of Van Durme, Loyez, and the other authors who have dealt so fully with this subject. I am in agreement with Van Durme's account, wherever I have had occasion to compare it with my material, but as I did not pay special attention to the nuclear phenomena at any stage, nor to nuclear or cytoplasmic changes during the later stages of oogenesis, maturation, fertilization, and segmentation, with which she is chiefly concerned, I am not in a position to substantiate her work as a whole. My observations on the other cytoplasmic inclusions, on the follicular epithelium, and on the zona pellucida arose naturally out of those on the Golgi apparatus, and are in the nature of notes not in themselves constituting a complete account of the structures dealt with. With this in view, the somewhat full summary of that part of Van Durme's paper which concerns the cytoplasmic changes was included, so as to furnish a framework into which my observations of a short period of oogenesis could be fitted, and to lend continuity to the whole.

(2) *The Oocytes of the 4 Days Old Chick.*

The oocytes of the 4 days old chick are to be found in the cortical cords of the ovary. They are not yet surrounded by a definite follicle layer, although follicle cells are scattered among them. In size they vary from 10 to 20μ in diameter, the nucleus averaging about 6μ in diameter. The Golgi apparatus is in the excentric peri-nuclear position, so characteristic of young oocytes (Plate 15, figs. 4 and 5). It consists of a number of rods or granules aggregated on the surface of a more or less flattened sphere, the flattest side of which is applied to the nuclear membrane. This Golgi apparatus is surrounded by a cloud of mitochondria, forming a cap over that pole of the nucleus. This is evidently an early stage of the yolk-forming crescent of D'Hollander. These oocytes are similar, therefore, to the primordial germ-cells, described by Woodger, so far as their cytoplasmic inclusions are concerned. There can be no doubt, as he pointed out, that in them the Golgi apparatus surrounds the centrosphere, or so-called *yolk-body of Balbiani* of D'Hollander, which Berenberg-Gossler has shown to contain a diploid centrosome.

(3) *The Oocytes of the 3 Weeks Old Chick.*

All the oocytes in the ovary of the chick 3 weeks after hatching are surrounded by a single layer of follicle epithelial cells. The oocytes vary in size from about 34 to 70μ in diameter. The nucleus varies in size from 20 to 35μ in diameter, according to the size of the oocyte; its diameter being thus almost two-thirds that of the entire cell in the smaller oocytes, the proportion gradually falling, as the size of the oocyte increases, until it is about one-half in the largest. In the smallest oocytes the nucleus contains a number of filaments or loops of chromatin, which appear granular in structure, and a conspicuous nucleolus. This chromatinic material is loosely arranged and fills the nucleus, coming close up to the nuclear membrane. This stage appears to be transitory, and gives rise to one in which the chromatinic material shrinks away from the nuclear wall and is separated from it on all sides by the karyolymphatic space.

The karyolymph is coagulated in the form of fine, scarcely stainable fibrils in the fixed material (Plate 15, figs. 1, 2, 3 and 6). The chromatin is in the form of loops, which are more or less connected and form a sort of reticulum. The nucleolus, which is eosinophil, measures on an average about 3μ in diameter. Thus the oocytes may be considered to be at the same stage as those described by VAN DURME at the outset of the first phase of yolk-formation of the intra-follicular growth period.

The Golgi apparatus is in the form of a number of rods or granules, more or less fused together, surrounding the archoplasmic sphere and applied to one side of the nucleus (Plate 15, fig. 1). It is usually spherical or sub-spherical in shape, but in some cases it is more spread out and forms a cap over one pole of the nucleus, and is closely applied to the nuclear membrane. Then it appears in longitudinal section as a crescent surrounding as much as one hemisphere of the nucleus (Plate 15, fig. 2), but in transverse section through that end of the nucleus the apparatus appears as a ring completely surrounding it (Plate 15, fig. 3).

The mitochondria are found throughout the cytoplasm, but chiefly around the nucleus and at that end at which the Golgi apparatus is found (Plate 15, figs. 1 and 2). Thus, except for their increase in size, the oocytes do not differ much from those of the 4 day chick and from the primordial germ-cells, so far as the cytoplasmic inclusions are concerned.

It is obvious that the body which VAN DURME, in agreement with D'HOLLANDER, considers as the yolk-nucleus of Balbiani in these oocytes is the centrosphere (or archoplasm), which I have shown to be surrounded by the Golgi apparatus. It is worth noticing that neither at this nor any other period of development do the oocytes, which have a marked polarity on account of the excentric position of the Golgi apparatus, display any definite orientation in relation to each other or to the gonad as a whole.

(4) *The Oocytes of the 6 Weeks Old Chick.*

The ovary of the chick 6 weeks after hatching has undergone considerable development. The cortex has become much thickened and folded, and the ovary is considerably

larger than that of the 3 weeks old chick. The oocytes range in size from about 38μ to 380μ in diameter. The diameter of the nucleus is approximately two-thirds the diameter of the cell in the smallest oocytes, but the proportion falls to one-half approximately in the oocytes from 50 to 100μ in diameter, and is not more than one-third in the largest. Thus not only the proportions, but the actual measurements of the oocytes from 38 to 70μ correspond with the range of sizes found in the oocytes of the 3 weeks old chick.

In the smallest oocytes the structure of the germinal vesicle and the arrangement of the chromatinic loops and nucleoli is the same as in those of the same size in the 3 weeks old chick. As the cell, and with it the germinal vesicle, grows the structure of the latter alters considerably, the nucleolus growing and dividing into three or more nucleoli and the loops of chromatin becoming much more loosely arranged, so that the entire vesicle appears to contain much less chromatin. These stages are figured and described by Van Durme (25) (Plate 19, figs. 24–41), as constituting the first stage of yolk-formation. I am of opinion that the nuclear space, filled with karyolymph, is very possibly produced by the action of the fixative, causing a partial plasmolysis of the delicate germinal vesicle, and in consequence giving origin to a fluid-filled cavity between the chromatin and the dense surrounding cytoplasm.

In the smaller oocytes from 38 to 75μ in diameter the arrangement of the Golgi apparatus and mitochondria is almost identical with that in the oocytes of the 3 weeks old chick. The Golgi apparatus contains a centrosome with diploid centrioles (Plate 15, fig. 6), which can be seen if a Da Fano preparation is stained with iron-hæmatoxylin. My observations, therefore, support the work of D'Hollander and Berenberg-Gossler, for although the former writer did not recognize the Golgi apparatus, there can be no doubt of its position around the centrosome when his figures are compared with the latter author's and with mine. In a few of the cells the mitochondria appear to be more compactly aggregated, into a spherical or ovate cloud, than was observed in the oocytes of the 3 weeks old chick (Plate 15, fig. 7).

The larger oocytes, from about 75μ in diameter and upwards, show a striking dissimilarity in the behaviour of their cytoplasmic inclusions to that observed in the smaller oocytes, or in any of those of the 3 weeks old chick. This behaviour is unique also in that it is not observed in oocytes of the same size in the adult ovary. The mitochondria become scattered throughout the cytoplasm and do not form the dense cloud surrounding the mitochondrial yolk-body that is typical of the oocytes of this size in the ovary of the adult fowl. The most remarkable changes are, however, those exhibited by the Golgi apparatus. The apparatus breaks away from its position in close apposition to the nuclear membrane and divides into two, three or more parts. These become scattered through the cytoplasm, but usually lie near or against the periphery of the cell. The individual Golgi elements, which are short, much curved, and twisted rod, or rings, increase enormously in number. Each part of the apparatus, consisting of a cloud of them, may measure 60μ or more in diameter, and there may be

several such clouds in one oocyte of, say, 300μ in diameter (Plate 16, figs. 10 and 11; Plate 19, figs. 29 and 31). These Golgi areas may be spherical (Plate 16, fig. 11) or irregular in shape, and the cytoplasm surrounding them generally appears darker than the remainder. The argentophil zone of cytoplasm appears to me to indicate some metabolic activity on the part of the Golgi elements, and is probably the same as the argentophil zones I described in the neurones of Helix (3). In DA FANO preparations toned and stained with MANN'S methyl blue eosin this cloud or zone around the Golgi focus stains intensely blue, in striking contrast to the remainder of the cytoplasm, which, after this method, appears amphophil.

In many of the cells the Golgi elements are all situated in the periphery, and may form a thick layer applied to the cell-membrane at one side (Plate 16, fig. 9). Or the several parts of the apparatus may be applied at different places in the periphery of the oocyte (Plate 16, fig. 10; Plate 19, fig. 31). The appearances produced are very striking and in many places suggest that the Golgi bodies of the follicle cells are involved also, and are passing into the periphery of the oocyte, there to augment the cloud of Golgi elements already present (Plate 16, figs. 8 and 12).

The cytoplasm of many of the cells does not appear homogeneous, but more or less granular, and the nucleus is often slightly crenated, thus strongly suggesting that these cells are in the early stages of atresia.

(5) *The Oocytes of the 11 Weeks Old Chick.*

The ovary of the chick 11 weeks after hatching is slightly larger and more developed than that of the 6 weeks old chick. The oocytes vary in size from about 44 to 380μ in diameter, a range almost exactly the same as that found in the 6 weeks chick. The size and structure of the nuclei, and their proportions in relation to the sizes of the oocytes, correspond exactly with those of the 6 weeks chick, the description of the nuclei of the latter applies equally well to those of the 11 weeks chick oocytes and need not be repeated here.

In the oocytes up to approximately 100μ the Golgi apparatus is typical and in the peri-nuclear position (Plate 18, fig. 27). It is thus similar in structure and position to that found in the smaller oocytes of the 6 weeks chick on the one hand (Plate 15, fig. 7) and the adult fowl on the other (Plate 17, figs. 14, 15 and 16; Plate 19, fig. 30, and text-fig. 4). In some cases a few other Golgi elements may be seen between the nuclear and cell membranes, where these are in close proximity (Plate 18, fig. 27). These are the Golgi type 2 elements, which, for convenience, I will defer further mention of until describing the oocytes of the adult fowl. The mitochondria are aggregated into a cloud, spherical in shape, situated close to the nucleus on one side (Plate 18, fig. 27). This cloud, which is found in almost all the oocytes up to 100μ in diameter, corresponds with the similar structure found in the smallest oocytes of the adult fowl (Plate 17, figs. 14, 15 and 16; Plate 19, fig. 30, and text-fig. 4), to be described presently, and with

the much less marked spherical aggregation of mitochondria already described as occurring in some of the smaller oocytes of the 6 weeks chick (Plate 15, fig. 7). This structure will be considered further in a later section.

All the oocytes over 100μ in diameter exhibit marked signs of atresia. The Golgi apparatus and the mitochondrial cloud can generally be made out in the oocytes up to 130μ in diameter, but even in these they show signs of dissolution. In the larger oocytes over 130μ in diameter no traces of these cytoplasmic inclusions can be discerned; the clouds of Golgi elements, so conspicuous in the oocytes of the same size in the 6 weeks chick, being entirely absent. The cytoplasm of all the oocytes over 100μ in diameter is much vacuolated and the nuclei are crenated. There can be no doubt, therefore, that these larger oocytes are in process of atresia and that the cytoplasmic inclusions have undergone dissolution.

(6) *The Oocytes of the Adult Fowl.*

The oocytes of the adult fowl, during the laying period, vary in size from about 50μ to that of maturity. Thus the smaller oocytes correspond in magnitude to those found in the 3, 6 and 11 weeks old chicks. The nuclear size in proportion to that of the cell, in the smaller oocytes, also corresponds to the proportions in the oocytes of the chicks. In the present description I am chiefly concerned with those up to 2·5 mm. in diameter, which are all within Van Durme's first period and the early stages of her second period of yolk-formation. The nuclear structure of the smaller oocytes is the same as already described in the chick material; the larger oocytes up to 2·5 mm. in diameter exhibit the same structure, but with a progressive diminution in the amount of chromophil material. I have not devoted much time to the study of the structure of the nuclei of these oocytes, but so far as my observations have gone they are in entire agreement with Van Durme's work, to whose paper (25) reference should be made for a detailed description. I published a preliminary account of my observations on the Da Fano material described in this section and the conclusions I then drew from them in 'Nature' for April, 1924 (2). The Nassonov material confirms my views, which were based on that prepared by the silver method.

The Golgi Apparatus.—The Golgi apparatus of the small oocytes, from 50 to 100μ in diameter, is spherical, and placed excentrically against the nucleus in the angle between the mitochondrial yolk-body and the nuclear membrane (Plate 17, figs. 13 and 15) towards the vegetative pole of the egg. In other sections taken in a plane at right angles to that of fig. 15 along the line *x-x*, the Golgi apparatus of the oocyte appears as if situated between the nuclear membrane and the mitochondrial yolk-body (Plate 17, figs. 14 and 16; Plate 19, fig. 30, and text-fig. 4). In some cells this apparatus may measure as much as 32μ in diameter. The individual elements, heavily impregnated with silver or osmium, cover the surface of the spherical apparatus. Each element appears to be ring-shaped. The shape, structure and size, in proportion to that of the cell, of this apparatus is very constant and characteristic.

I stained some DA FANO preparations by the iron-hæmatoxylin method, having made drawings first of certain identifiable oocytes. It was possible to distinguish then with certainty the remains of the Golgi apparatus of the oocytes, described above, from which all traces of silver had been removed. It appeared then as a darkly staining minutely fibrous area of the cytoplasm, not easily distinguishable, without the help of the drawings, from the remains of the mitochondrial structures. In the centre of this area two dark granules can be discerned in most cases (Plate 18, fig. 25) and in some it can be seen that the diploid granules are surrounded by a clear vesicle. I have no hesitation in stating that I consider this to be the diploid centrosome and that the Golgi apparatus of the oocyte encloses it and the surrounding centrosphere or archoplasm. The Golgi apparatus of the oocyte of the adult and the contained centrosomal mechanism are in every way similar to those which I have described above in the oocytes of the chick at various ages, from which they undoubtedly originate. I propose to call this true Golgi apparatus of the oocyte the Golgi type 1 or G.T.1 for convenience.

It may be of interest to compare with this G.T.1 of the bird oocyte a somewhat similar structure found by Prof. GATENBY and myself in the ovarian oocyte of the frog (*Rana temporaria*), in some material we prepared by the cobalt silver nitrate method, which has not been described previously so far as I am aware. This structure (Plate 17, fig. 17) is situated at one side of the nucleus in the small oocyte, and consists of a sphere, on the surface of which are studded a number of small ring-shaped elements, well impregnated with the silver. This, therefore, bears a certain similarity to the G.T.1 of the bird's oocyte and may represent the Golgi apparatus and centrosphere.

Shortly after the oocyte of the fowl has attained a diameter of about 150μ, the G.T.1 undergoes a remarkable change, resulting in its complete dispersal throughout the cytoplasm in the form of fine granules which impregnate with the silver or osmium. This change is heralded by the breaking up of the individual elements of the G.T.1 *in situ* into granules (Plate 17, fig. 18). It is possible that this appearance is due to the fixative, but as I have found it in several cells, all of which were approximately the same size, which were surrounded by earlier stages in which the impregnation was perfectly normal, in both silver and osmium preparations, it would appear justifiable to assume that it is not an artefact. As the fragmentation of the G.T.1 elements proceeds the resulting granules begin to pass out into the cytoplasm in all directions, but chiefly over the surface of the mitochondrial yolk-body, to which they seem attracted, probably by some surface action. This process is continued until the entire G.T.1 is completely dispersed throughout the cytoplasm in granular form, in all oocytes larger than about 200μ in diameter.

Another set of elements, which impregnate with silver and osmium, but are quite separate and distinct from the G.T.1, can be seen in the small oocytes; even in the smallest in the majority of cases. These consist of twisted and branched rods which, in oocytes up to about 125μ in diameter, are chiefly at the animal pole of the cell,

being scattered in the peripheral cytoplasm in this region and often partly aggregated into a loose irregular mass close to the nuclear membrane (Plate 17, figs. 14, 15, and 16; Plate 19, fig. 30, and text-fig. 4), as I described above in the small oocytes of the 11 weeks old chick. The elements of this apparatus differ in shape and in position in the cell from those of the G.T.1, from which they are easily and constantly distinguishable. Therefore I will call them, for convenience, the Golgi type 2 or G.T.2, as, on account of their reactions, they are entitled to be classified obviously as Golgi bodies in the present state of our knowledge.

Many of the individual elements of the G.T.2, especially when very close to the periphery, show a remarkable resemblance to the entire apparatus seen within each of the follicle cells surrounding the oocyte. In some cases they seem to be half in the follicle cell and half in the oocyte. In some of the follicle cells the apparatus appears to be dividing into two. The appearance strongly suggests that the Golgi apparatus of the follicle cell, after enlarging somewhat, divides into two, one portion being extruded then from the follicle cell into the oocyte, where it forms part of the G.T.2. In slightly larger oocytes, about 140μ in diameter, similar G.T.2 elements can be seen scattered around the periphery of the cell, and are more numerous than previously. Again, their appearance is suggestive of a follicular origin. More striking still is the appearance presented by later stages up to 800μ in diameter. In them a single layer of G.T.2 elements, which have increased enormously in numbers, can be seen just within the cell membrane throughout the entire periphery of the cell. Each element has a complex structure consisting of a number of blackened rods or banana-shaped bodies on the surface of a less deeply impregnated spherical or ovate mass of protoplasm. They are sub-equal in size, and spaced in such a manner that one is opposite almost every follicle cell in an extraordinary regular manner (Plate 18, fig. 23; Plate 20, figs. 33, 35). Their structure is similar to that of the apparatus seen within each follicle cell.

In many cases one can observe what appears to be every stage in the enlargement of the Golgi apparatus in the follicle cells, its subsequent division into two and the passage of one-half through the cell membrane into the oocyte (Plate 18, fig. 21). This appearance persists in the oocytes long after the fragmentation of the G.T.1 is complete and after the mitochondrial yolk-body has disappeared. The process of intrusion of elements from the follicle ceases at the time when the one layered follicle becomes many layered and commences to secrete the zona striata, but the peripheral arrangement of the intruded elements persists for some time after and can be seen in oocytes of even 800μ in diameter. In oocytes above this size they, too, have disappeared, either fragmenting and producing the finely granular material, impregnated with silver or osmium, scattered throughout the cytoplasm, as did the G.T.1, or undergoing a more complete dissolution, resulting in their dispersion and loss of affinity for the metal. The fact that in some oocytes, after the zona striata has commenced to form, the peripheral elements can be seen to be shrunken, only slightly impregnated, and more or less flattened against the membrane, supports the latter conclusion. On the other hand, in the larger oocytes of, for instance,

$2\frac{1}{2}$ mm. in diameter, in which enough yolk has not yet been formed to make impregnation difficult, the amount of these granules in the cytoplasm is many times that in the oocytes just after the fragmentation of the G.T.1. If these are Golgi granules, as I believe, they must either have originated entirely from the granules resulting from the breaking up of the G.T.1 by a subsequent process of multiplication, or else from the breaking up of the G.T.2.

The appearances suggesting the intrusion of the G.T.2, into the normal oocytes of a certain size within the adult ovary, are very similar to those which I observed in the larger oocytes of the 6 weeks old chick. In the latter, however, the arrangement of the elements, which are much more numerous than in the same sized oocytes in the adult ovary, is variable and lacks the extraordinary regularity so characteristic of them.

It is a fairly frequent occurrence to find two small oocytes situated in such close proximity as to touch each other without even the intervention of the follicular epithelium. In such oocytes an arrangement of Golgi elements, somewhat different to that observed in others of the same size, is present. In each a group of elements is situated between the nucleus and area of contact with the other cell (Plate 17, fig. 19). I am unable to say whether such paired oocytes ever reach maturity, and cannot, therefore, express an opinion as to whether this arrangement of the Golgi elements is to be considered normal, or an abnormality produced by circumstances which will result in the destruction of one, or both, cells.

The Mitochondria and Mitochondrial Yolk-Body.—In the smallest oocytes of the adult fowl the mitochondria can be found scattered throughout the cytoplasm of the cell, but the majority are clustered together to form a dense cloud, usually spherical, close to the nucleus. This *mitochondrial cloud*, which I have already described in the oocytes of the 6 and 11 weeks old chicks, lies at the side of the excentric nucleus farthest from the periphery of the cell, at the vegetative pole. It is often in contact with the nuclear membrane (Plate 17, figs. 14, 15 and 16, text-fig. 4), and may extend from it to the periphery of the cell. It is of considerable size, often larger than the nucleus itself. From it mitochondria radiate out into the surrounding cytoplasm. The individual mitochondria are rod-shaped or spherical and small. They exhibit the characteristic staining reactions, which it is hardly necessary to enumerate here. After Da Fano's method they appear golden in colour in untoned sections, and stain well after toning. In material prepared by Nassonov's method they are well fixed, and stain well with acid fuchsin.

They increase in number with the growth of the cell, probably by fission. The mitochondrial cloud is at its maximum stage of development in oocytes of 100 to 140μ in diameter. At this time a more definite and homogeneous structure differentiates, in the middle of the mitochondrial cloud, which I propose to call *the mitochondrial yolk-body* (Plate 17, fig. 13; Plate 19, fig. 32). On account of its exact similarity in staining reactions, its place of origin in the centre of the mitochondrial cloud, and the fact that there are much fewer individual mitochondria apparent in the cell immediately after its

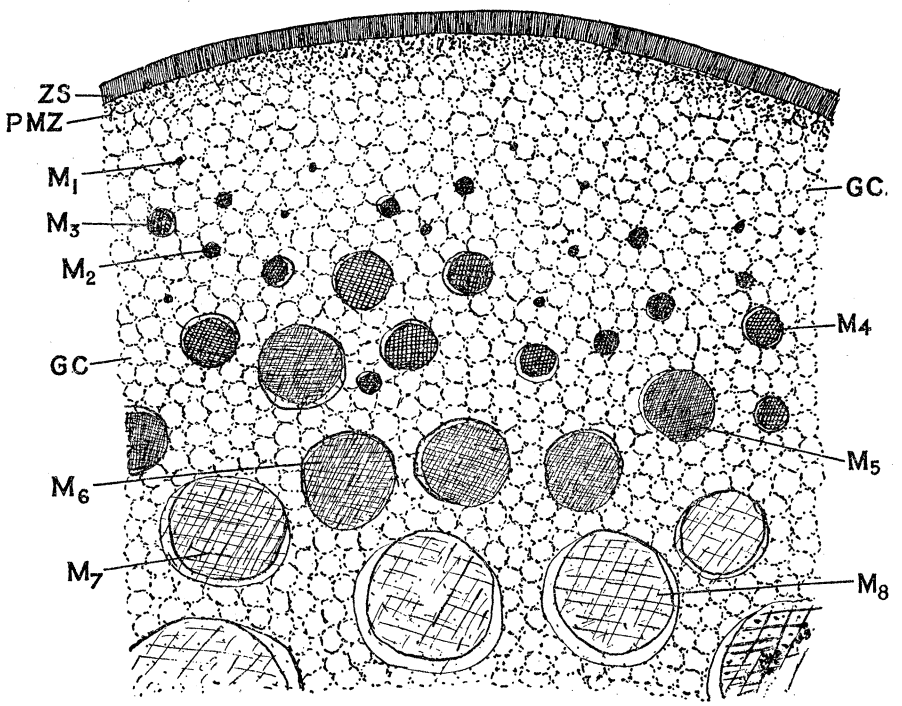
formation, I conclude that it is formed directly from the mitochondria by a process of fusion, more or less complete, of a large number. It is spherical in shape and varies in size from 50–70μ in diameter. It consists of a central easily stainable portion, very homogeneous in texture, and of a narrow outer zone, which is much less stainable. One, two or more small vacuoles, all approximately of the same size, can be seen in the central region. With iron-hæmatoxylin or other suitable stains one or more minute densely staining granules can often be seen in or around each vacuole. It would be easy to mistake these vacuoles, each with its associated granules, for centrosomes, were it not for their variation in number and the mitochondrial origin of the structure as a whole.

This mitochondrial yolk-body is usually in contact with the nuclear membrane on one side, and is surrounded on the other by a broad zone of mitochondria, representing that portion of the mitochondrial cloud which has not been used up in its production (Plate 17, fig. 13; Plate 19, fig. 32). This mitochondrial zone is often considerably thicker at one place than over the remainder of the surface of the mitochondrial yolk-body (Plate 18, fig. 28). The G.T.1 is situated in the angle between the nucleus and the yolk-body, and passes out over the surface of the latter when fragmenting, as already described (Plate 17, fig. 18). The mitochondrial yolk-body, although so definite and conspicuous in the oocytes at this stage, is transitory. In oocytes of about 220μ in diameter it begins to disappear. First, the surrounding zone of mitochondria becomes dispersed throughout the cytoplasm. Then the yolk-body itself commences to break up, often moving out, as it does so, from the nucleus. It becomes less definite and less stainable and finally, by the time the oocyte has attained a diameter of about 260μ, disappears altogether, breaking up into its constituent parts, the individual mitochondria of which it is formed probably resuming their identity and becoming dispersed, with the others, throughout the cytoplasm.

