

LISTS OF PAPERS PUBLISHED BY THIS OFFICE.

"A Study of the Cytoplasmic Inclusions and Mitochondrial Phenomena During the Oogenesis of the Mouse",
"Quart. Journ. Micr. Sci.", vol. 71, 1933.

A STUDY OF THE CYTOPLASMIC INCLUSIONS DURING OOGENESIS,
SPERMATOGENESIS, MATURATION, FERTILISATION AND THE FIRST
CLEAVAGE DIVISION OF THE EGG OF THE MOUSE (MUS MUSCULUS)

"Presence of the Sperry Middle-Plate in the Fertilized Egg of the Mouse (*Mus musculus*)",
"Natur", vol. 145, 1940.

by

"A Study of the Cytoplasmic Inclusions during Maturation, Fertilisation and the First Cleavage Division of the Egg of the Mouse".

Accepted for publication in Quart. Journ. Micr. Sci., 1940.

R.A.R. GRESSON.

SUBMITTED AS ADDITIONAL EVIDENCE OF RESEARCH.

"The Effect of Increased Daily Illumination and of reversed Day and Night on the Oestrous Cycle of the Mouse (*Mus musculus*)",
"Proc. Roy. Soc. Edin.", vol. 60, 1940.

THESIS PRESENTED
FOR THE DEGREE OF D.Sc.



LISTS OF PAPERS EMBODIED IN THIS THESIS.

- "A Study of the Cytoplasmic Inclusions and Nucleolar Phenomena During the Oogenesis of the Mouse",
'Quart.Journ.Micr. Sci.', vol.75, 1933.
- "Effect of Ultra-centrifuging the Oocytes of the Mouse",
'Nature', vol.142, 1938.
- "A Cytological Study of the Centrifuged Oocyte of the Mouse",
'Quart. Journ. Micr. Sci.', vol.81, 1940.
- "Presence of the Sperm Middle-Piece in the Fertilised Egg of the Mouse (Mus musculus)",
'Nature', vol.145, 1940.
- "A Study of the Cytoplasmic Inclusions during Maturation, Fertilisation and the First Cleavage Division of the Egg of the Mouse".
- Accepted for publication in Quart. Journ.Micr.Sci.1940.

SUBMITTED AS ADDITIONAL EVIDENCE OF RESEARCH.

- "The Effect of Increased Daily Illumination and of Reversed Day and Night on the Oestrus Cycle of the Mouse (Mus musculus)",
'Proc. Roy. Soc. Edin.', vol.60, 1940.

ACKNOWLEDGMENTS.

The Thesis embodies the results of my own independent research, and is composed by me. The illustrations, with the exception of the photomicrographs, are my own original drawings.

All the research, with the exception of the work with the ultra-centrifuge and the preliminary observations on the centrifuged ovaries, was carried out in the Department of Zoology, University of Edinburgh.

The work with the ultra-centrifuge was carried out in the University Department of Zoology, Trinity College, Dublin. It is a pleasure to express my thanks to Professor J. Brontë Gatenby for granting me facilities to work in his Laboratory, and for the interest he has shown in this part of the work.

It is with pleasure that I thank Professor James Ritchie for granting me research facilities in the Department of Zoology, University of Edinburgh, and for the interest he has shown in my work.

I am indebted to Mr R.J. Fant, Department of Zoology, University of Edinburgh, for taking the photomicrographs and for photographing my original drawings. I take this opportunity of expressing my thanks for his help.

Most of the work was aided by a grant from the Earl of Moray Endowment of the University of Edinburgh.

INTRODUCTION.

A large number of papers have been published on mammalian oogenesis and spermatogenesis, but comparatively little work has been carried out with the aid of modern technique for the demonstration of the Golgi material. The writer, therefore, considered it desirable that investigations be undertaken, using modern osmic and silver methods, on the germ-cells of a mammal, and further, that this study should be made on the male and female germ-cells of the same species, so that a complete account be given for one species of mammal at least.

The literature on the behaviour of the Golgi material and mitochondria during the maturation and fertilisation of the mammalian egg is scanty. Although the sperm tail has been identified in the fertilised ovum of several mammals little attempt has been made to study its structure. Consequently, it was decided to follow the work on gametogenesis with a study of maturation and fertilisation with particular reference to the entry of the sperm middle-piece, and the part, if any, played by the Golgi material and mitochondria of the sperm during fertilisation.

The present investigations were undertaken, therefore, primarily in order to follow the history of the Golgi/

Golgi material and mitochondria during the oogenesis and spermatogenesis of the mouse, and to determine the part which these bodies play in the development of the ripe germ-cells and during the stages of fertilisation. The mouse was chosen as the egg contains but little yolk, the presence of which, in the eggs of many animals, tends to make observations on the cytoplasmic inclusions very difficult.

As the result of the present work it is possible to give a complete account of the behaviour of the Golgi material and mitochondria of the male and female germ-cells of the mouse during gametogenesis and fertilisation, and further it is claimed that Golgi material and mitochondria are introduced into the fertilised egg by the sperm, and that, consequently, the male, as well as the female, contributes to the cytoplasmic inclusions of the offspring.

During the course of the investigations further light was shed on certain other problems relating to the germ-cells. These matters, additional to the original purpose of the research, are dealt with in the appropriate sections of the thesis.

The writer's findings, with the exception of the work on spermatogenesis, have been published or accepted for publication. The first paper, on the oogenesis of the mouse, appeared in 1933. Owing to certain circumstances it was impossible to proceed immediately, as was/

was originally intended, with further investigations on the gametogenesis and fertilisation of the mouse, and it was not until 1937 that the work was again taken up. The original findings on the cytoplasmic inclusions of the oocyte were then checked, using fresh material and some additional methods of technique.

It is now possible to correlate the whole work and to draw certain conclusions, which was not possible in the separate papers. The writer therefore, considers it desirable that his findings be presented in a typed thesis, rather than in a series of separate papers. The observations and conclusions are presented, as far as possible, in the original form in which they appeared in the papers but it has been possible to eliminate a certain amount of repetition in the different sections of the work, and to add certain additional matter, chiefly in the form of discussion and conclusions. A discussion and a summary is included in each section of the thesis. A general discussion, including certain conclusions drawn from the whole work is given in a separate section.

Reference to previous papers on mammals and certain other animals, having a bearing on the present findings, will be found in the appropriate sections of this work. Although the writer consulted a large number of papers, works on gametogenesis and fertilisation/

fertilisation having no direct reference to the present investigations are not mentioned. Reference to these will be found in the papers cited.

The writer believes that the Golgi material is to be regarded as a substance which undergoes changes of form and distribution correlated with the growth and phases of activity of the cell, rather than as having a definite structure. Consequently, wherever possible, it is referred to as the Golgi material or Golgi substance. The term Golgi element or Golgi body, however, is often used to describe the individual bodies of the localised or dispersed conditions