Fat.—In the small oocytes, in sections of Da Fano material, a zone of small vacuoles can be seen around the nucleus in the vicinity of the Golgi elements. In larger oocytes another zone of these vacuoles is apparent in the periphery. In osmium preparations these vacuoles appear as blackened spheres of fat. They undoubtedly correspond to the "boules graisseuses" forming the "capuchon nucléaire graisseuse" and the "couche corticale granulo-graisseuse" of Van Durme. After careful examination I have come to the conclusion that they are not formed directly from the Golgi elements or mitochondria. They occur, however, in the region occupied by the Golgi apparatus and at the stage when it is present, and are, I believe, formed in the ground cytoplasm, possibly under the influence of the Golgi elements of both types.

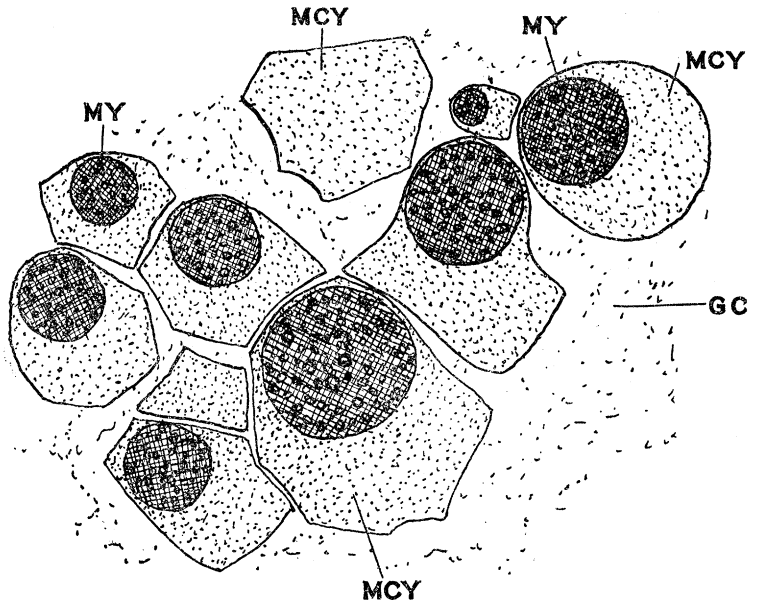
Yolk Spheres.—In oocytes 1 to 3 mm. in diameter, yolk is being formed rapidly. The central regions of the cytoplasm are full of yolk, which appears as vacuoles or spheres according to the methods of fixation employed. These can be observed in process of formation in the peripheral cytoplasm. In material fixed in Champy's fluid or in formol corrosive bichromate and stained by the Champy-Kull method, mitochondria can be seen in this region as fine scattered granules, staining deeply with the acid fuchsin—a

very characteristic reaction. They appear to swell up and transform directly into the yolk-spheres, every stage in the process being observable in this peripheral zone of cytoplasm (Plate 20, fig. 36, and text-fig. 1).



TEXT-FIG. 1.—Portion of the periphery of a 3 mm. oocyte. Fixed in FLEMMING'S fluid without acetic acid and stained with iron-hæmatoxylin. Magnification, 560 diameters approximately. Somewhat diagrammatic. *Z.S.*, zona striata. *P.M.Z.*, peripheral mitochondrial zone. *G.C.*, ground cytoplasm. M_1 *to* M_8, successive stages in the transformation of mitochondria into M-yolk spheres.

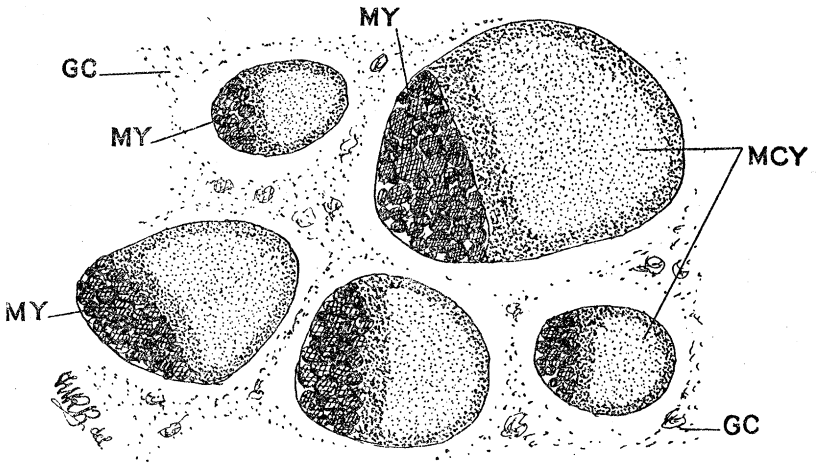
As the mitochondria swell a clear zone is formed around each, so that it appears as a spherical body in the centre of a vacuole. This vacuole enlarges as the mitochondrion increases in size. In the smaller vacuoles a light coagulum is found, which becomes increasingly dense in the larger ones. It stains with the toluidine blue. In oocytes 3 mm. in diameter and larger the greater part of the oocyte is occupied by these yolk-vacuoles, closely packed together and consequently polygonal in section, composed of a dense basophil coagulum surrounding the swollen mitochondrion (text-fig. 2, Plate 20, fig. 37). The mitochondria in oocytes 3 to 8 mm. in diameter attain a size of as much as 20μ in diameter and appear complex in structure, each containing many globules (Plate 20, fig. 37). The coagulum, formed in the vacuole around each transforming mitochondrion, does not appear to be formed from it by a direct transformation, but both the mitochondrion and the ground cytoplasm may play a part in its production. These appearances are well shown in chrome-osmium material that has been stained in iron-hæmatoxylin. In accordance with a scheme previously suggested, I propose to call the swollen mitochondria *mitochondrial-yolk*, or M-yolk, as a convenient distinction from the other cytoplasmic inclusions of the oocyte. The coagulum forming



TEXT-FIG. 2.—Portion of the cytoplasm of an oocyte fixed in formol corrosive bichromate and stained by CHAMPY-KULL'S method. Magnification, 1,000 diameters approximately. Somewhat diagrammatic. *M.C.Y.*, M-C-yolk formed in vacuole around M-yolk sphere. *M.Y.*, M-yolk sphere.

the zone around them I will call *mitochondrial-ground-cytoplasmic-yolk*, or M-C-yolk. Finally, I will call the fat, which is produced in the ground-cytoplasm, *ground-cytoplasmic-yolk* or C-yolk.

In the oocytes of about 3 mm. in diameter, which were centrifuged as described, fixed in F.W.A. and stained by the CHAMPY-KULL method, each M-yolk sphere went to the top of the surrounding zone of M-C-yolk but did not break out of the vacuole (text-fig. 3). It is clear, therefore, that the M-yolk which is probably fatty in nature, is lighter than the M-C-yolk formed in the vacuole around it.



TEXT-FIG. 3.—M-yolk spheres with the surrounding M-C-yolk vacuoles from the middle of a 3 mm. oocyte which was centrifuged for ½ hour at approximately 6,000 revolutions per minute (radius of revolution, 5½ inches), fixed in FLEMMING'S fluid without acetic acid and stained by CHAMPY-KULL'S method. Magnification, 560 diameters approximately. Somewhat diagrammatic. *G.C.*, ground cytoplasm. *M.C.Y.*, M-C-yolk. *M.Y.*, M-yolk sphere.

(7) *The Egg-Membranes.*

The young oocyte of the fowl is enclosed in a very thin, poorly staining membrane, which is the true cell-membrane. In the chick, at about the fourth day after hatching, the oocyte becomes surrounded by epithelial elements, which constitute the primordium of the follicular epithelium. Outside the follicle is the theca, formed of a sheath of connective tissue elements.

The theca of the bird can be divided into two more or less distinct layers :—the theca externa or outer loose layer, containing many blood vessels, and the theca interna or inner more fibrous layer,* with few blood vessels (Plate 18, fig. 22). The chief elements of the theca, externa and interna, are typical fibrous connective tissue cells, with elongated nuclei and Golgi bodies. Amongst these are to be found other elements, including the remarkable glandular looking cells, the so-called *luteal cells*. At first the cells of the follicular epithelium are few in number and flattened in shape. They form a scarcely continuous sheath over the surface of the oocyte. They rapidly increase in number, become more or less cubical in shape, and form a single continuous epithelial layer over the entire surface of the oocyte (Plate 18, fig. 21). The surface of contact of the follicular epithelium, or membrana granulosa, with the theca interna is sharply defined at all stages, and presents an appearance suggestive of the existence of a thin basement membrane (Plate 18, fig. 26). The follicle cells at rest contain a large reticulate nucleus, with one, two or three nucleoli (Plate 18, fig. 20). The cytoplasm is smooth and homogeneous in character and does not stain deeply. The mitochondria are granular and scattered throughout the cytoplasm (Plate 18, fig. 20).

The Golgi apparatus is in the excentric peri-nuclear position, situated at the side of the nucleus or between it and the surface of the oocyte (Plate 18, fig. 20). It is spherical or ovate and is usually slightly smaller than the nucleus. It consists of an argentophil reticulum covering over the surface of the less stainable archoplasm. In some cells the apparatus is considerably larger than others and appears to be in the act of constricting into two. When this is the case the apparatus is found to be close to the membrane separating the cell from the oocyte, and in many cases one half appears to be in process of extrusion into the oocyte. The G.T.2 elements, in every way similar to those contained in the follicle cells, can be observed in the peripheral cytoplasm at this stage. This secretion of the Golgi apparatus from the follicle cell into the oocyte appears to cease when the latter attains a diameter of about 650μ. At this stage changes are observable in the follicular epithelium.

In material fixed in CHAMPY'S fluid and stained with iron-hæmatoxylin it can be seen that some of the cells stain more deeply than others (Plate 18, figs. 23 and 26). Both the cytoplasm and nuclei of these chromophil cells show great affinity for the stain, the whole cell going quite black and standing out in marked contrast to the other lightly stained cells of the follicle (Plate 20, fig. 34). Intermediate stages, exhibiting every

* The theca interna of the bird is quite unlike that of the mammal, in which the cells are glandular.

grade of chromophility, are present. At the same time, as these chromophil cells are differentiating, a rapid multiplication of follicle cells is taking place and transforming the single-layered into a many-layered follicle. This results from the mitotic division of the follicle cells themselves. Mitoses are plentiful and the spindles are oriented in all directions and are not related to a plane tangential to the surface of the oocyte (Plate 18, fig. 26). At this stage the chromophil cells can be seen to extend in most cases from the basement membrane next the theca to the membrane next the oocyte ; the ends of the cells being more or less flattened over these surfaces (Plate 20, fig. 34), while the other follicle cells, which are arranged in two or more layers, are situated between them. Four or five layers may be formed in the follicle by the time the oocyte has attained a diameter of about 3 mm. By this time the chromophil cells have lost almost all signs of their cellular nature, and only occasionally can the remains of a nucleus be discerned in them. They appear to have become completely broken down and to have formed an intercellular substance of colloidal nature. This intercellular substance retains the staining affinities of the altered chromophil cells of the follicle, going black with iron-hæmatoxylin and red with acid-fuchsin.

Shortly after the intrusion of the G.T.2 has ceased, and at the time when the follicle is becoming many layered, a new membrane is formed around the egg. This membrane appears as a thin radially striated band around the periphery of the oocyte, which gradually thickens, until, in the oocyte of about 3 mm. in diameter, it is as much as 7μ in thickness. The appearance, particularly in semi-tangential sections, strongly supports the view that the zona is formed on the outside of the true cell membrane of the oocyte from an intercellular cement, possibly identical with that formed from the chromophil cells. It is finely, though clearly, striated in a radial direction but I am unable to decide whether these striæ are produced by prolongations of the follicle cells extending into the periphery of the oocyte, or are not. Sometimes the zona striata appears to be two-layered (Plate 18, fig. 22). Immediately within it is a highly granular zone of the peripheral cytoplasm containing numerous mitochondria, and, in silver and osmium preparations, the fine granules which, I believe, represent the Golgi bodies at this stage. I do not think, however, that this zone is sufficiently definite to be considered as another membrane.

During the subsequent growth of the egg-cell the various membranes become stretched and, in consequence, thinned. Finally, the follicle becomes one-layered once more and its individual cells flattened over the surface of the maturing egg, as in the young oocyte. The zona striata also becomes stretched and thin as the end of the growth stage is attained.

5. Discussion.

(1) *The Growth of the Oocytes.*

The measurements of the oocytes, at the various stages given in the text, seem to provide evidence for some deductions of interest in connection with the growth and

survival of the oocytes in the ovary of the bird. The following are the approximate diameters of the oocytes, in the ovary at the various stages studied, presented in tabular form.

TABLE I.

Ovarian oocytes of	4 days chick	.. .. ..	10–20μ in diameter.
,, ,,	3 weeks ,,	.. .. ..	34–70μ ,,
,, ,,	6 ,, ,,	.. .. ..	38–380μ ,,
,, ,,	11 ,, ,,	.. .. ..	44–380μ ,,
,, ,,	adult fowl ..	.. .. ..	50–<μ ,,

It will be noticed that even the smallest oocytes of the 3 weeks chick are considerably larger than the largest of the 4 days chick. I consider that this period of growth of all the oocytes intervening between these stages is the extra-follicular growth period of D'HOLLANDER. All the oocytes present in the ovary at this time enter upon this phase of development. It will be seen also that in the older ovaries the smallest oocytes found do not vary much in size, although a slight but definite increase is to be observed in the minimum size in successive stages. In fact the smallest oocytes of the laying hen are only one-half as large again in diameter as the smallest in the 3 weeks chick. It is obvious, therefore, that many of the oocytes remain throughout the development of the fowl, and even for some time after sexual maturity, at almost the same size as they had attained in the ovary of the 3 weeks old chick at the close of the extra-follicular growth period. Others, however, appear to grow more rapidly, as is shown by the maximum measurements for the 3, 6, and 11 weeks chicks. The difference between the maximum size of the oocytes of the 3 and 6 weeks chicks is enormous, being more than 500 per cent.; however, the maximum size of the oocytes of the 6 and 11 weeks chicks is the same. From this it is clear that many of the oocytes enter upon a period of rapid growth between the third and sixth week after hatching. The cytological evidence shows that, in the 6 weeks chick, all the oocytes over 75μ in diameter exhibit marked abnormalities and signs of atresia. In the 11 weeks chick all the oocytes over 100μ exhibit a more advanced stage of atresia. I conclude, therefore, that all those oocytes, which enter upon the period of rapid growth between the third and sixth week, are abnormal and fated to become atretic without ever reaching maturity. They have, in fact, entered precociously upon the growth stage (the intra-follicular growth stage of D'HOLLANDER) and by the sixth week all are showing signs of atresia. As the atretic condition is much more advanced in all the oocytes over 100μ in diameter in the 11 weeks chick, we can assume that no more oocytes enter upon a period of precocious growth between the sixth and eleventh week after hatching. Further, although the smallest precocious oocytes of the 6 weeks chick grow a little during this period, as is shown by the increase of $33\frac{1}{3}$ per cent. in the minimum size, the larger ones do not grow at all, as the maximum size remains constant. If Table I is corrected in view

of these results so as to show only the measurements of oocytes, which we have no evidence for considering to be abnormal, the following results :—

TABLE II.

Normal ovarian oocytes of	4 days chick	..	..	10–20μ in diameter.
,, ,, ,,	3 weeks ,,	..	..	34–70μ ,, ,,
,, ,, ,,	6 ,, ,,	..	..	38–75μ ,, ,,
,, ,, ,,	11 ,, ,,	..	..	44–100μ ,, ,,
,, ,, ,,	adult fowl	..	..	50–<μ ,, ,,

All the oocytes in the ovary have completed the extra-follicular growth period before the third week after hatching. They have attained then a diameter of 34–70μ and are surrounded by a follicle and theca. The majority of the oocytes remain at approximately the same size, exhibiting a slight but definite growth, until the ovary approaches sexual maturity. Then they enter in succession upon the intra-follicular growth period, which culminates in maturation, though at all stages occasional follicles may become atretic. On account of the absence of all the larger sizes of follicles from the ovaries of birds during non-laying periods, it seems probable that once the intra-follicular growth stage is entered upon growth is rapid and continuous until the egg is mature, and that no resting stage, such as that between the extra-follicular and intra-follicular growth periods, can intervene. However, at one period, between the sixth and eleventh week, a considerable number of precocious oocytes enter upon a second period of rapid growth, the intra-follicular growth period. All these are abnormal and become atretic; they exhibit an extraordinary behaviour of the cytoplasmic inclusions, especially a great development and activity of the Golgi elements.

It is a remarkable and significant fact that, at one period of the ontogeny of the individual, a number of oocytes enter upon this precocious growth period, which inevitably results in their destruction. It is possible that, although they are never functional in the fowl, they may be of phylogenetic significance and represent an early development of germ-cells, which, in some more or less remote ancestor, were of functional importance.

If the primordial germ-cells fail to reach maturity in the fowl, as FIRKET believes, these precocious oocytes may be the last survivors of them, making a final abortive attempt to form definite ova. It must be admitted that the primordial germ-cells, at some time in the history of the race, were of functional importance and gave rise to definitive germ-cells, even if they are now functionless. The precocious oocytes of the 6 weeks chick would, on this view, be vestigial structures, representing the oogenesis of some ancestral form, which have been functionally replaced in the course of evolution by the neo-formation of the definitive oocytes.

From the fact that all the oocytes, in the ovaries of fowl from 3 weeks old and

upwards which I have examined, have completed the extra-follicular growth period, I can say that I have not observed any neo-formation of germ-cells, in these stages, in any of my material. It must be remembered, however, that in cases of sex-reversal, a neo-formation apparently occurs and has been described by GATENBY (10), but this may constitute a later differentiation of primordial elements, already present in the region of the gonad.

(2) *The Golgi Apparatus.*

So far as I am aware I have described for the first time, in my letter to ' Nature ' (2) and in the present paper, the Golgi apparatus of the definitive oocytes of the bird. VON BERENBERG-GOSSLER (1) and WOODGER (28) were the first to describe it in the primordial germ-cells. The latter worker has allowed me, with great kindness, to examine some of his material of embryonic gonads of the chick. In it the arrangement of the cytoplasmic inclusions, in the primordial germ-cells, closely resembles that which I have described in the oocytes of the 4 days old chick. The Golgi apparatus is similar in position, structure and proportions and has been shown by BERENBERG-GOSSLER to contain a diploid centrosome. The cytological evidence does not, therefore, support FIRKET'S view that the primordial germ-cells do not form definitive oocytes. The similarity in structure and position of the Golgi apparatus of the oocytes of chicks, of four days and older, and the G.T.1 of the adult oocytes is so apparent that I do not think there can be any doubt that they are the same, especially as I have demonstrated in it a diploid centrosome, and that the G.T.1 of the oocytes of the adult ovary is the true Golgi apparatus of the oocyte.

I consider, therefore, that the true Golgi apparatus of the oocyte of the fowl (G.T.1) surrounds the centrosphere, which contains a diploid centrosome, and is situated in a juxta-nuclear position until it breaks up and disperses in the cytoplasm. It increases in size and in the number of its component elements as the oocyte grows and may attain a diameter of 32μ ; the largest size, so far as I know, ever recorded for the Golgi apparatus, in the peri-nuclear condensed state, in any cell, it being impossible to do more than roughly estimate its size when in the dispersed condition. I believe that the structure D'HOLLANDER describes and calls the yolk-body of Balbiani, and which he shows to be the centrosphere, in the oogonia, represents the G.T.1 from which the argento-osmiophil elements have been removed by the action of the fixatives he employed.

The similarity of the G.T.1 of the bird oocyte with the structure I have referred to in the oocyte of **Rana**, and which I think is the Golgi apparatus and centrosphere, is remarkable. It is also, I think, the first time that two types of Golgi structures have been described within the one cell, and that the passage of Golgi elements from one cell to another has been shown. My reasons for assuming the G.T.2 to be distinct from the G.T.1 and to have passed into the oocyte from the follicle cells, are as follows :—

1. The elements of the G.T.2 constantly differ in shape from the elements of the G.T.1 *in the same cell.*

2. The elements of the G.T.2 constantly differ in position from the elements of the G.T.1, the former being peripheral, the latter juxta-nuclear, until they fragment and spread out. In some stages an element of the G.T.2 can be observed opposite almost every follicle cell.

3. The elements of the G.T.2 can be observed after the G.T.1 has fragmented and is only represented by fine scattered granules.

4. The elements of the G.T.2 closely resemble in size and structure the Golgi apparatus of each follicle cell.

5. The elements of the G.T.2 can be observed, half in the follicle cell and half in the oocyte, apparently in process of passage through the egg-membrane. I do not wish to lay particular stress on this reason, as it may be objected that the action of the fixative produces this appearance, although it is only observable in cells of a certain stage and is not seen in neighbouring cells of an earlier or later stage.

6. The Golgi apparatus of the follicle cells has been shown to enlarge and divide into two, apparently prior to the passage of one portion into the oocyte.

7. The G.T.2 is not apparent in oocytes before the follicle is formed around them.

8. The great increase in amount of the G.T.2 during growth of the egg has to be accounted for.

9. After the formation of the zona striata the G.T.2 does not increase in amount, and soon becomes dispersed, simultaneously with which marked histological changes in the follicle occur.

These reasons appear to me to amount almost to a proof of my assumptions, regarding the nature and origin of the G.T.2. In a recent paper, on the " luteal cells " of the bird, Miss FELL (8) figures the periphery of an oocyte in a DA FANO preparation (fig. 5). In it black bodies can be seen, closely resembling those in one of my figures (Plate 18, fig. 23), but she does not refer to them in the text. I feel confident that these bodies represent the G.T.2.

The Golgi apparatus of the follicle cell probably contains the centrosome. The latter has been demonstrated by VAN DURME (26) in the position that I have shown is occupied by the former. It is possible, therefore, that the centrosome of the follicle cell divides, when the apparatus does, and that one of the two resulting passes into the oocyte with the Golgi elements. I am unable to state definitely whether this is the case or not, but I can say that, even after suitable staining, I have never observed any bodies, which appeared to be of this nature, in the region of the oocyte in which the G.T.2 is observable.

The fragmentation and dispersal of the G.T.1 throughout the cytoplasm, at a certain definite stage, is an interesting phenomenon and is comparable to the dispersal of the individual elements in the oocytes of many other groups, as, for instance, the mollusca described by GATENBY (11 and 12). In the larger oocytes of Rana, Golgi elements, in the form of short argentophil rods, can be observed scattered between the yolk-spheres throughout the cytoplasm. This dispersal of the Golgi material is probably

closely connected with yolk-formation and the accumulation of deutoplasmic material in the oocyte.

The association of the fragments of the G.T.1 with the mitochondrial yolk-body, as they begin to spread out, is probably a surface tension phenomenon and of no special significance. The number of Golgi granules in the cytoplasm, after the fragmentation of the G.T.1, increases considerably with the growth of the egg, which suggests that they are added to by the fragmentation of the elements of the G.T.2. This, however, may not be the case, as I have pointed out in the text; the increase may result from the multiplication of the granules themselves by a process of fission, and the G.T.2 may undergo more complete dissolution. The G.T.2 becomes dispersed throughout the cytoplasm in either event, and we must suppose that it, also, plays a part, directly or indirectly, in the formation of yolk, which is proceeding rapidly at this period.

The fate of the centrosphere and centrosome of the egg, which I have shown to be enclosed by the G.T.1, is a matter of considerable interest. The problem is: What becomes of them when the G.T.1 fragments and becomes dispersed? The ordinary methods of fixation, although they do not preserve the Golgi apparatus, usually show up the centrosphere. This body, however, disappears, as shown by VAN DURME, at the time when I have shown that the G.T.1 becomes dispersed. Small portions of it probably remain attached to the Golgi elements, as they spread out, as GATENBY and others have described in the oogenesis of many other forms, using the name "archoplasm" for the substance of the centrosphere. The real point at issue is the fate of the centrosome after the presumed dispersal of the "archoplasm" with the elements of the G.T.1. The amount of deutoplasm, already present at this stage in the oocytes, renders this question peculiarly difficult to answer by direct observation. I can only state that I have failed to distinguish the centrosome in the oocyte at any subsequent stage. It is, therefore, a matter of speculation as to whether the centrosome persists throughout the remainder of the growth stage and gives origin to those of the maturation spindles.

In *Limnæa stagnalis*, GATENBY (12) and HIRSCHLER (14) have shown that the Golgi elements of the oocyte persist through oogenesis and are divided during segmentation among the resulting blastomeres. Therefore, the embryological continuity of the Golgi elements in this form has been demonstrated. In the bird the accumulation of yolk has, so far, baffled all my attempts to demonstrate the persistence of the Golgi granules in the larger oocytes. It is highly probable, however, that this is due to the altered mechanical and physico-chemical conditions resulting from the enormous increase in quantity of the yolk in the cell.

WOODGER (28), in the primitive-streak stages of the chick, finds only scattered argentophil granules of different sizes in the cells, but in later stages the Golgi apparatus assumes the typical form of granules or short rods arranged around a sphere at one side of the nucleus. In reference to the Golgi apparatus, in earlier stages than those he described in his paper, he states *in literis* :—"I consider it to be diffuse, but I have felt

it incautious to assert this dogmatically in my paper. As a matter of fact, I have sections of unincubated blastoderms in which the cells are filled with *exceedingly fine grains,* just as you find in the oocytes after the formation of the zona pellucida. At this stage they are infinitely finer than those I have figured in the primitive-streak stage."

In view of WOODGER'S work on embryogeny and mine on oogenesis, it seems that the Golgi apparatus of the young oocytes persists during maturation and fertilization as fine scattered granules which are included in every blastomere at segmentation. They remain in this condition in the embryonic cells up to the later primitive-streak stages, and then gradually segregate to form the typical juxta-nuclear apparatus of each of the differentiating cells. It would appear from this evidence that the Golgi bodies of the oocyte of the fowl persist throughout development and form the apparatus of each embryonic cell, as GATENBY and HIRSCHLER have shown to be the case in the mollusc.

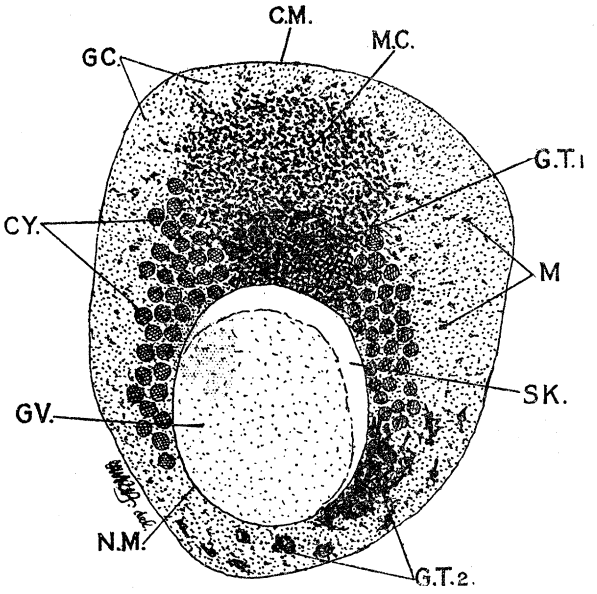
The re-formation of the Golgi apparatus of the embryonic cells from fragments which have persisted from the early stages of oogenesis is thus very different from its re-formation in the glandular epithelial cells of the oviduct of the adult fowl after secretion, which I have described elsewhere (5). In them the Golgi apparatus appears to arise *de novo* around the centrosome. Therefore the Golgi apparatus in the cell can reform in more than one way.

The extraordinary behaviour of the cytoplasmic inclusions, in the precocious oocytes of the chick, is difficult to interpret. In them the activity of the mitochondria is much less marked than in normal oocytes of the same size, but the activity of the Golgi apparatus is increased enormously and elements of the G.T.1 and G.T.2 are mixed up, apparently, in scattered masses in the cytoplasm, often close to and more or less spread over a portion of the periphery. The amount of Golgi elements present in some of these cells is very much greater than in normal oocytes. These scattered masses of Golgi bodies, on account of the increased chromophility of the cytoplasm in their neighbourhood, appear to be centres of considerable metabolic activity. Whether these appearances are purely abnormalities, or whether, if the precocious oocytes alone originate from the primordial germ-cells, they represent peculiarities of these cells which do not correspond to those of the normal cells, I cannot guess. VAN DURME records the occasional division of the yolk-body in the oocytes of the bird, as in those of mammals, into two, three, or four derived bodies. I have not observed this in any normal oocytes, and think that VAN DURME, lacking preparations showing the Golgi apparatus, has misinterpreted the condition in the precocious oocytes as a normal division, or has considered that the *centrosphere* and the *mitochondrial yolk-body* have originated by such a division.

(3) *The Mitochondrial Yolk-body.*

In the oocytes of the 4 days old chick the mitochondria are scattered around the nucleus, but chiefly in the vicinity of the Golgi apparatus (Plate 15, figs. 4 and 5), in a manner like that recorded in the primordial germ-cells. In the 3 weeks old chick the arrangement is similar (Plate 15, figs. 1, 2, and 3). In some of the larger oocytes of

the 6 weeks old chick the mitochondria are more or less grouped together into a cloud at one side of the nucleus (Plate 15, fig. 7). This *mitochondrial cloud* is usually spherical in shape, larger, and much denser in all the normal oocytes of the 11 weeks old chick (Plate 18, fig. 27) and in those of the adult fowl which are under 100 to 140μ in diameter (Plate 17, figs. 14, 15, and 16; Plate 19, fig. 30, and text-fig. 4). In some of these the mito-



TEXT-FIG. 4.—Diagram of young oocyte about 100μ in diameter from adult ovary. Magnification, 660 diameters approximately. *C.M.*, cell membrane. *C.Y.*, C-yolk spheres. *G.C.*, ground cytoplasm. *G.T.*1, Golgi apparatus, type 1. *G.T.*2, Golgi apparatus, type 2. *G.V.*, germinal vesicle. *M.*, mitochondria. *M.C.*, mitochondrial cloud. *N.M.*, nuclear membrane. *S.K.*, space possibly occupied by karyolymph in living cell.

chondrial cloud extends from the nuclear membrane to the periphery of the cell. It is composed of closely clustered mitochondria in the form of short rods and spheres which exhibit the characteristic reactions. These mitochondria are scattered also throughout the cytoplasm. Sometimes the mitochondrial cloud has a diameter almost half that of the entire cell. This cloud corresponds to the *couche vitellogène ou palléale* of VAN DURME. In the oocytes, about 100 to 140μ in diameter, the spherical *mitochondrial yolk-body* differentiates in the centre of the mitochondrial cloud (Plate 17, fig. 13; Plate 19, fig. 32), the remains of which form a zone around it, which is sometimes of very considerable thickness on one side (Plate 18, fig. 28). I have described this body and its reactions in the text and have stated my reasons for considering it to have originated from the mitochondria by a process of partial fusion of a large number in the middle of the mitochondrial cloud. The following are my reasons for believing that it is not an artefact, produced by the action of the reagents on the mitochondrial cloud causing an artificial fusion :—

1. It is demonstrated by all the methods I have used.

2. It is found only in oocytes of a certain size and always in them (with the exception of the precocious oocytes of the chick).

3. It may be present in one cell but absent from neighbouring ones at a slightly earlier or later stage, although, in them, the mitochondrial cloud is well developed.

VAN DURME describes this body under the name of *le corps vitelline de Balbiani* and identifies it with the centrosphere, described by D'HOLLANDER under the same name. She fails also to distinguish between it and the true centrosphere, which her methods should have demonstrated, although they would not have shown the surrounding Golgi elements. In sections I have prepared by BOUIN'S fixation, which she used chiefly, it is difficult to distinguish between these two bodies, which I have shown to be distinct by the silver and osmium methods. LOYEZ is more discerning and states that two different formations have been described under the title of yolk-body (*corps vitelline*). She says that it is the centrosome and the remains of the attraction sphere which constitute a sort of yolk-body in the very young oocytes of the birds and reptiles. In the later eggs of the reptiles there is present a vesicle from which are detached globules which spread out and appear to furnish substances used in the formation of the yolk. Mlle. LOYEZ thinks that the latter body (which appears to be the *mitochondrial yolk-body*) originates from the nucleus, but she does not express a definite opinion on this point. It is clear, therefore, that the term *yolk-body of Balbiani* has been used by many authors both for the *centrosphere* with or without the remains of the Golgi apparatus which surrounds it, and for the *mitochondrial yolk-body*, and I suggest that the latter terms be employed in future for these distinct structures and that the former term be rejected.

The fact that a mitochondrial yolk-body, the origin and nature of which has not been previously recognized, is present in the oocyte of the bird at a certain stage suggests that a similar structure may be present in the oocytes of other vertebrates also, and may have been described under the term of *yolk-body of Balbiani* and confused with the centrosphere. In the Ascidians JAN HIRSCHLER (15) has described yolk-nuclei (Dotterkerne) in the oocytes. They develop from small *mitochondrial bodies* (Mitochondrienkörper) which also produce the mitochondria (Mitochondrien). In the younger stages there are several of these yolk-nuclei. They develop stalk-like processes which become attached to the nuclear membrane and they gradually become hollow. In later stages there is only one or rarely two yolk-nuclei, which lose contact with the nucleus, break down into several pieces and finally disappear completely. They are thus transitory cytoplasmic structures, like the mitochondrial yolk-body of the bird. In the latter I have never observed more than one mitochondrial yolk-body in the oocyte, but it is applied, usually, like those of the Ascidians, on one side to the nuclear membrane. On account of their mitochondrial nature, their relation to the nuclear membrane, and their transitory presence during yolk-formation, it is possible that these structures in the Ascidian and Avian oocytes are homologous.

Finally, in oocytes of about 260μ in diameter the mitochondrial yolk-body resolves itself into a cloud of mitochondria, similar to that from which it was formed, which

become dispersed throughout the cytoplasm. We may therefore presume that the individual mitochondria, from which it was formed, probably retain their identity throughout its transitory existence, although they appear to be completely fused, and subsequently become distinguishable as such once more.

It is difficult to guess the function of so transitory, and yet so definite, a cell organ, but it is significant that it is present at the commencement of the stage of rapid growth and extensive yolk-formation. It may be concerned with the latter process. Possibly the yolk-nuclei of the Ascidians furnish a key to the solution of this problem by their relation to the nucleus. If so, we would suppose that the mitochondrial yolk-body of the Avian oocyte functions as an intermediate organ between the nucleus and the cytoplasm, conveying substances from the one to the other and possibly altering and elaborating them in the process.

(4) *The Mitochondria.*

The number of mitochondria in the oocyte increases rapidly during growth until the formation of the mitochondrial yolk-body. The number present in the cell is then reduced for the time being, owing to the metamorphosis of a large proportion of the whole into the latter body. This metamorphosis amounts to more than a localized concentration of the mitochondria, as they appear to become completely fused together to form the yolk-body. The fusion, although it appears complete, is probably only partial, as the yolk-body later breaks up into a cloud of separate mitochondria.

The increase in the individual mitochondria proceeds, however, and their numbers are greatly augmented by those derived from the mitochondrial yolk-body, when it breaks up. Then they become distributed throughout the cytoplasm, but are most plentiful in a peripheral zone, the *zone mitochondriale corticale* of VAN DURME. I have not observed any signs of intrusion of mitochondria into the oocyte from the follicle cells, and I think that their increase in number is due simply to fission within the cell.

Mlle. LOYEZ (17) states that corpuscles of nucleolar or chromatinic origin can be observed in the cytoplasm of the oocyte of the bird. I have observed in no case, in the oocytes of the fowl, anything that could be interpreted as nuclear extrusions, and would suggest that the bodies observed by Mlle. LOYEZ were really mitochondrial in nature, such as the pseudo-chromosomes of D'HOLLANDER (7).

VAN DURME (25) describes the spreading out of the mitochondria in the cytoplasm. Soon a narrow cortical mitochondrial zone, an endoplasmic zone with a large meshed mitochondrial network, and an exoplasmic zone with a smaller meshed network appear. In the neighbourhood of the first of these zones a second centre of yolk-formation appears, in which new mitochondrial structures are formed. These two centres of yolk-formation, one in the endoplasm near the nucleus, the other in the exoplasm near the cortical mitochondrial layer, persist throughout all the stages of yolk-formation. At first the former centre is very active, then the latter becomes so. Fat globules, representing

the first of the nutritive yolk, appear under the influence of these centres, and form a nuclear hood (*capuchon nucléaire graisseuse*) and a cortical fatty layer (*couche corticale graisseuse*).

Presently clear yolk vesicles appear in the neighbourhood of the cortical granulo-fatty zone (*zone granulo-graisseuse corticale*) and around the nucleus. These form a peripheral and a peri-nuclear vacuolated zone which gradually extend inwards and outwards respectively, and join at the animal pole forming a vacuolated nuclear hood. The first yolk-spheres appear in the middle of the exoplasm and then in the endoplasm, by a direct transformation of the larger mitochondria and by a special differentiation in the middle and at the expense of the contents of the clear yolk vesicles. The cortical vacuolar zone changes into a compact layer (*couche corticale compacte*), characterized by a dense yolk *gangue* studded with a multitude of small clear vacuoles enclosing yolk spheres. The rapid formation of more vacuoles and spheres speedily disperses these and transforms the dense cortical layer into the peripheral yolk layer (*couche vitelline périphérique*), with yolk granules of different sizes.

I have described and figured in the text the transformation of mitochondria into M-yolk (see also Plate 20, fig. 36), and the formation of the M-C-yolk in the vacuole surrounding each. Van Durme admits that the mitochondria transform directly into yolk and that some is formed from clear yolk vesicles also, but she does not distinguish sharply between these. I believe that the clear yolk vesicles and the M-C-yolk produced from them are formed around the transforming mitochondria, but are quite distinct. Otherwise my observations confirm hers.

The yolk of an egg (18) is composed of about 50 per cent. of water, 16 per cent. of protein, 23 per cent. of fat, and 11 per cent. of lipoids, with small quantities of salts, cholesterol, lutein, etc. The proteins are chiefly composed of vitellin, but small quantities of livetin are also present (20). The lipoids are chiefly lecithin. The lecithin and vitellin appear to be in a kind of loose combination, lecitho-vitellin. It is probable therefore that the M-C-yolk, formed in the vacuoles around the mitochondria, is composed during life of a colloidal solution of lecitho-vitellin in water and that the M-yolk consists of the fat with any of the lipoids, which are not in combination with the proteins, dissolved in it.

(5) *The Follicle and its Function.*

It is obvious that the oocyte of the vertebrate must derive the substances necessary for its growth from without, through the medium of the membrana granulosa or follicular epithelium, since it is entirely surrounded by this layer of cells. There has been, however, considerable doubt as to the exact method by which the nutritive substances pass through the follicle into the oocyte. In the present paper I claim to have shown that in the bird each follicle cell secretes a portion of the Golgi apparatus into the cell. I have not observed any similar process of secretion of the other cell elements into the egg by these cells. It is clear that these Golgi elements represent only a part of the

entire amount of substances which pass from the follicle to the egg, the remainder probably passing through in a fluid form. The extrusion of the Golgi apparatus from the follicle cells is comparable to the process which I have described in the ciliated epithelial cells of the oviducal gland of the fowl during secretion (5). In the latter the greater part of the Golgi apparatus is secreted from each cell into the lumen of the oviduct. A Golgi apparatus is then formed *de novo* around the centrosphere. In the follicle cells the apparatus divides into two, only one half being intruded into the oocyte. It is probable, as I pointed out in the paper referred to, that the Golgi apparatus is solid or semi-solid, because it retains its shape and structure after extrusion from the cell.

It is also of interest to compare these results with those obtained by Mlle. LOYEZ (17) in the Reptiles. She finds that the rôle of the large follicle cells is the furnishing of substances, for the formation of yolk, to the egg. The products, which they cast into the cytoplasm, pass out by way of canaliculiform prolongations, and may be fluid, semi-fluid or granular. She states that the contents of the nucleus and of the entire cell may pass into the yolk without having undergone previously any essential modification, or the cell may undergo first a sort of fatty degeneration and the resulting corpuscles, which become finer and finer, then pass as such into the yolk. I have not observed similar processes in the bird, but LOYEZ'S work furnishes an example of the secretion of substances into the oocyte by the follicle cells.

Previously to LOYEZ'S work, as far back as 1870, WALDEYER (27) admitted that granules pass from the follicle cells into the yolk in reptiles. The secretion of substances by the follicle cells into the egg in vertebrates is, therefore, by no means a new idea, but I think I am justified in saying that the passage of the Golgi apparatus from one cell to another and its persistence in the latter has not hitherto been described. Indeed, it is extremely rare for any protoplasmic body to pass from one cell into another and to live there ; the migration of the fusion nuclei in the Carpogonia of the Rhodophyceæ, and the survival of the head and middle piece of the fertilizing sperm in the cytoplasm of the egg are examples. Another remarkable case is furnished by the placenta of *Perameles* in which, as FLYNN has shown, trophoblast cells pass into, and fuse with, the syncytial layer formed from the uterine epithelium, whilst their nuclei persist in the latter layer in an active functional condition.

Until the cessation of the passage of the Golgi elements into the oocyte the follicle is composed of a single layer of cells, apparently all similar. At this stage, when the oocyte is about 650μ in diameter, the follicle cells can be seen, in chrome-osmium material, to be differentiating into two kinds, one of which remains like the earlier follicle cells, while the other stains deeply. Intermediate stages are plentiful, and I have no hesitation in saying that the latter originate from the former. At this time the follicle becomes many-layered, so far as the clear cells are concerned, but the dark cells can be seen to stretch across the whole breadth of the follicle in many places (Plate 18, fig. 26; Plate 20, fig. 34). HOLL (16) described and figured, in the bird, these two kinds of cells. He thought that those with denser protoplasm were supporting

elements (Stützzellen), and those with clear protoplasm were playing a part in the nutrition of the egg (Nährzellen). He said that the former were present already in the follicle while it was single-layered, but became more numerous with the growth of the egg. MERTENS (19) also claimed to have seen these two kinds of cells in *Pica caudata* (Ray). I think that HOLL is right, undoubtedly, in attributing a nutritive function to the clear cells.

Mlle. LOYEZ (17) denies the existence of these two kinds of cells and states that she has observed a similar appearance to that described by HOLL, which she considers an artefact produced by the shrinkage due to fixation. I do not think this is the case, as the two kinds are shown by some methods, not by others, and are present in the best fixed and least shrunk portions, as well as in those which are obviously distorted.

These chromophil cells, which originate from the clear cells of the follicle, appear to break down completely, to form an intercellular substance, in the follicle. It is possible that this intercellular substance is the same as the cement from which the terminal bars are produced, according to VAN DER STRICHT (24) and Miss THYNG (23), but I do not wish to express a final opinion on its origin or function at present. I intend to extend my researches on these questions and offer my views tentatively.

Mlle. LOYEZ showed that in the Reptiles the follicular epithelium is first a single layer of small cells. In the " Sauriens " (Lizards) and " Ophidiens " (Snakes) it becomes polymorph and contains :—(1) small cells which can divide mitotically ; (2) intermediate cells, arising from the differentiation of the small cells in the inner layer of the follicle ; (3) large pear-shaped cells resulting from the development of the intermediate elements. Possibly the two kinds of cells of the follicle of the bird are homologous to the large and small cells of the lizards and snakes.

The zona striata commences to form in the bird at the time that the follicle becomes many layered. It is interesting that GATENBY (13) has shown that, in Ornithorhynchus also, the zona pellucida commences to form at the time that the follicle changes from the single to the double-layered condition. Thus, in the bird, the intrusion of the G.T.2, the differentiation of the two kinds of cells, and the assumption of the many-layered condition of the follicle are immediately followed by the formation of the zona striata, and all four processes probably bear an intimate relation to each other.

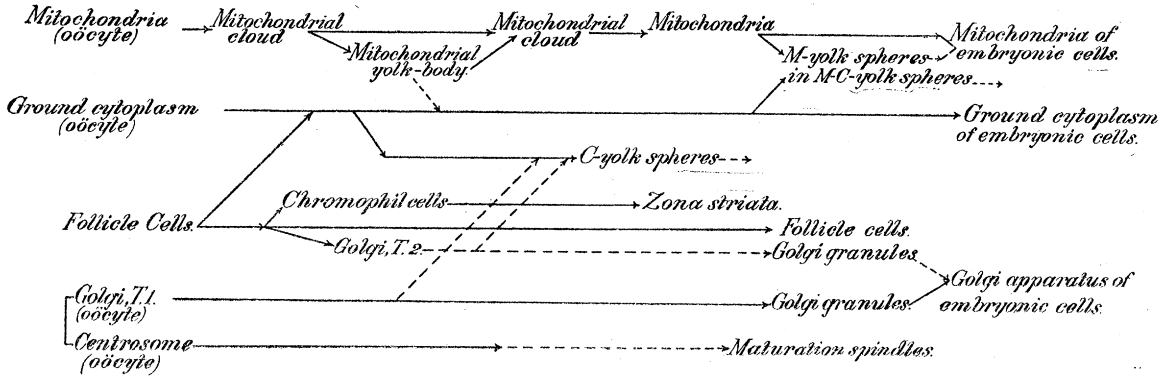
VAN DER STRICHT (24), with reference to mammals, Miss THYNG (23), in the case of turtles, and GATENBY (13), in that of Ornithorhynchus, are of opinion that the zona pellucida is formed by the follicle, probably by the intercellular substance and terminal bars. I think it probable that, in the bird also, the zona striata is formed from the intercellular substance or cement, which may result in part from the degeneration of the chromophil cells of the follicle. In the bird the zona striata is clearly radially striated, but I am unable to say whether it is traversed by canalicular prolongations of the follicle cells as has been described in the reptiles by LOYEZ (17) and THYNG (23) and in the mammals by VAN DER STRICHT (24), but I have not observed the passage of any bodies from the follicle to the egg through this membrane.

The dense layer of mitochondria and Golgi granules, in the peripheral zone of the oocyte immediately underneath the zona striata, probably represents the *zone mitochondriale corticale* of VAN DURME. Possibly this layer is concerned with the elaboration of fluid substances supplied to the oocyte by the follicle.

During the later stages of growth the follicle becomes single-layered again, the individual cells being more or less flattened over the surface of the egg. The zona striata also becomes stretched and thin.

(6) *Yolk-formation.*

In text-fig. 5 I have attempted to show graphically the relations of the various structures described in this paper. The C-yolk spheres are the first deutoplasmic



TEXT-FIG. 5.—Graphic representation of yolk-formation in the fowl during the intra-follicular growth stage of the oocyte. Broken lines represent possible relations.

globules to appear in the growing oocyte and are formed in the ground cytoplasm in the vicinity of the Golgi elements, and possibly under their influence. The M-yolk spheres, with the M-C-yolk formed around them, constitute the only other deutoplasmic bodies I have observed in the oocytes I have worked on, which, it will be remembered, were all under 8 mm. in diameter. I consider, for the reasons I have given, that all the M-yolk spheres are formed by a direct transformation of mitochondria. I do not know whether, when the yolk is used up in embryogeny, the M-yolk spheres disappear completely or transform back again into mitochondria. GATENBY (12) finds that the latter is the case in *Limnæa stagnalis*. He says :—" Careful examination of the mitochondria in the unsegmented egg and in the advanced differentiating organ or germ-layer seems to establish the fact that the mitochondria shrink gradually in size *pari passu* with the differentiating somatic or germ-cells during any stages I have examined." If this is the case also in the bird the M-yolk spheres would be entitled to be considered as living cell-bodies, and not as deutoplasmic globules. In any case there are probably a large number of mitochondria which persist unchanged throughout oogenesis and in the embryonic cells after segmentation. During oogenesis the amount of mitochondria and Golgi T.1 in the oocyte increases enormously, and a considerable

quantity of Golgi T.2 is intruded into the cell. Although these structures are living protoplasmic bodies, not deutoplasm, they certainly constitute part of the yolk of the egg, in the broader sense of the term.

In concluding this paper I feel that much work remains to be done before our knowledge of the oogenesis of the bird can be considered complete. It is a process extraordinarily complex and in many ways unique, resulting in the production of the largest single animal cell known.

6. Summary.

1. *The Golgi apparatus type* 1 of the oocyte is demonstrated for the first time. It is shown to surround the centrosphere.

2. An intrusion into the oocyte of *Golgi apparatus type* 2 from the follicle cells takes place.

3. The Golgi apparatus, type 1, and possibly type 2, breaks up into fine granules and becomes dispersed throughout the cell during oogenesis.

4. It is probable that these Golgi granules persist as such during maturation and fertilization, become included in the embryonic cells at segmentation, and produce the Golgi apparatus of each embryonic cell by a process of condensation.

5. The mitochondria increase in number in the oocyte and form the *mitochondrial cloud*. The transitory *mitochondrial yolk-body* differentiates in the middle of this cloud. Hitherto it has been confused with the centrosphere, with which it has no connection, and both structures have been described as the yolk-body of Balbiani.

6. The mitochondrial yolk-body breaks up again into a cloud of mitochondria, which become dispersed through the cytoplasm.

7. Some of the mitochondria transform directly into yolk-spheres (M-yolk). Another kind of yolk (M-C-yolk) is formed in vacuoles surrounding each M-yolk sphere.

8. Fat globules (C-yolk) are formed in the ground cytoplasm, possibly under the influence of the Golgi elements.

9. At the time that the intrusion of the G.T.2 ceases two kinds of cells differentiate in the follicle, which becomes many-layered, and the zona striata commences to form. One of these types of cells stains deeply. I think that these dark cells transform into an intercellular substance, possibly homologous to that described in the mammal by Van der Stricht as forming the terminal bars, which gives rise to the zona pellucida.

10. At a certain stage between the third and sixth week after hatching a number of oocytes in the ovary of the chick enter upon a period of precocious growth. These precocious oocytes exhibit remarkable abnormality in the behaviour of their cytoplasmic inclusions and finally become atretic. This may or may not represent the final degeneration of the primordial germ-cells.

Addendum.

Since this paper went to press, Prof. Gatenby has kindly afforded me the opportunity of consulting a copy of the manuscript of a paper by D. R. Bhattacharya on "Les Inclusions cytoplasmiques dans l'Oogénèse de certains Reptiles," to be published

shortly as a Thèse de Paris. He worked on *Testudo græca* and *Uromastix hardwicki* in both of which he finds that Golgi bodies pass from the follicle cells into the oocytes in a manner similar to that which I have described in the fowl. The Golgi bodies from the follicle cells pass through canalliculi in the zona radiata into the oocyte. BHATTACHARYA, having had access to a copy of the manuscript of this paper, apparently misunderstands my views on the nature of the mitochondrial yolk-body and the centrosphere. He says:—"D'après BRAMBELL ce corps vitellin mitochondrial est separé et distinct du noyau vitellin de Balbiani, qui est situé en relation avec la centrosphère," and again:—"Le travail de BRAMBELL chez la Poule essaie de démontrer que le noyau vitellin de Balbiani et le corps vitellin mitochondrial soit des formations distinctes et différentes. Le premier comprend la centrosphère et le corps de Golgi; le second ne contient ni corps de Golgi ni centrosphère mais des corps mitochondriaux ou chondriome."

I consider certainly that the mitochondrial yolk-body is a structure separate and distinct from the centrosphere, with the Golgi apparatus around it, but I do not apply to either of these structures the term 'yolk-nucleus of Balbiani.' I believe that this term has been applied in the past to both these structures in the oocyte of the fowl without distinction, and, I advocate that it be no longer used and that the terms 'mitochondrial yolk-body' and 'centrosphere' be used instead.

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8. Explanation of Plates.

Plates 15 to 18 were drawn with the help of a camera lucida. I am indebted for the microphotographs reproduced on Plates 19 and 20, which are not touched up in any way, Mr. F. J. Pittock.

Key to Lettering.

A.G., argentophil granules in oocyte of Rana.
C.M., cell membrane of oocyte.
C.S., centrosome.
C.S.P., centrosphere.
C.Y.V., vacuoles left after removal of C-yolk spheres.
C.Z., clear outer zone of mitochondrial yolk-body.
D.C., dark cell of follicle.
D.C.S., diploid centriole.
F., follicle.
G., Golgi elements.
G.C., ground cytoplasm.
G.E., Golgi elements of oocyte of Rana.
G.F., Golgi elements clustered in abnormal focus of activity.
G.Fo., Golgi apparatus of follicle cell.
G.Fo.$_1$, Golgi apparatus of follicle cell being intruded into the oocyte.
G.G., Golgi granules.
*G.T.*1, Golgi apparatus type 1 of oocyte.
*G.T.*1 *E.*, stray elements of the G.T.1.
*G.T.*2, Golgi apparatus, type 2.
G.T.C., Golgi apparatus of theca cells.
M., mitochondria of oocyte.
M_{1-8}, stages of transformation of mitochondria into M-yolk spheres.
M.C., mitochondrial cloud.
M.F., mitochondria of follicle cell.
M.Y.B., mitochondrial yolk-body.
M.Y., M-yolk sphere.
N., nucleus of oocyte.
N.D.C., nucleus of dark cell of follicle.
N.F., nucleus of follicle cell.
N.M., nuclear membrane of oocyte.
N.P.C., nucleus of pale cell of follicle.
P.C., pale cell of follicle.
P.C.$_1$, pale cell of follicle dividing.
P.M.$_2$ or *P.M.Z.*, peripheral mitochondrial zone.
S., centrosphere of oocyte of Rana.
S.K., space possibly occupied by Karyolymph in live cell.
T.E., theca externa.
T.I., theca interna.
V., vacuole in mitochondrial yolk-body.
Z.S., zona striata.

PLATE 15.

FIG. 1.—Oocyte from ovary of 3 weeks old chick. DA FANO preparation. × 2170.

FIG. 2.—Oocyte from ovary of 3 weeks old chick. Golgi apparatus (*G.T.*1) forming a cap over the nucleus. DA FANO preparation. × 2170.

FIG. 3.—Oocyte from ovary of 3 weeks chick. Golgi apparatus forming a ring around the nucleus owing to plane of section being at right angles to that in previous figure and along a line *x*—*y*. DA FANO preparation. × 2170.

FIG. 4.—Oocyte from ovary of 4 days chick. DA FANO preparation. × 2000.

FIG. 5.—Oocyte from ovary of 4 days chick. DA FANO preparation. × 4540.

FIG. 6.—Oocyte 75μ in diameter from ovary of 6 weeks chick, showing centrosome (*C.S.*), containing diploid centrioles, in centrosphere (*C.S.P.*). DA FANO preparation stained with iron-hæmatoxylin. × 1470.

FIG. 7.—Oocyte from ovary of 6 weeks chick, showing the Golgi apparatus (*G.T.*1) and first appearance of mitochondrial cloud (*M.C.*). DA FANO preparation. × 1470.

PLATE 16.

FIG. 8.—Oocyte from the ovary of 6 weeks chick showing abnormal behaviour of Golgi elements (*G.*) and intrusion from follicle cells. DA FANO preparations. × 675.

FIG. 9.—Oocyte from ovary of 6 weeks chick showing abnormal distribution of Golgi elements (*G.*) which are clustered at one side of the cell. DA FANO preparation. × 675.

FIG. 10.—Oocyte from ovary of 6 weeks chick showing abnormal arrangement of Golgi elements which form several foci (*G.F.*). DA FANO preparation. × 340.

FIG. 11.—Portion of similar oocyte to that shown in previous figure. Golgi focus (*G.F.*) drawn on large scale. DA FANO preparation. × 1470.

FIG. 12.—Portion of periphery of oocyte and follicle cells from ovary of 6 weeks chick showing apparent intrusion of Golgi of follicle (*G.Fo.*) into abnormal oocyte, and peripheral arrangement of the Golgi elements (*G.*) in the latter. DA FANO preparation. × 1470.

PLATE 17.

(All the figures are on the same scale. × 640.)

FIG. 13.—Oocyte from the ovary of an adult fowl, showing both types of Golgi apparatus (*G.T.*1 and *G.T.*2) the mitochondrial cloud (*M.C.*) and the mitochondrial yolk-body (*M.Y.B.*), with an outer clear zone (*C.Z.*), containing several vacuoles (*V.*). DA FANO preparation untoned and unstained. Same cell as Plate 19, fig. 32.

FIG. 14.—Oocyte from the ovary of an adult fowl, showing both types of Golgi apparatus (*G.T.*1 and *G.T.*2), the mitochondrial cloud (*M.C.*) at its maximum development before the differentiation of the mitochondrial yolk-body, and the vacuoles (*C.Y.V.*) from which the C-yolk spheres have been dissolved out by the reagents. DA FANO preparation.

FIG. 15.—Oocyte from ovary of an adult fowl, showing both types of Golgi apparatus (*G.T.*1 and *G.T.*2) and the mitochondrial cloud (*M.C.*). DA FANO preparation.

FIG. 16.—Oocyte from the ovary of an adult fowl showing the same structure as fig. 14. This is the same cell as Plate 19, fig. 30. DA FANO preparation.

FIG. 17.—Portion of small oocyte of *Rana temporaria* showing supposed centrosphere (*S.*) and Golgi elements (*G.E.*), and argentophil granules (*A.G.*) in the ground cytoplasm. DA FANO preparation.

FIG. 18.—Portion of oocyte from the ovary of an adult fowl, showing fragmentation of the Golgi apparatus type 1 (*G.T.*1), the association of the resulting Golgi granules (*G.G.*) with the mitochondrial yolk-body (*M.Y.B.*) as they become dispersed through the cytoplasm. Two vacuoles (*V.*) with associated granules are seen in the mitochondrial yolk-body. DA FANO preparation.

FIG. 19.—Two oocytes touching, from the ovary of an adult fowl, showing unusual arrangement of the Golgi apparatus (*G.*). DA FANO preparation.

PLATE 18.

FIG. 20.—Two cells from the follicle, in the single-layered stage, from the ovary of the adult fowl, showing the Golgi apparatus (*G.Fo.*) and mitochondria (*M.F.*). DA FANO preparation. × 2700.

FIG. 21.—Portion of periphery of oocyte and follicle, showing intrusion of the Golgi apparatus of the follicle cells (*G.Fo.* and $G.Fo._1$) into the oocyte to form the Golgi apparatus type 2 (*G.T.*2). DA FANO preparation × 1300.

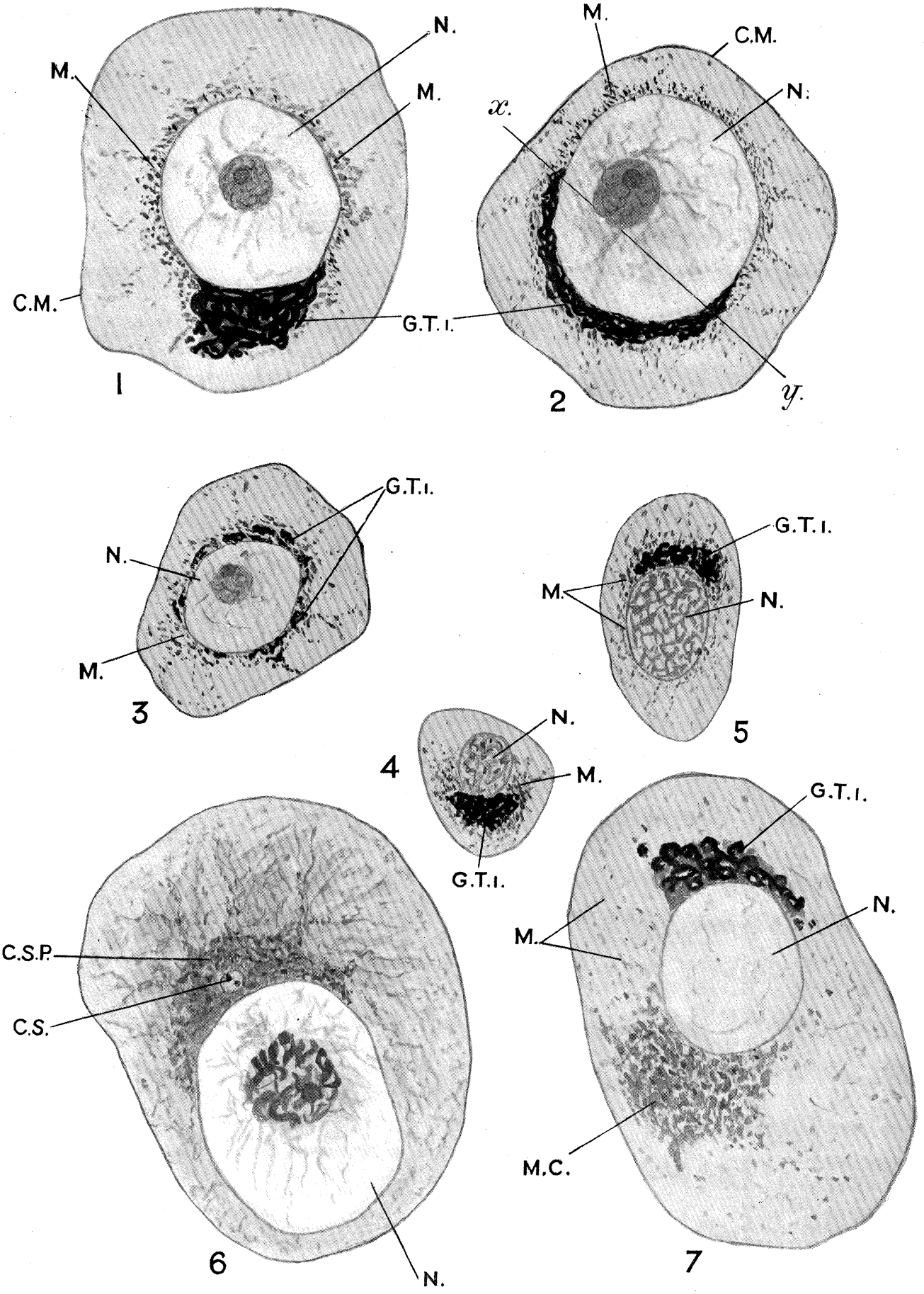
FIG. 22.—Portion of theca, follicle, and periphery of oocyte from the ovary of an adult fowl. Showing the follicle in the many-layered condition (*F.*), the zona striata (*Z.S.*), and the peripheral mitochondrial zone (*P.M.Z.*). DA FANO preparation. × 640.

FIG. 23.—Portion of follicle and periphery of oocyte from the ovary of an adult fowl at the stage where the intrusion of the Golgi bodies from the follicle cells has ceased. The Golgi bodies which have been intruded (*G.T.*2) are arranged opposite the follicle cells. The dark cells (*D.C.*) and the pale cells (*P.C.*) are differentiating in the follicle. DA FANO preparation toned and stained with safranin and light green. × 1350.

FIG. 24.—Portion of follicle and periphery of oocyte from the ovary of an adult fowl, showing the zona striata. DA FANO toned preparation. × 1350.

FIG. 25.—Portion of oocyte from the ovary of an adult fowl, showing the mitochondrial yolk-body (*M.Y.B.*), and the centrosphere (*C.S.P.*), containing a diploid centriole (*D.C.S.*), from which the Golgi apparatus has been removed. DA FANO preparation stained with iron-hæmatoxylin. × 640.

FIG. 26. Portion of follicle and periphery of oocyte from the ovary of an adult fowl, showing the zona striata (*Z.S.*). The follicle is assuming the many-layered


N.
M.
M.
C.M.
G.T.I.
1
M.
C.M.
N.
x.
y.
2
G.T.I.
N.
M.
3
G.T.I.
M.
N.
5
N.
M.
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6
G.T.I.
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M.C.
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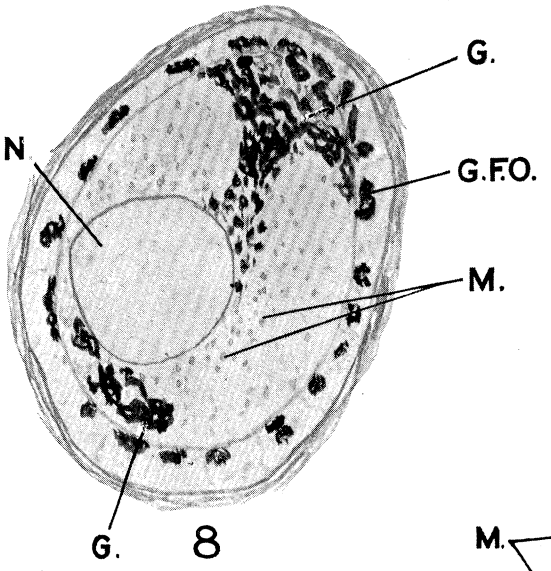
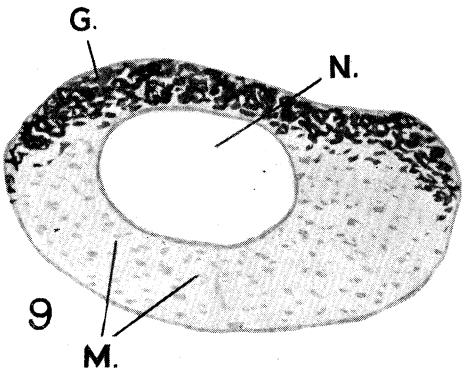
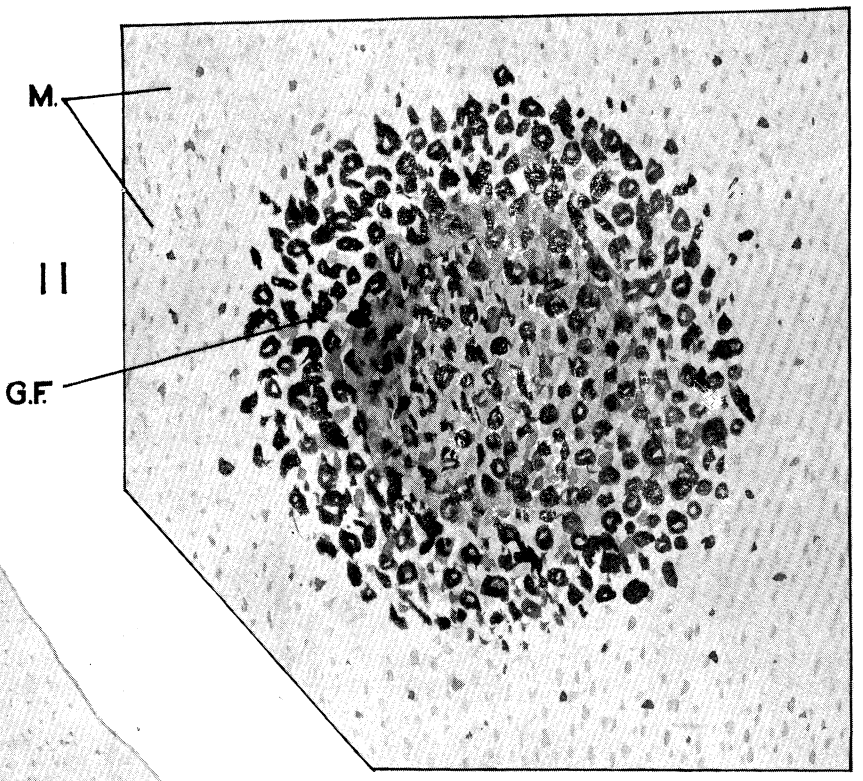

G.
N
G.F.O.
M.
G.
8


G.
N.
9
M.


M.
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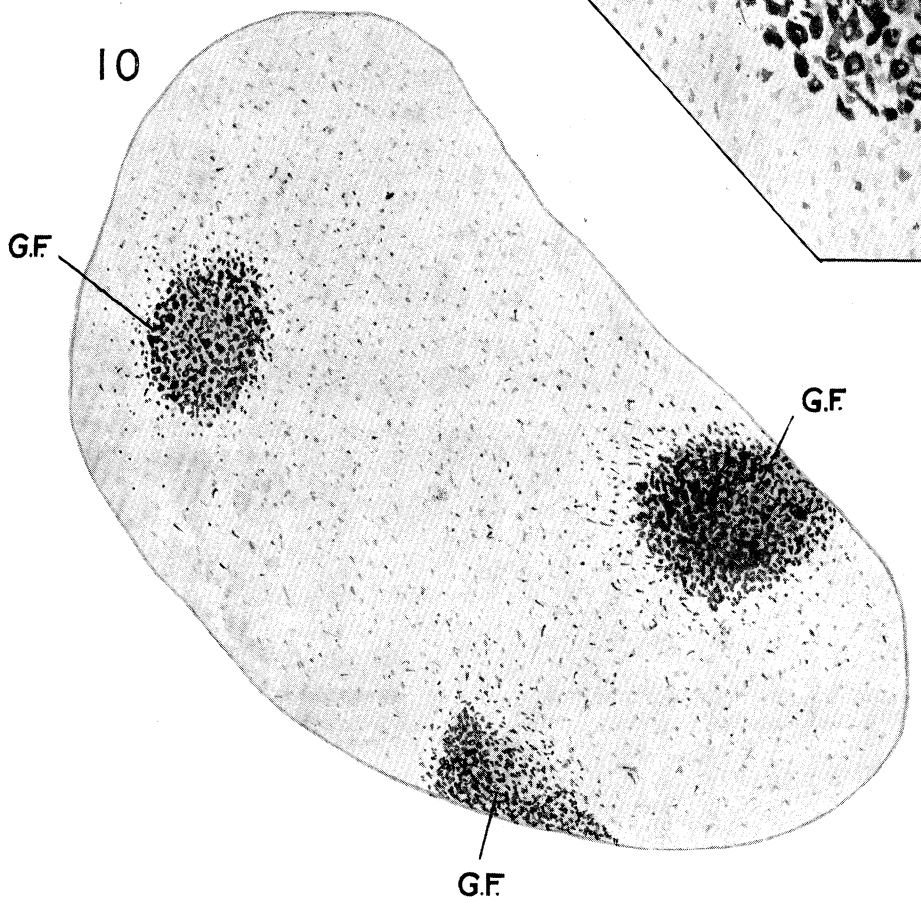
G.F.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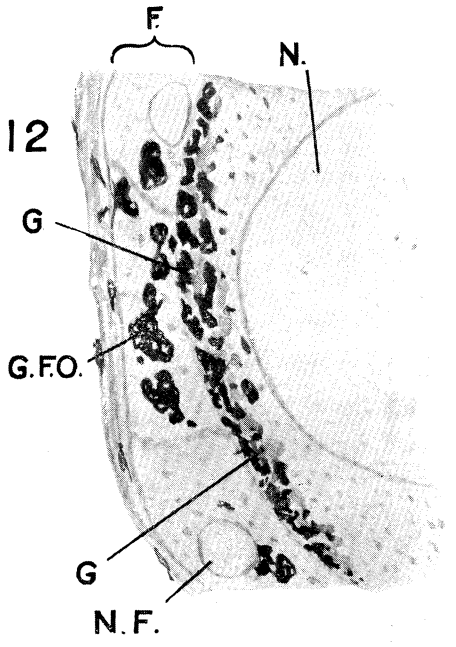
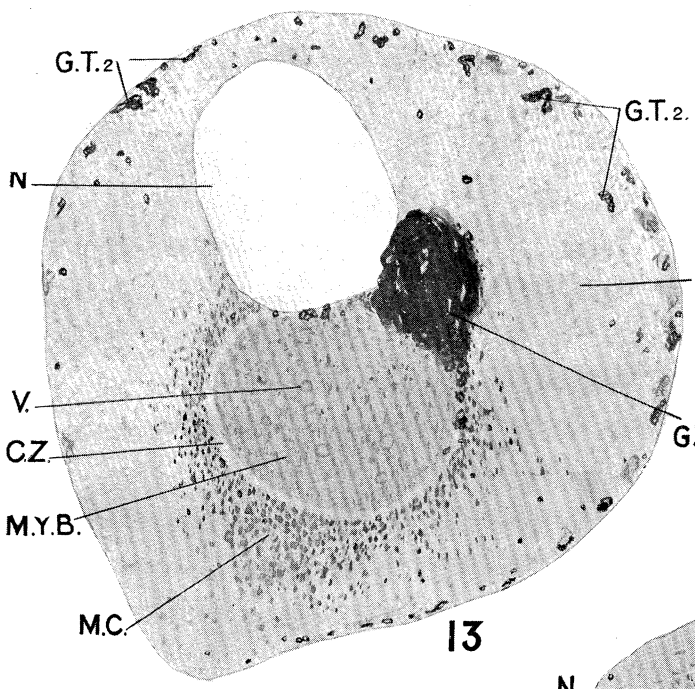
G.F.
G.F.
G.F.


F.
N.
12
G
G.F.O.
G
N.F.



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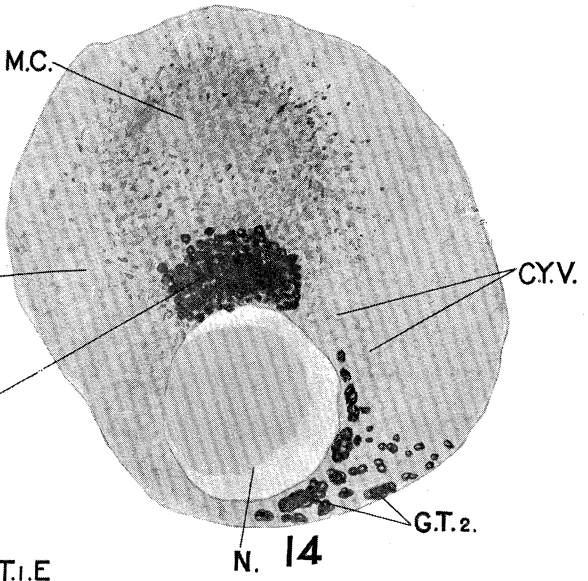

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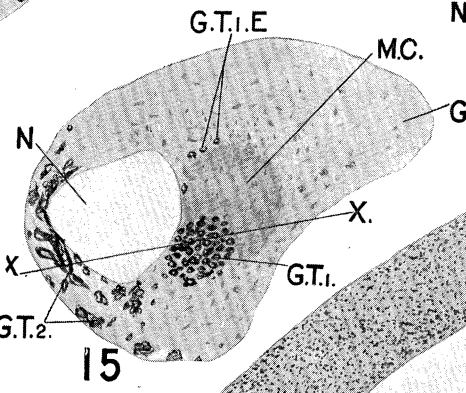

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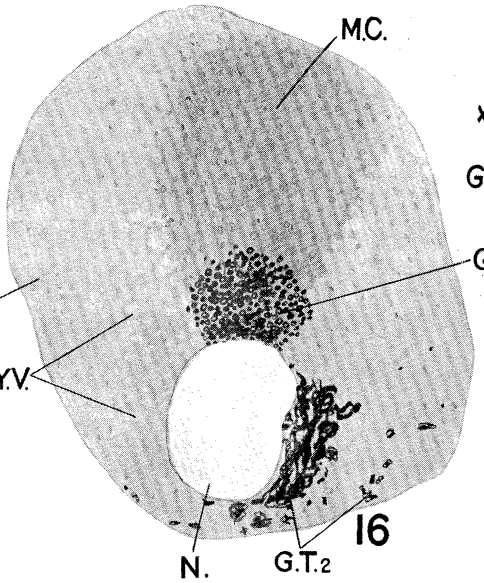

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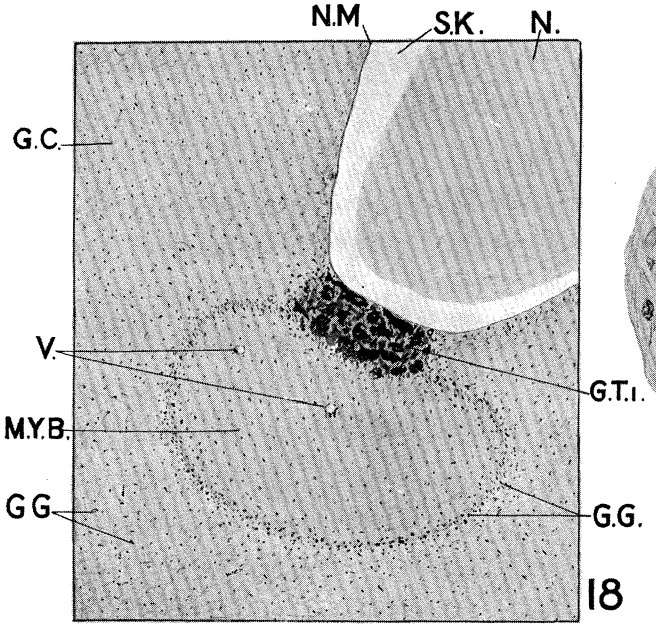

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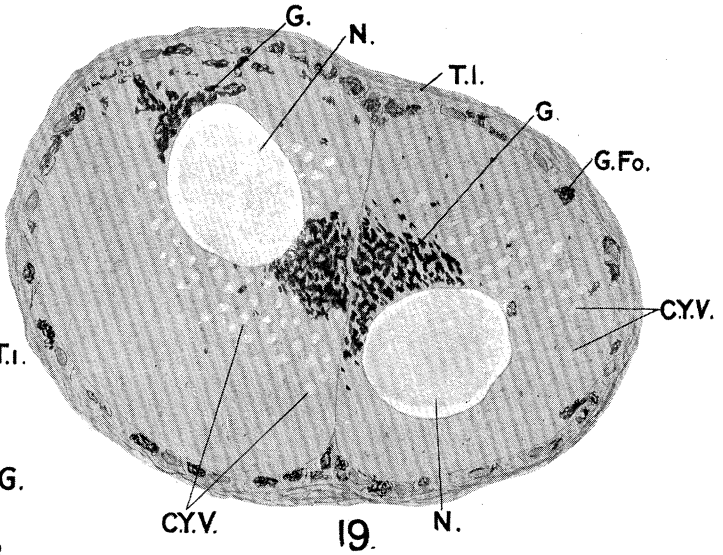

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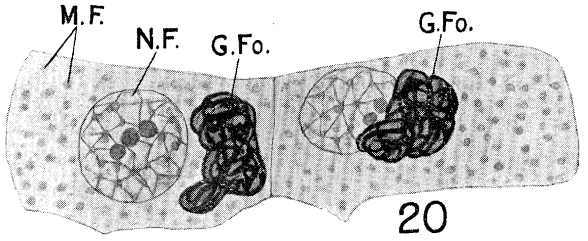

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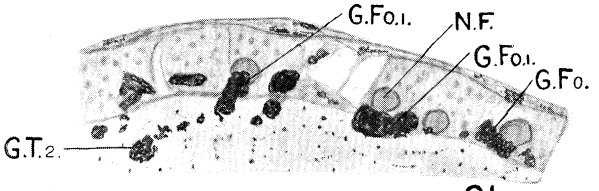

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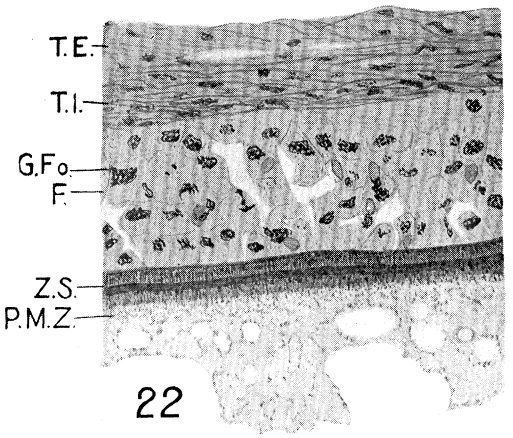

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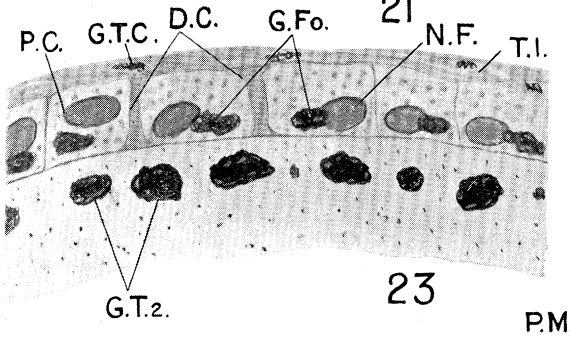

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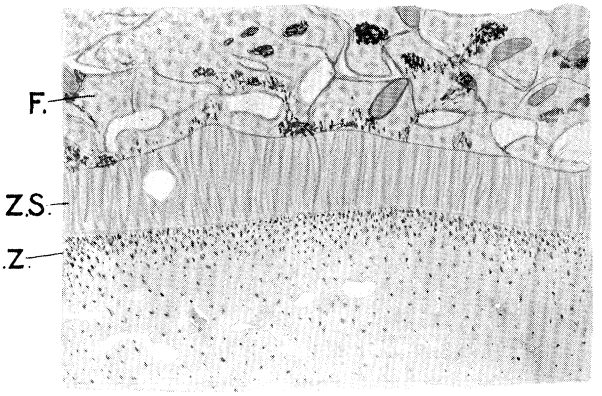

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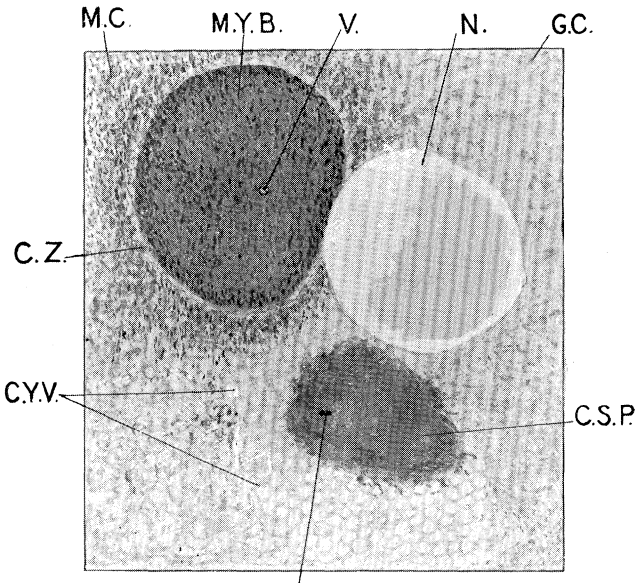

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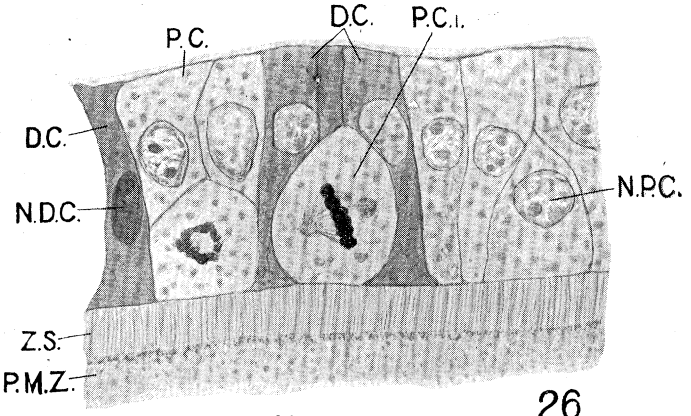

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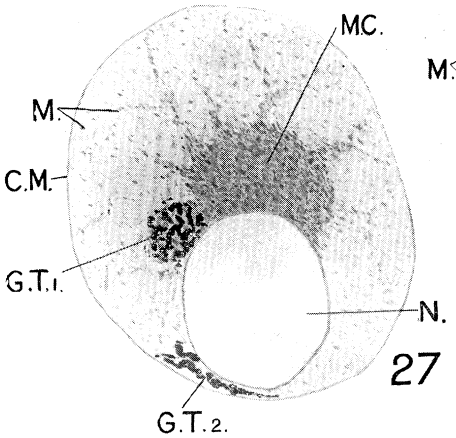

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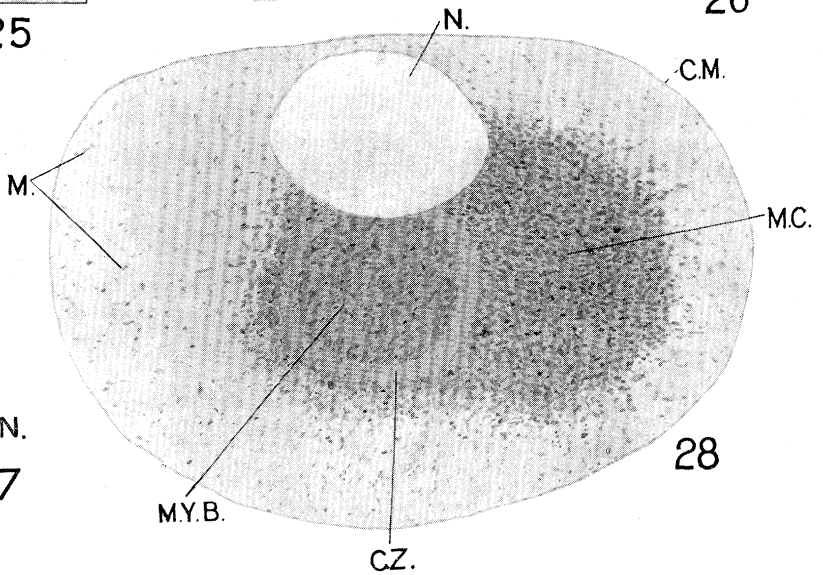

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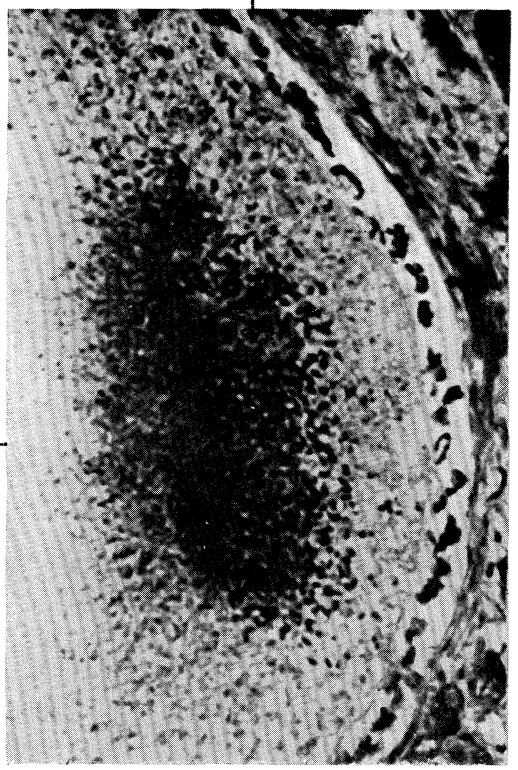

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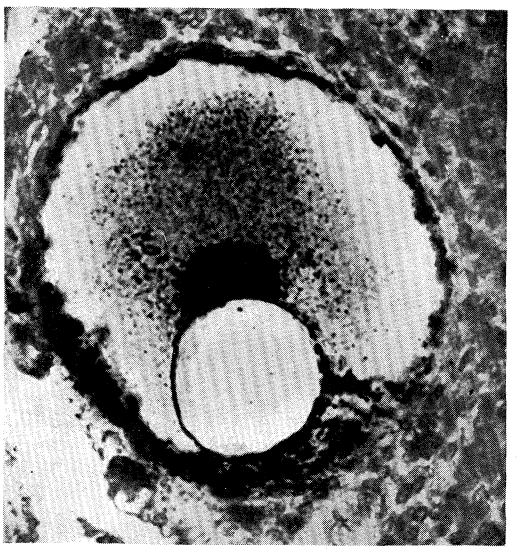

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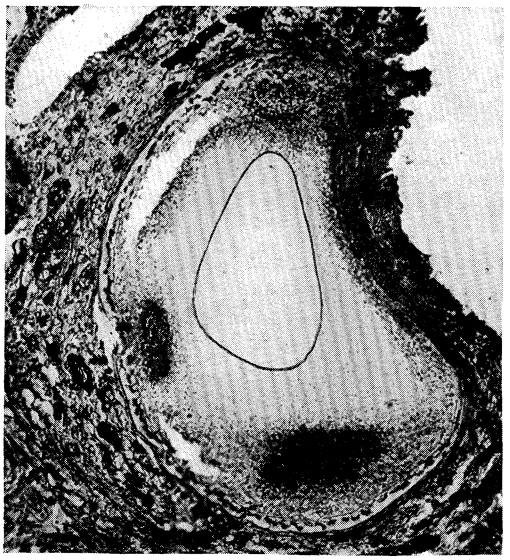

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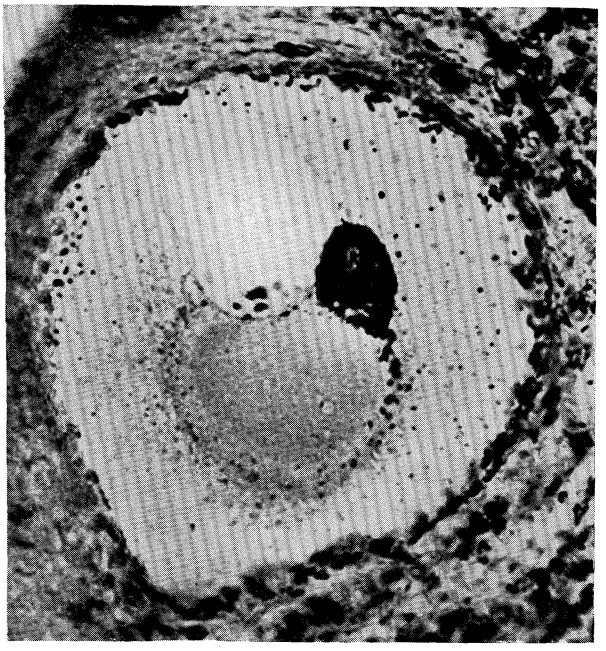

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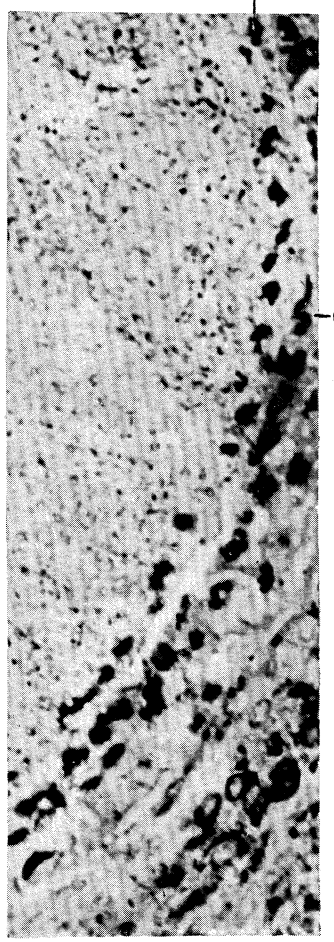

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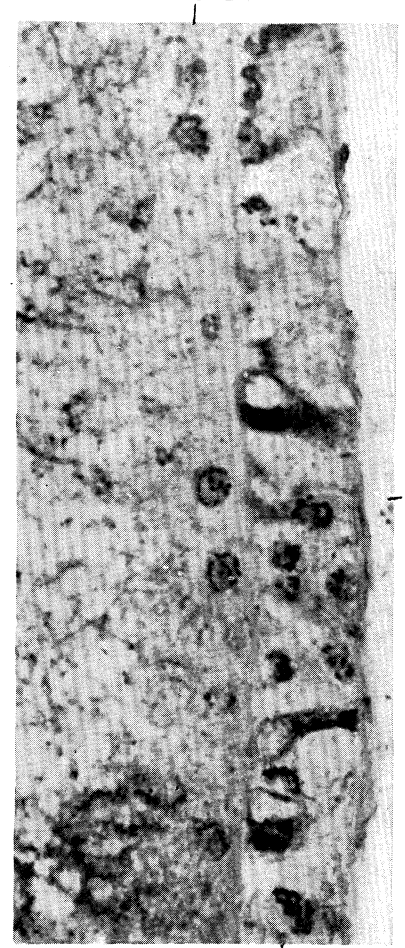

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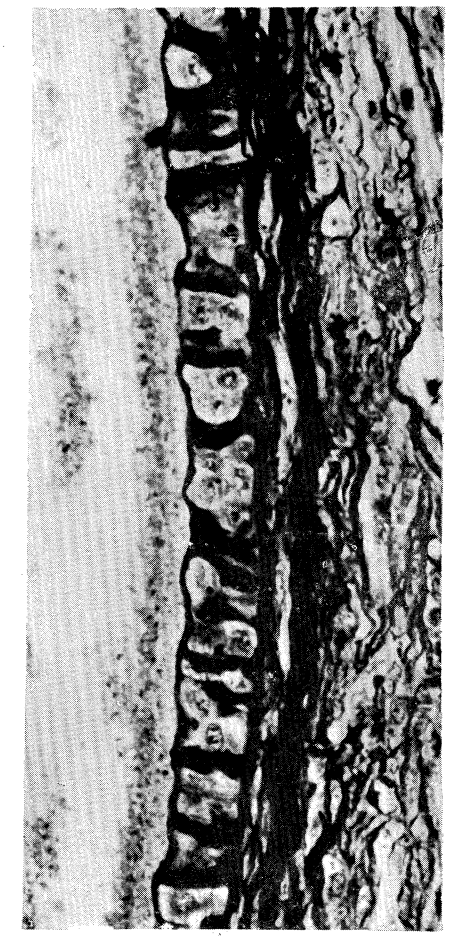

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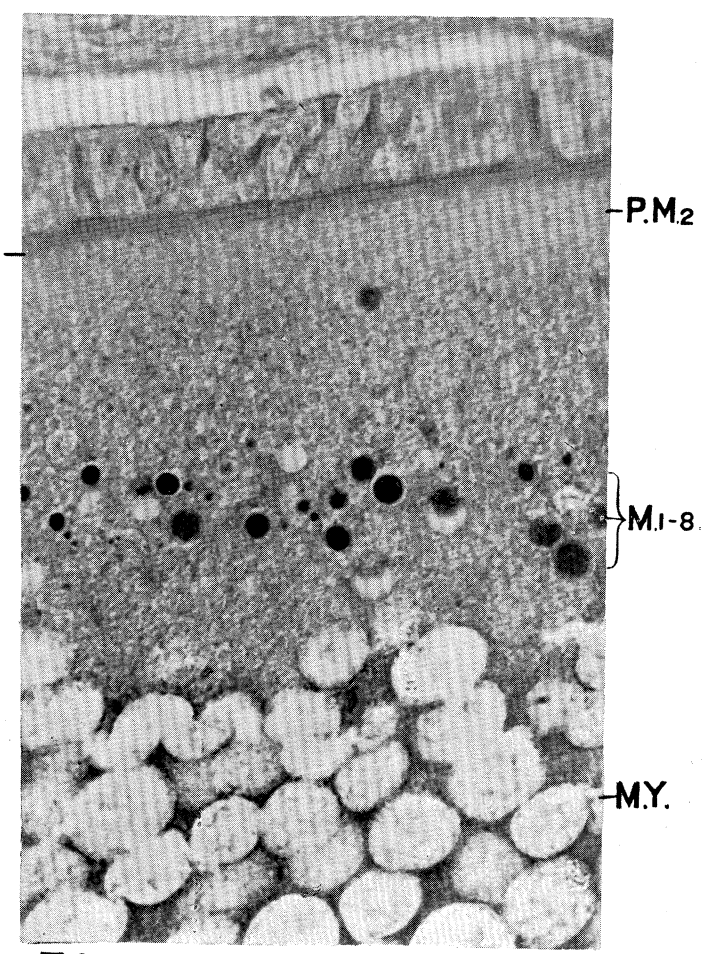

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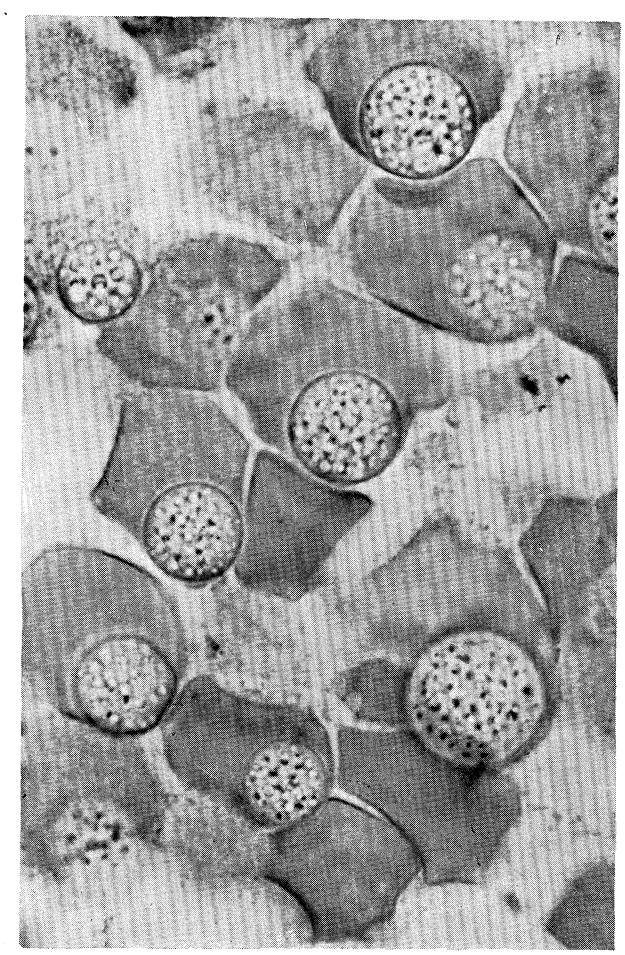

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condition. The dark cells (*D.C.*) and the pale cells (*P.C.*) are distinguishable and one of the latter is in the metaphase of mitosis (*P.C.*1). CHAMPY-KULL preparation. × 1350.

FIG. 27. Oocyte from the ovary of an 11 weeks chick, showing the mitochondrial cloud (*M.C.*), the Golgi T.1 (*G.T.*1) and the first of the Golgi T.2 (*G.T.*2) to be intruded. DA FANO preparation. × 740.

FIG. 28. Oocyte from the ovary of an adult fowl, showing the mitochondrial cloud (*M.C.*) and the mitochondrial yolk-body (*M.Y.B.*). DA FANO preparation. × 590.

PLATE 19.

FIG. 29.—Portion of the abnormal oocyte shown in fig. 31 from the ovary of a 6 weeks chick, showing a Golgi focus (*G.F.*). DA FANO preparation. × 670.

FIG. 30.—The same oocyte as drawn in fig. 16. × 500.

FIG. 31.—Abnormal oocyte from the ovary of a 6 weeks chick, showing Golgi foci. Similar to another oocyte drawn in fig. 10. DA FANO preparation. × 190.

FIG. 32.—The same oocyte as drawn in fig. 13. × 500.

PLATE 20.

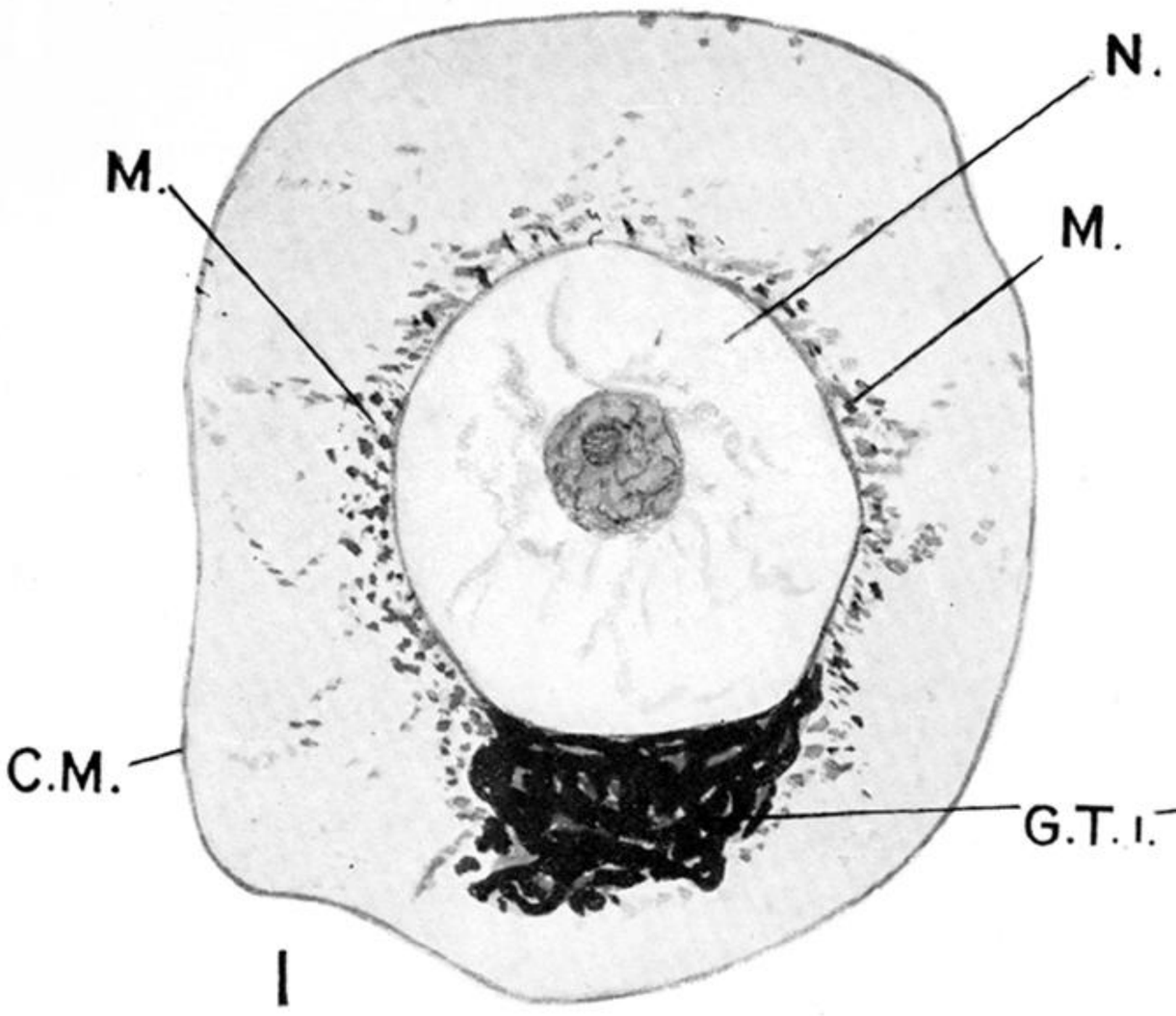
FIG. 33.—Portion of oocyte and follicle from the ovary of an adult fowl showing the Golgi T.2 (*G.T.*2) in the periphery of the oocyte, and the Golgi apparatus (*G.Fo.*) in each follicle cell. NASSONOV preparation. × 950.

FIG. 34.—Portion of follicle from an ovary of an adult fowl, showing the dark cells and the pale cells. Fixed in CHAMPY'S fluid and stained with iron-hæmatoxylin. × 500.

FIG. 35.—Portion of oocyte and follicle from the ovary of an adult fowl showing the Golgi granules in the cytoplasm, the Golgi T.2 (*G.T.*2) in the periphery of the oocyte, and the Golgi apparatus (*G.Fo.*) in each follicle cell. DA FANO preparation. × 500.

FIG. 36.—Portion of follicle and periphery of oocyte from the ovary of an adult fowl showing the transformation of mitochondria into M-yolk spheres. (For explanation compare with text-fig. 1.) Fixed in CHAMPY'S fluid and stained with iron-hæmatoxylin. × 500.

FIG. 37.—Portion of the cytoplasm of an oocyte fixed in formol corrosive bichromate and stained by CHAMPY-KULL method. (For explanation compare text-fig. 2.) × 950.



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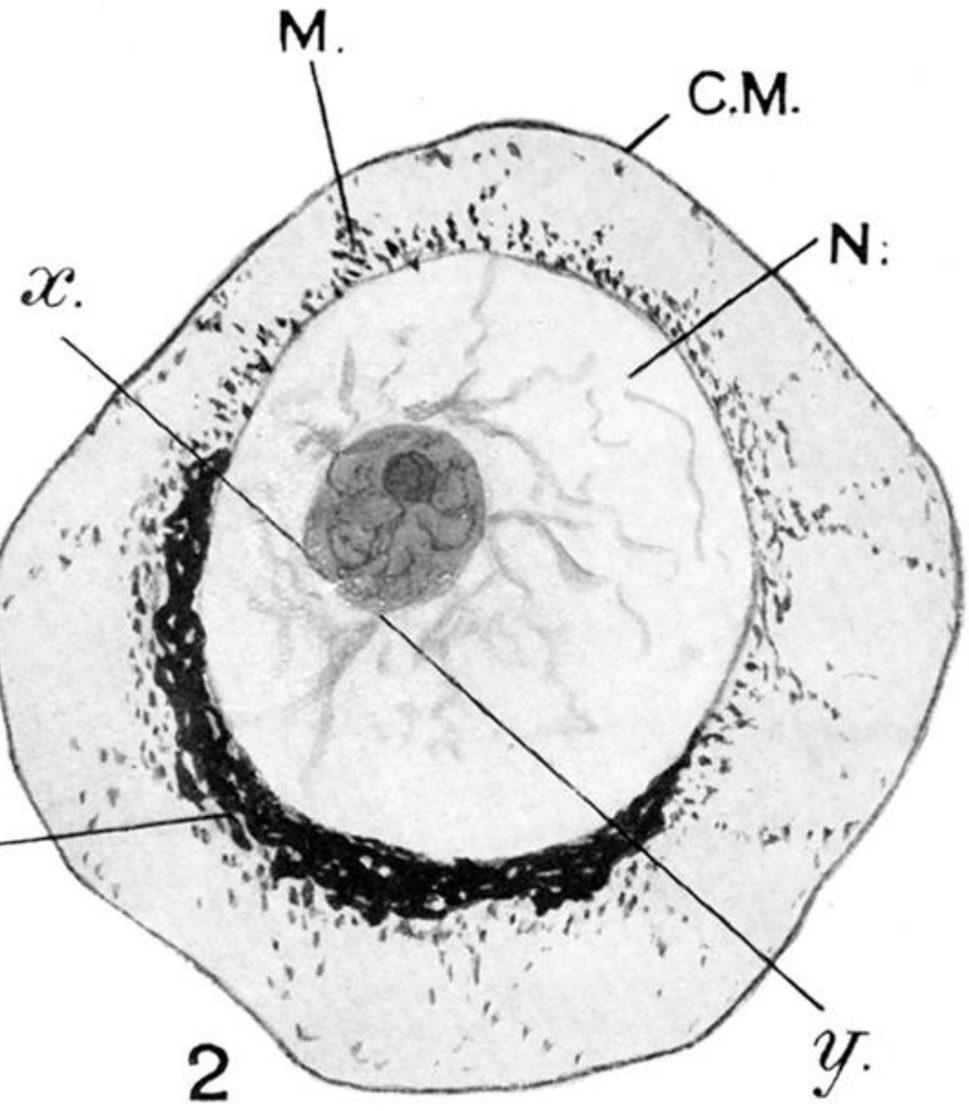

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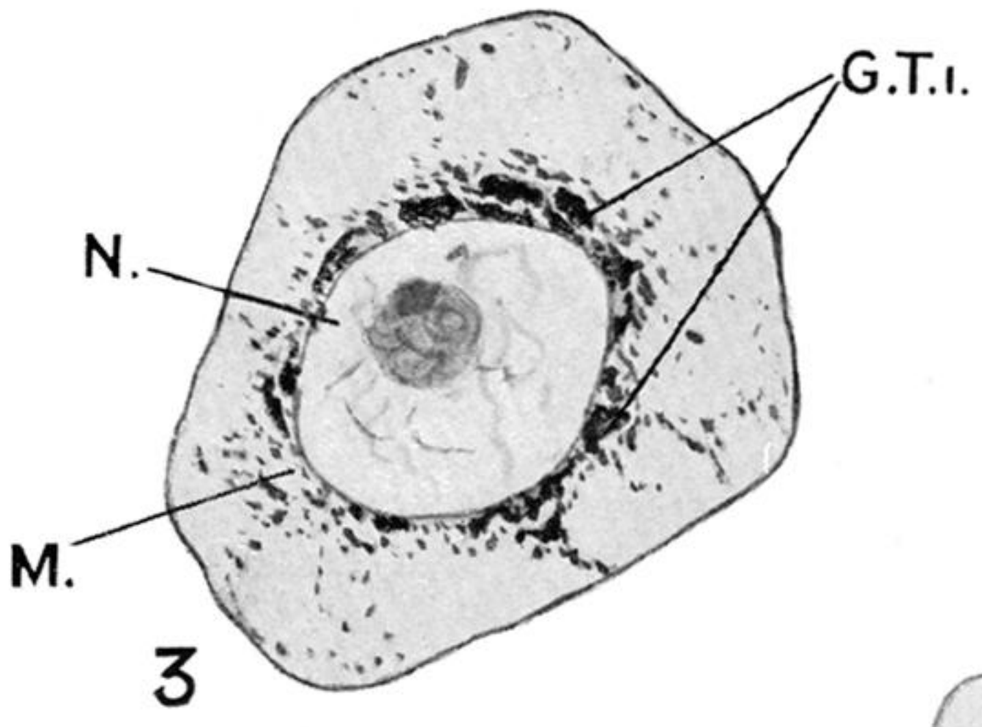

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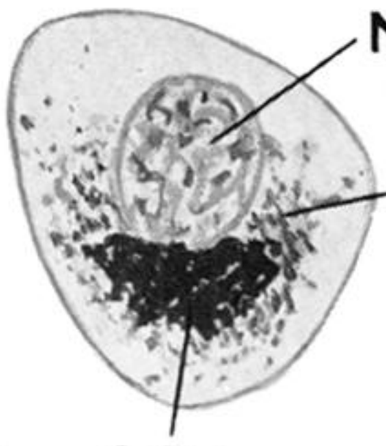

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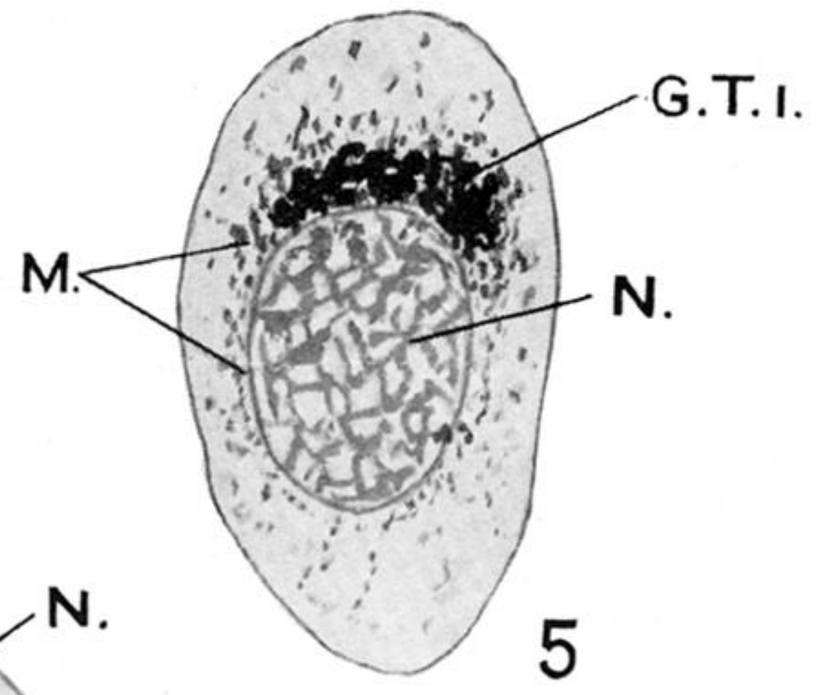

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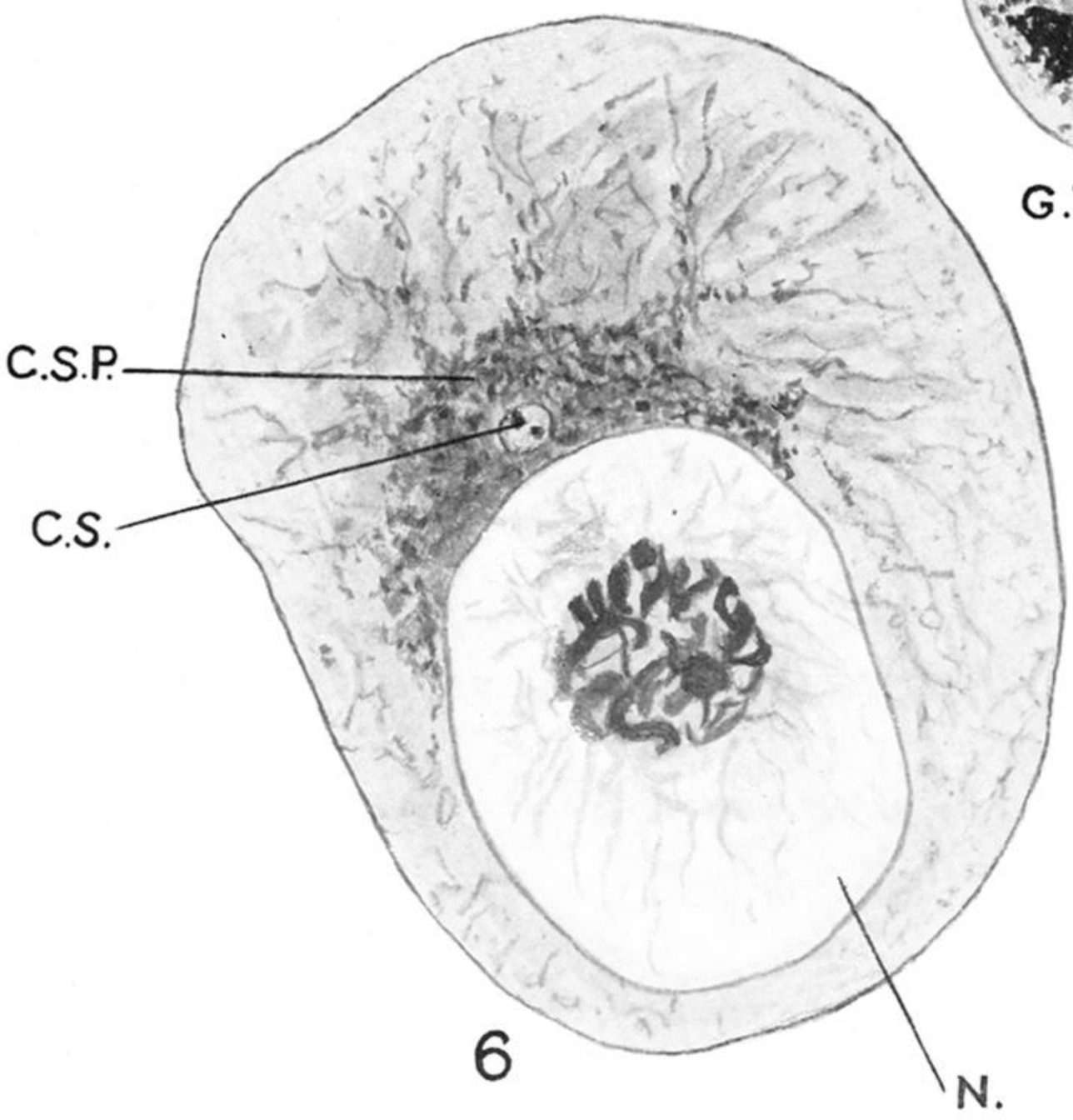

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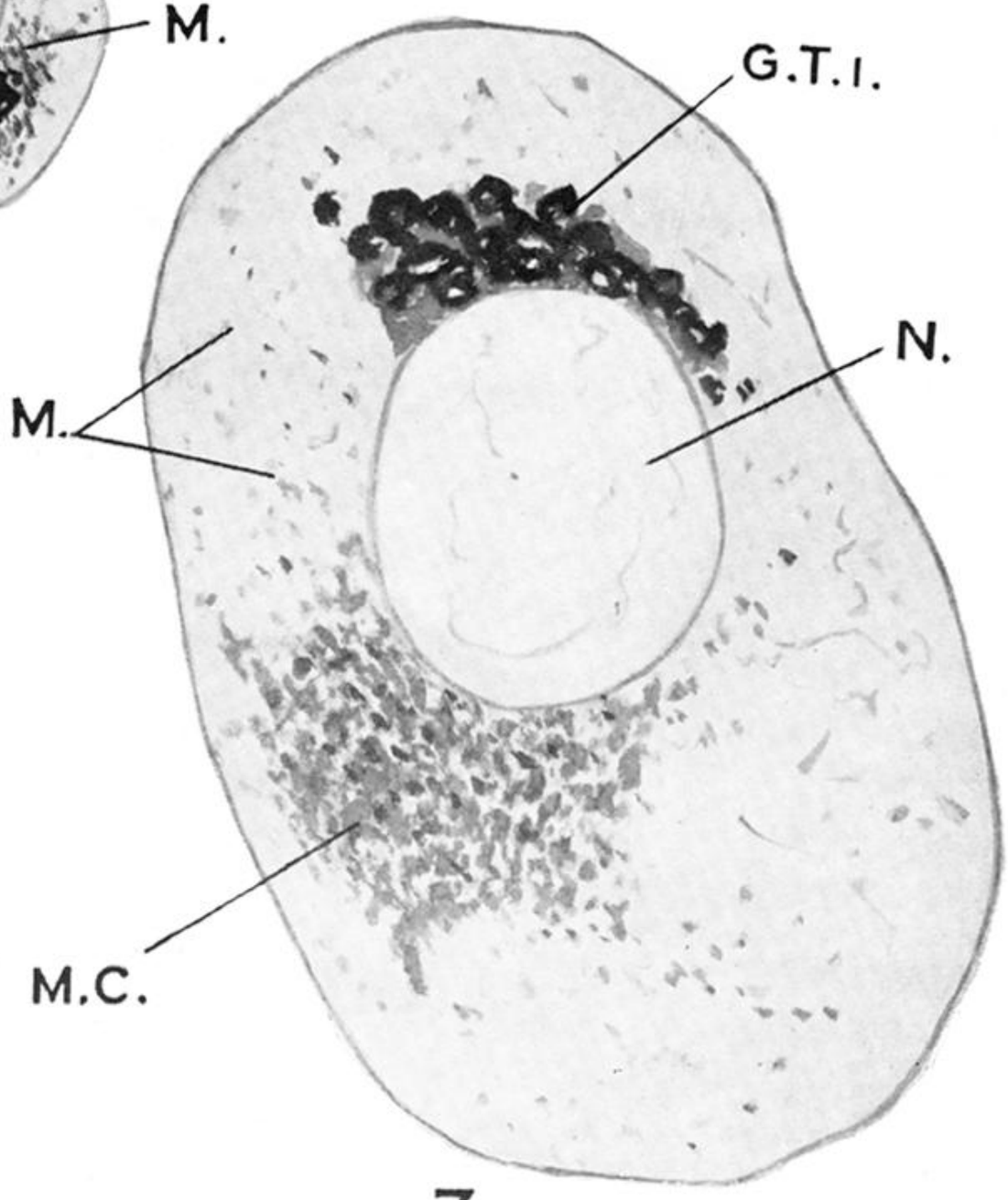

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PLATE 15.

FIG. 1.—Oocyte from ovary of 3 weeks old chick. DA FANO preparation. × 2170.

FIG. 2.—Oocyte from ovary of 3 weeks old chick. Golgi apparatus (*G.T.*1) forming a cap over the nucleus. DA FANO preparation. × 2170.

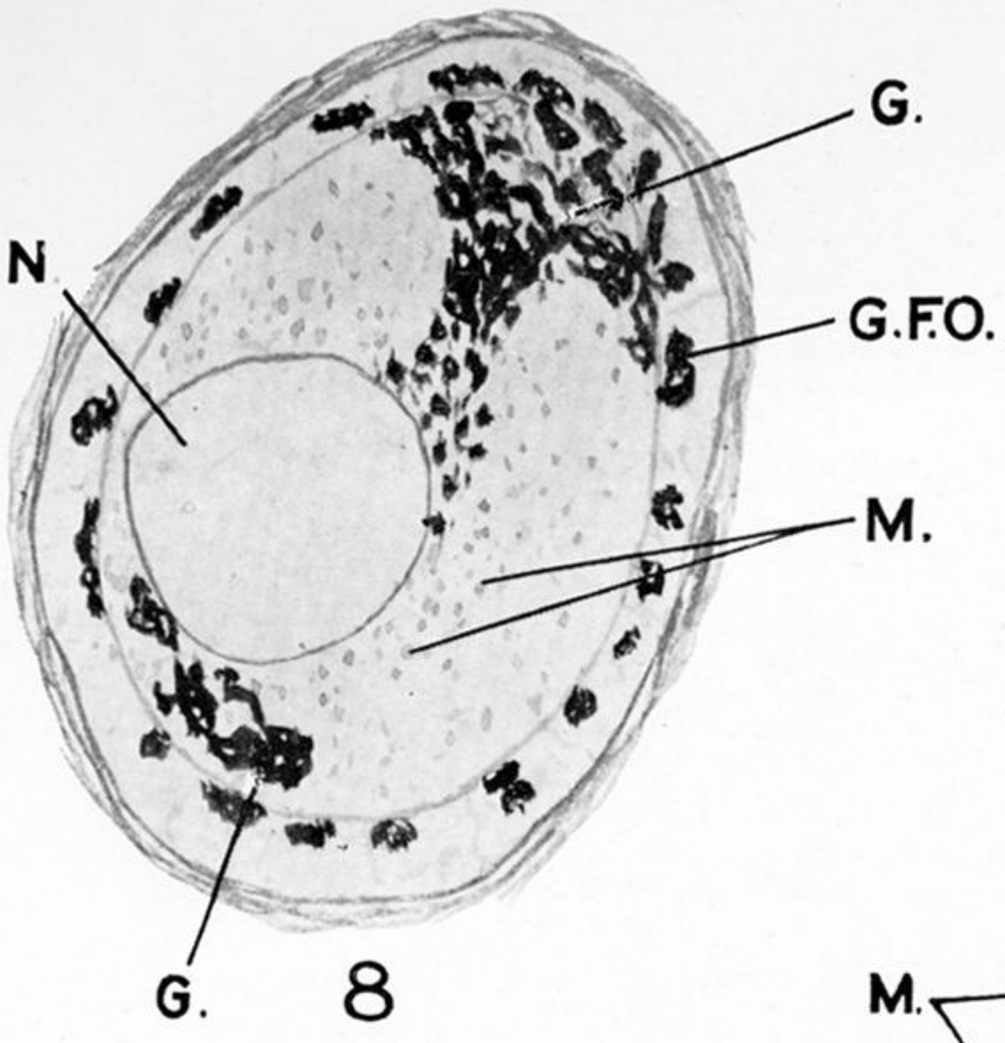
FIG. 3.—Oocyte from ovary of 3 weeks chick. Golgi apparatus forming a ring around the nucleus owing to plane of section being at right angles to that in previous figure and along a line *x*—*y*. DA FANO preparation. × 2170.

FIG. 4.—Oocyte from ovary of 4 days chick. DA FANO preparation. × 2000.

FIG. 5.—Oocyte from ovary of 4 days chick. DA FANO preparation. × 4540.

FIG. 6.—Oocyte 75μ in diameter from ovary of 6 weeks chick, showing centrosome (*C.S.*), containing diploid centrioles, in centrosphere (*C.S.P.*). DA FANO preparation stained with iron-hæmatoxylin. × 1470.

FIG. 7.—Oocyte from ovary of 6 weeks chick, showing the Golgi apparatus (*G.T.*1) and first appearance of mitochondrial cloud (*M.C.*). DA FANO preparation. × 1470.



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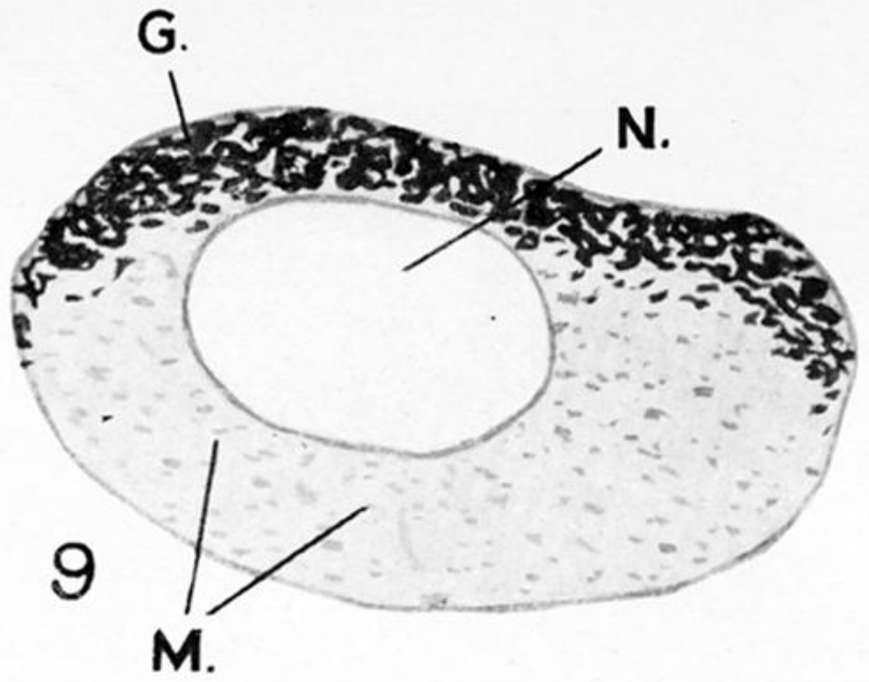

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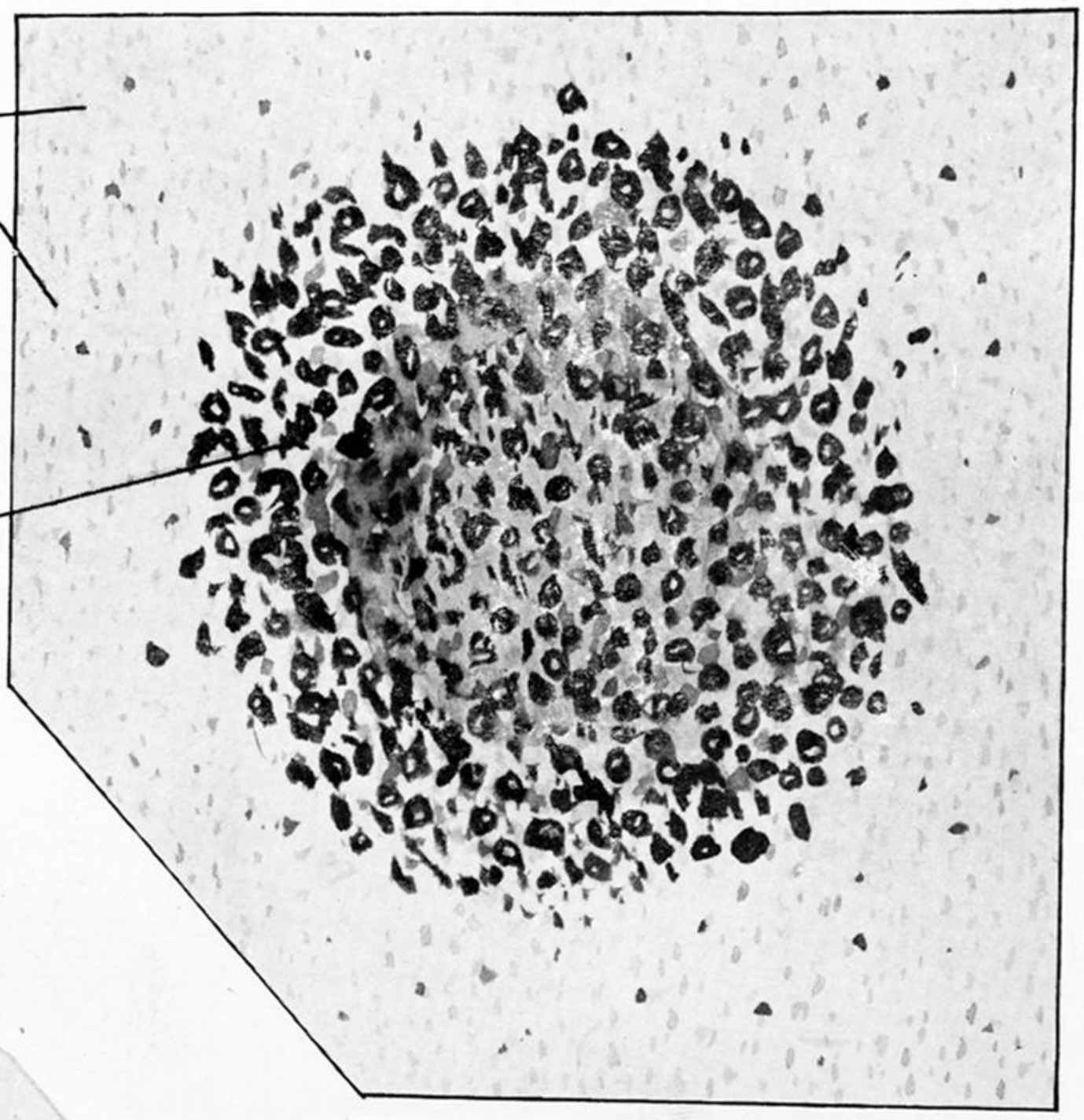

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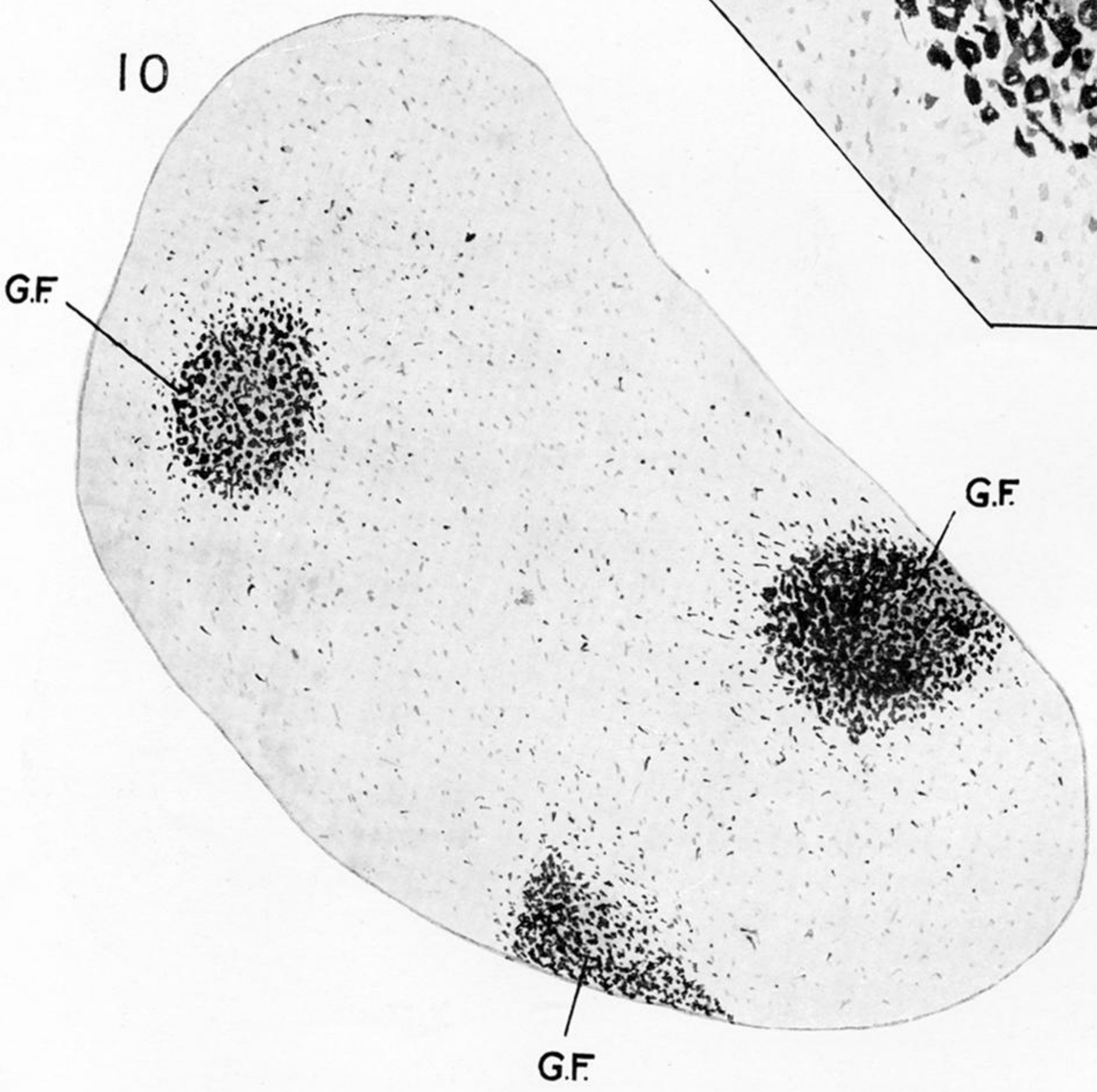

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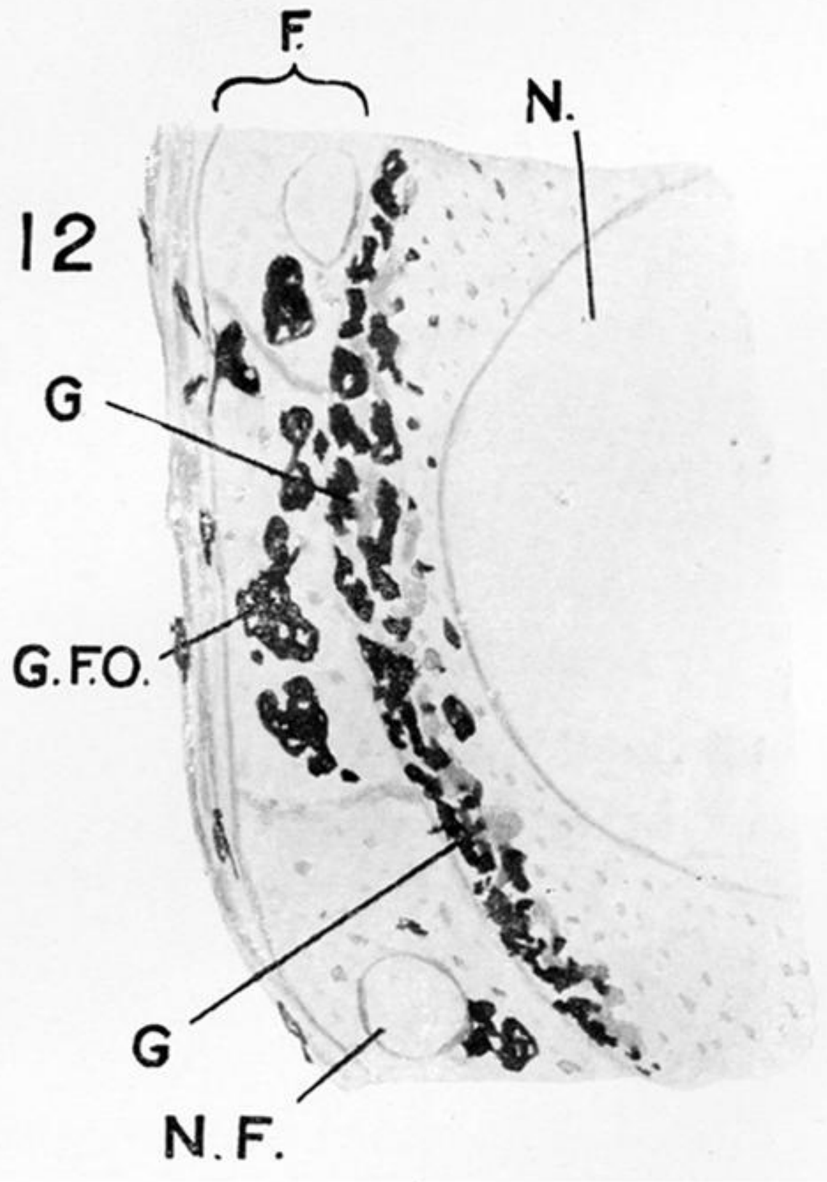

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PLATE 16.

FIG. 8.—Oocyte from the ovary of 6 weeks chick showing abnormal behaviour of Golgi elements (*G.*) and intrusion from follicle cells. DA FANO preparations. × 675.

FIG. 9.—Oocyte from ovary of 6 weeks chick showing abnormal distribution of Golgi elements (*G.*) which are clustered at one side of the cell. DA FANO preparation. × 675.

FIG. 10.—Oocyte from ovary of 6 weeks chick showing abnormal arrangement of Golgi elements which form several foci (*G.F.*). DA FANO preparation. × 340.

FIG. 11.—Portion of similar oocyte to that shown in previous figure. Golgi focus (*G.F.*) drawn on large scale. DA FANO preparation. × 1470.

FIG. 12.—Portion of periphery of oocyte and follicle cells from ovary of 6 weeks chick showing apparent intrusion of Golgi of follicle (*G.Fo.*) into abnormal oocyte, and peripheral arrangement of the Golgi elements (*G.*) in the latter. DA FANO preparation. × 1470.

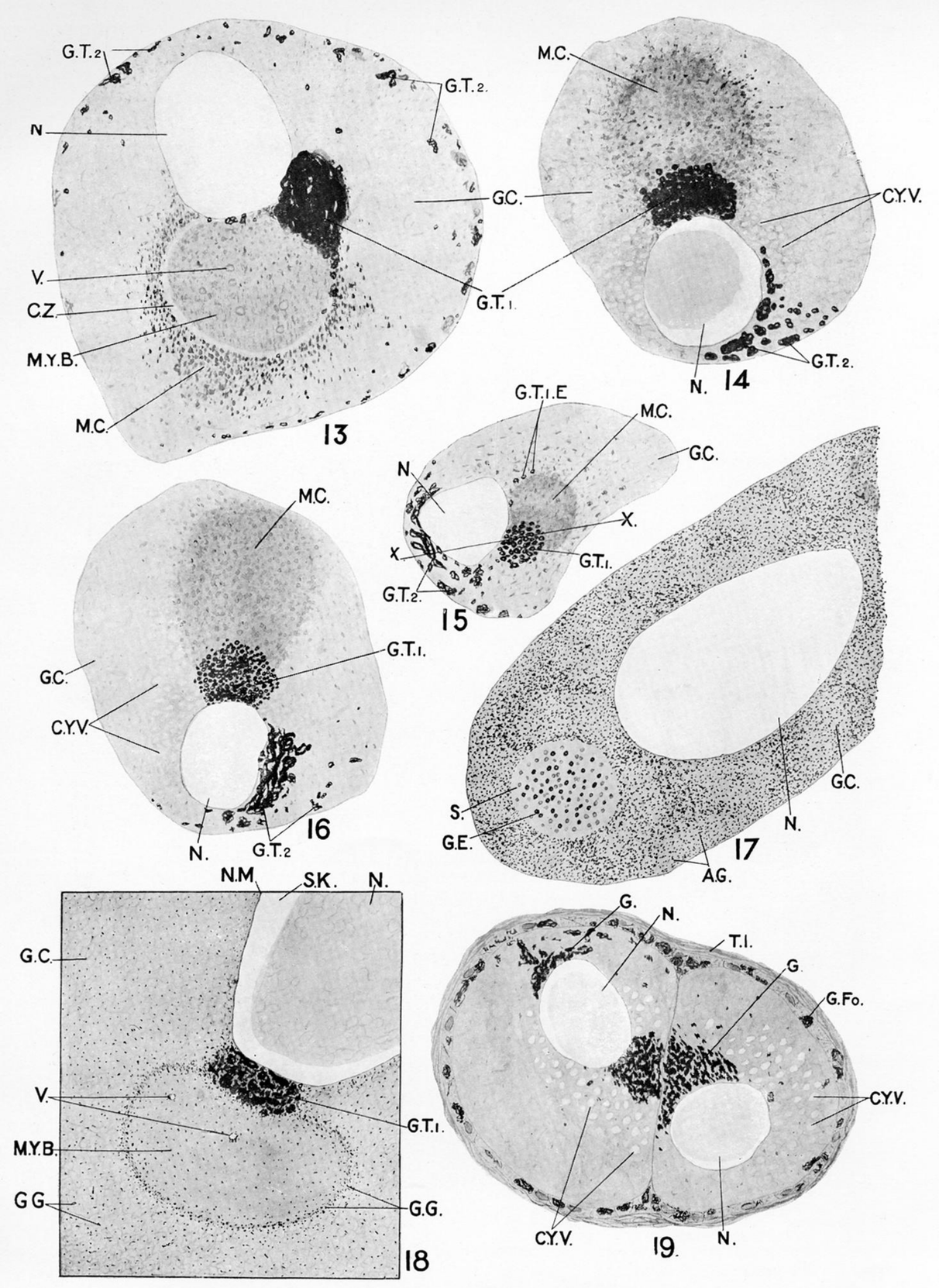


PLATE 17.

(All the figures are on the same scale. × 640.)

FIG. 13.—Oocyte from the ovary of an adult fowl, showing both types of Golgi apparatus (*G.T.*1 and *G.T.*2) the mitochondrial cloud (*M.C.*) and the mitochondrial yolk-body (*M.Y.B.*), with an outer clear zone (*C.Z.*), containing several vacuoles (*V.*). DA FANO preparation untoned and unstained. Same cell as Plate 19, fig. 32.

FIG. 14.—Oocyte from the ovary of an adult fowl, showing both types of Golgi apparatus (*G.T.*1 and *G.T.*2), the mitochondrial cloud (*M.C.*) at its maximum development before the differentiation of the mitochondrial yolk-body, and the vacuoles (*C.Y.V.*) from which the C-yolk spheres have been dissolved out by the reagents. DA FANO preparation.

FIG. 15.—Oocyte from ovary of an adult fowl, showing both types of Golgi apparatus (*G.T.*1 and *G.T.*2) and the mitochondrial cloud (*M.C.*). DA FANO preparation.

FIG. 16.—Oocyte from the ovary of an adult fowl showing the same structure as fig. 14. This is the same cell as Plate 19, fig. 30. DA FANO preparation.

FIG. 17.—Portion of small oocyte of *Rana temporaria* showing supposed centrosphere (*S.*) and Golgi elements (*G.E.*), and argentophil granules (*A.G.*) in the ground cytoplasm. DA FANO preparation.

FIG. 18.—Portion of oocyte from the ovary of an adult fowl, showing fragmentation of the Golgi apparatus type 1 (*G.T.*1), the association of the resulting Golgi granules (*G.G.*) with the mitochondrial yolk-body (*M.Y.B.*) as they become dispersed through the cytoplasm. Two vacuoles (*V.*) with associated granules are seen in the mitochondrial yolk-body. DA FANO preparation.

FIG. 19.—Two oocytes touching, from the ovary of an adult fowl, showing unusual arrangement of the Golgi apparatus (*G.*). DA FANO preparation.

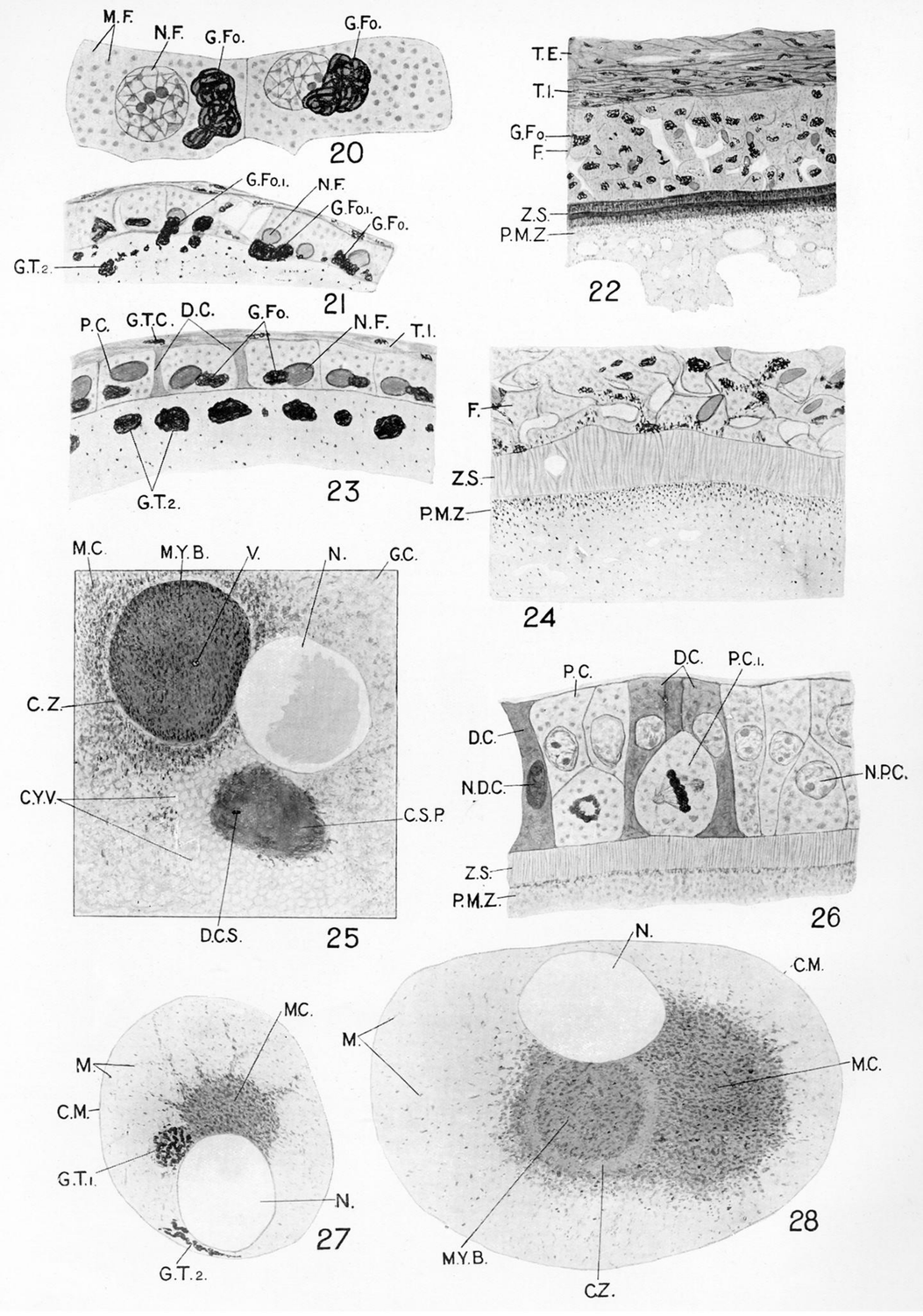


PLATE 18.

FIG. 20.—Two cells from the follicle, in the single-layered stage, from the ovary of the adult fowl, showing the Golgi apparatus (*G.Fo.*) and mitochondria (*M.F.*). DA FANO preparation. × 2700.

FIG. 21.—Portion of periphery of oocyte and follicle, showing intrusion of the Golgi apparatus of the follicle cells (*G.Fo.* and $G.Fo._1$) into the oocyte to form the Golgi apparatus type 2 (*G.T.*2). DA FANO preparation × 1300.

FIG. 22.—Portion of theca, follicle, and periphery of oocyte from the ovary of an adult fowl. Showing the follicle in the many-layered condition (*F.*), the zona striata (*Z.S.*), and the peripheral mitochondrial zone (*P.M.Z.*). DA FANO preparation. × 640.

FIG. 23.—Portion of follicle and periphery of oocyte from the ovary of an adult fowl at the stage where the intrusion of the Golgi bodies from the follicle cells has ceased. The Golgi bodies which have been intruded (*G.T.*2) are arranged opposite the follicle cells. The dark cells (*D.C.*) and the pale cells (*P.C.*) are differentiating in the follicle. DA FANO preparation toned and stained with safranin and light green. × 1350.

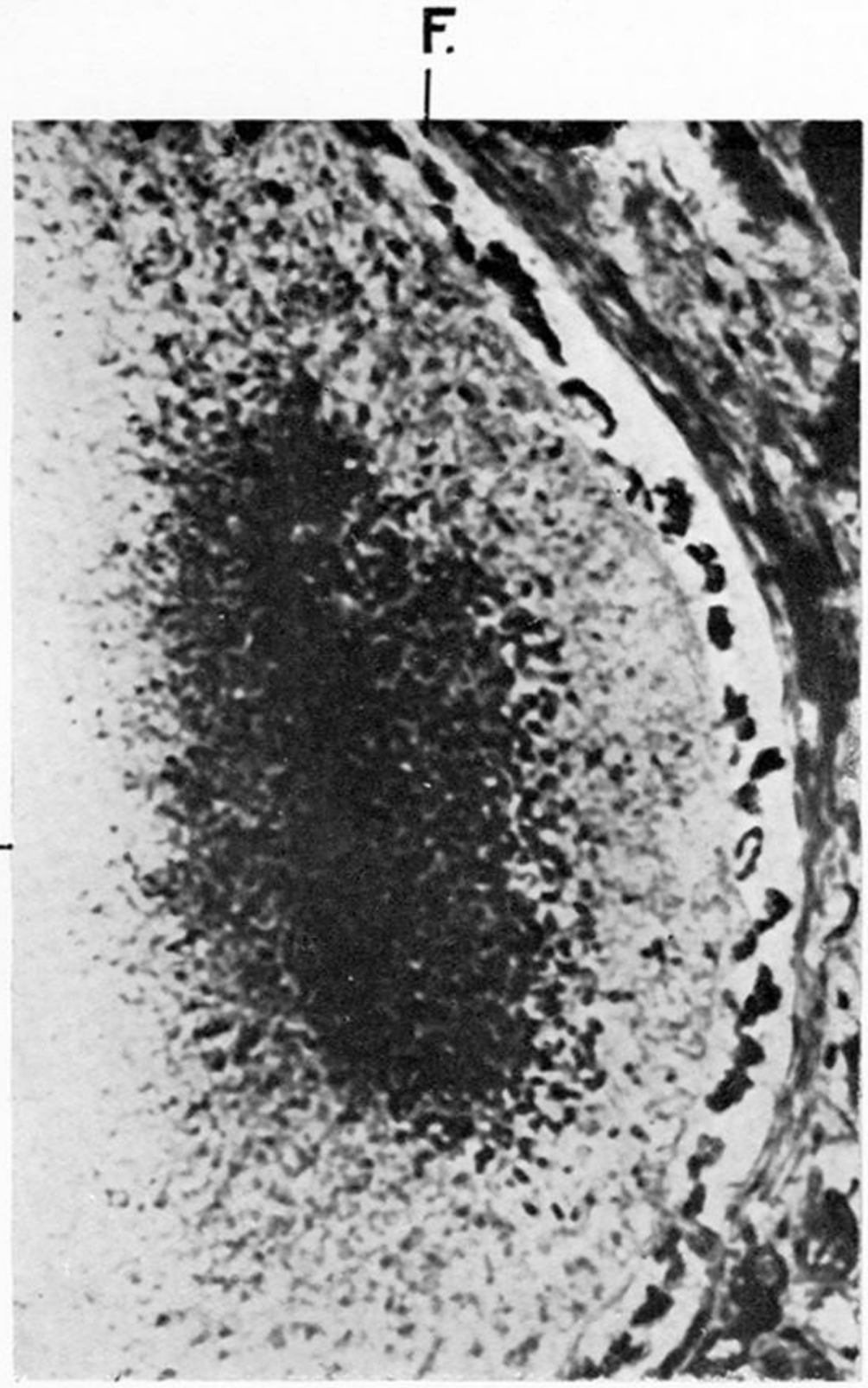
FIG. 24.—Portion of follicle and periphery of oocyte from the ovary of an adult fowl, showing the zona striata. DA FANO toned preparation. × 1350.

FIG. 25.—Portion of oocyte from the ovary of an adult fowl, showing the mitochondrial yolk-body (*M.Y.B.*), and the centrosphere (*C.S.P.*), containing a diploid centriole (*D.C.S.*), from which the Golgi apparatus has been removed. DA FANO preparation stained with iron-hæmatoxylin. × 640.

FIG. 26. Portion of follicle and periphery of oocyte from the ovary of an adult fowl, showing the zona striata (*Z.S.*). The follicle is assuming the many-layered condition. The dark cells (*D.C.*) and the pale cells (*P.C.*) are distinguishable and one of the latter is in the metaphase of mitosis (*P.C.*1). CHAMPY-KULL preparation. × 1350.

FIG. 27. Oocyte from the ovary of an 11 weeks chick, showing the mitochondrial cloud (*M.C.*), the Golgi T.1 (*G.T.*1) and the first of the Golgi T.2 (*G.T.*2) to be intruded. DA FANO preparation. × 740.

FIG. 28. Oocyte from the ovary of an adult fowl, showing the mitochondrial cloud (*M.C.*) and the mitochondrial yolk-body (*M.Y.B.*). DA FANO preparation. × 590.



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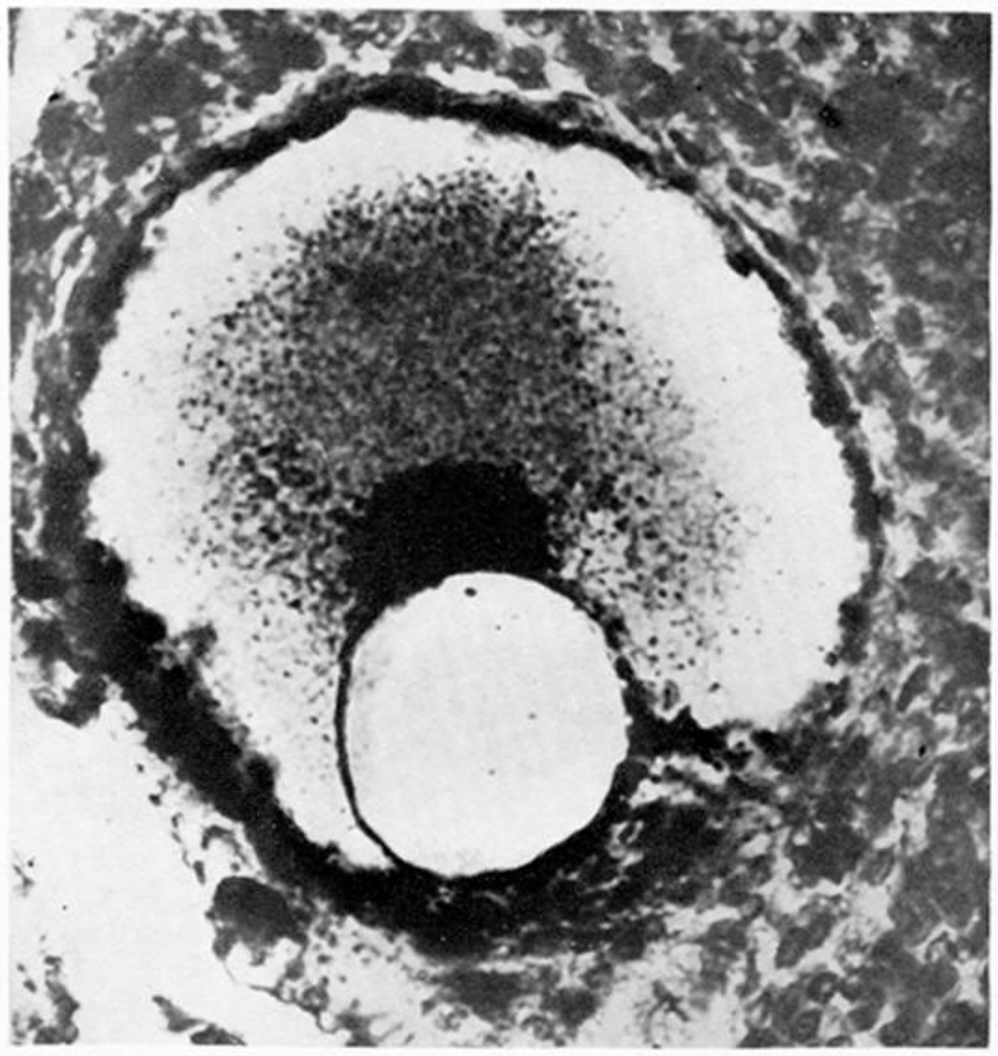

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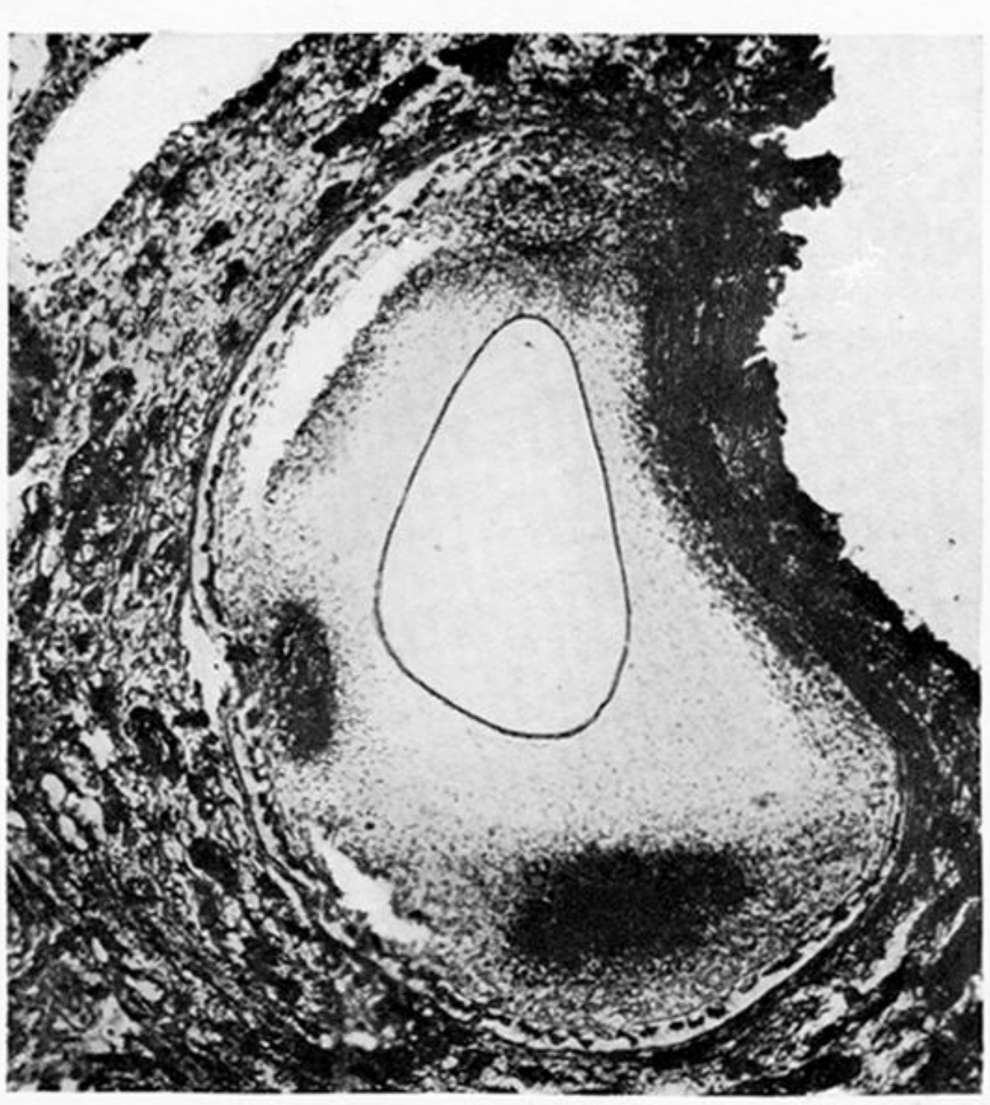

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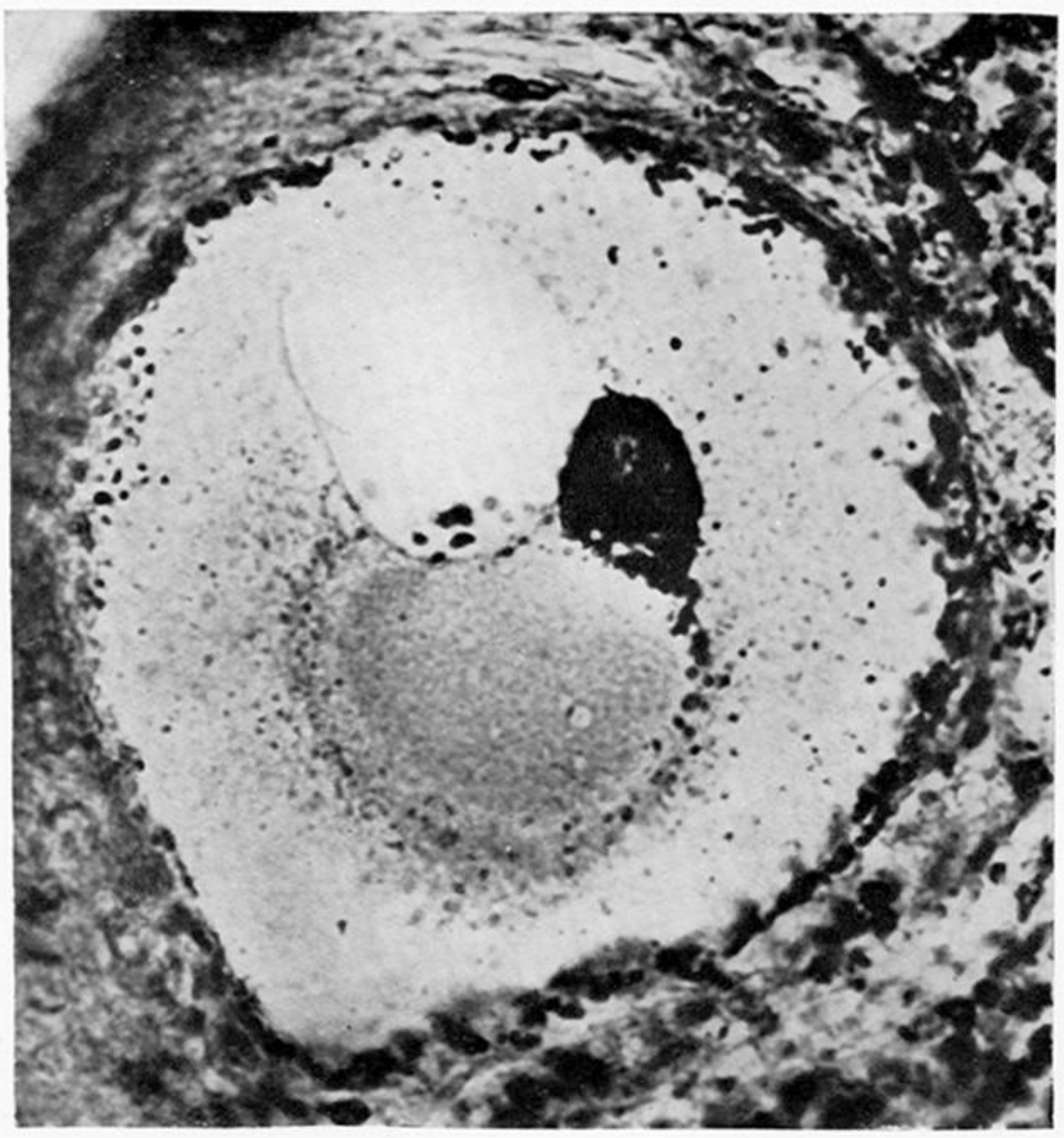

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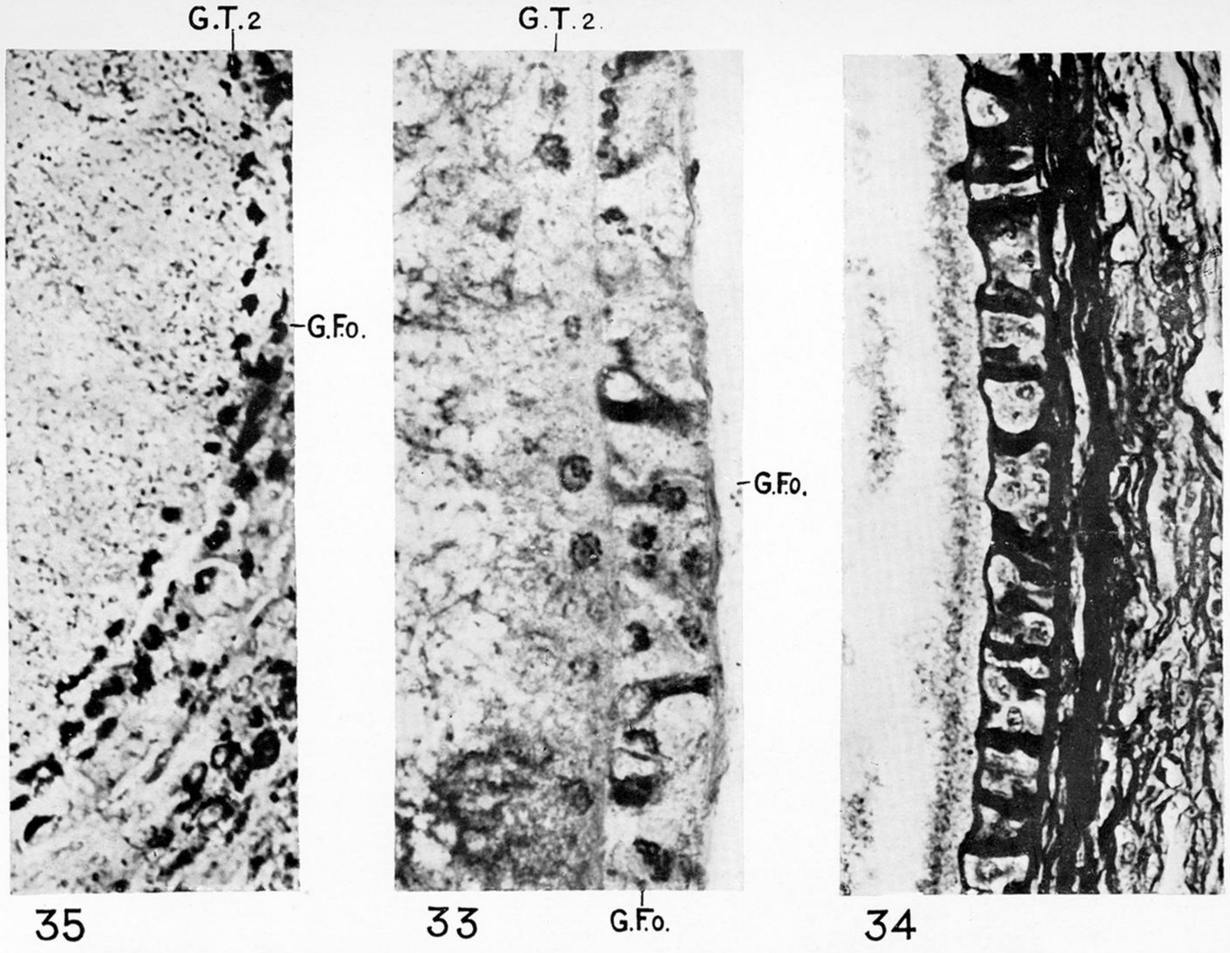
PLATE 19.

FIG. 29.—Portion of the abnormal oocyte shown in fig. 31 from the ovary of a 6 weeks chick, showing a Golgi focus (*G.F.*). DA FANO preparation. × 670.

FIG. 30.—The same oocyte as drawn in fig. 16. × 500.

FIG. 31.—Abnormal oocyte from the ovary of a 6 weeks chick, showing Golgi foci. Similar to another oocyte drawn in fig. 10. DA FANO preparation. × 190.

FIG. 32.—The same oocyte as drawn in fig. 13. × 500.



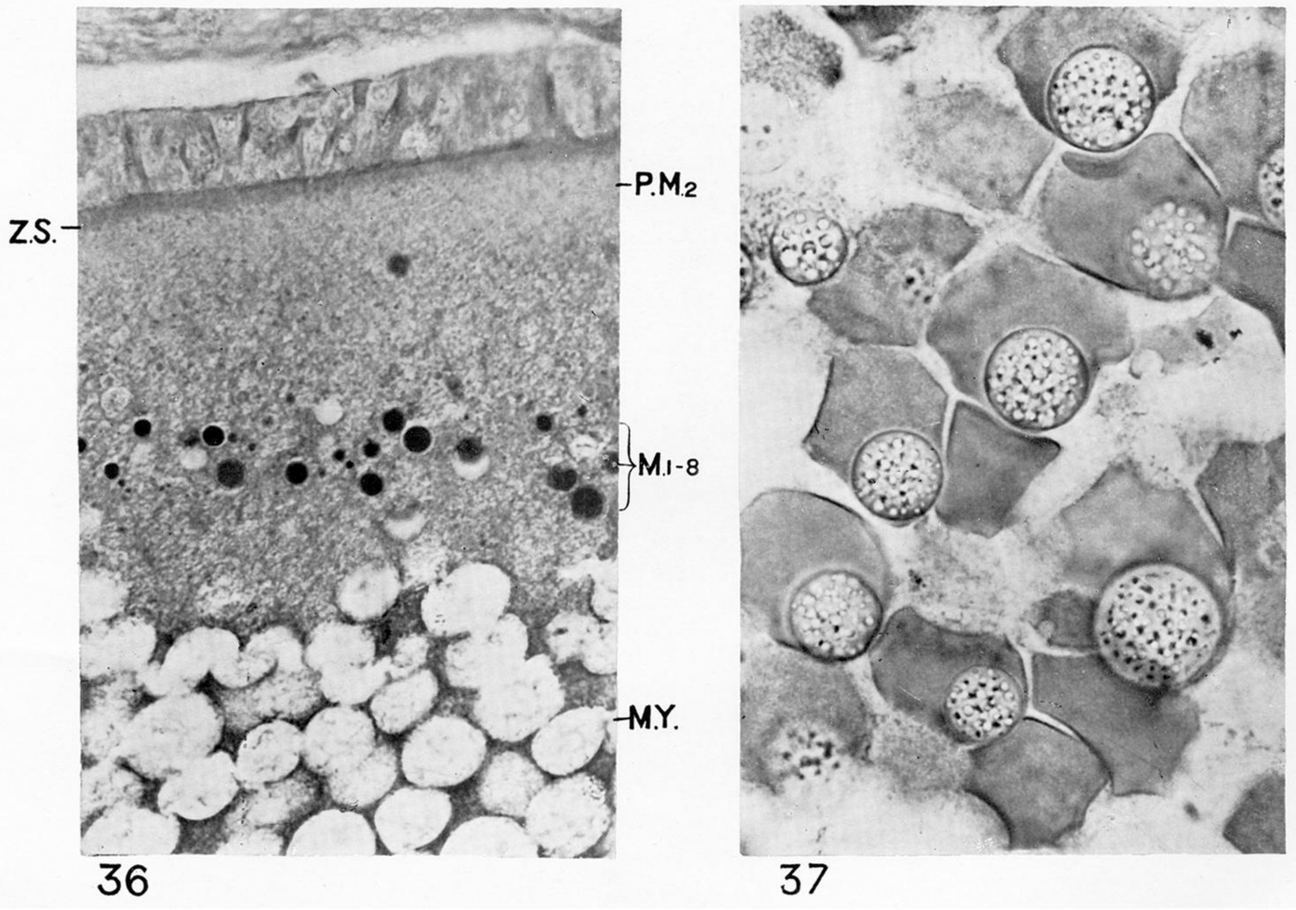


PLATE 20.

FIG. 33.—Portion of oocyte and follicle from the ovary of an adult fowl showing the Golgi T.2 (*G.T.2*) in the periphery of the oocyte, and the Golgi apparatus (*G.Fo.*) in each follicle cell. NASSONOV preparation. × 950.

FIG. 34.—Portion of follicle from an ovary of an adult fowl, showing the dark cells and the pale cells. Fixed in CHAMPY'S fluid and stained with iron-hæmatoxylin. × 500.

FIG. 35.—Portion of oocyte and follicle from the ovary of an adult fowl showing the Golgi granules in the cytoplasm, the Golgi T.2 (*G.T.2*) in the periphery of the oocyte, and the Golgi apparatus (*G.Fo.*) in each follicle cell. DA FANO preparation. × 500.

FIG. 36.—Portion of follicle and periphery of oocyte from the ovary of an adult fowl showing the transformation of mitochondria into M-yolk spheres. (For explanation compare with text-fig. 1.) Fixed in CHAMPY'S fluid and stained with iron-hæmatoxylin. × 500.

FIG. 37.—Portion of the cytoplasm of an oocyte fixed in formol corrosive bichromate and stained by CHAMPY-KULL method. (For explanation compare text-fig. 2.) × 950.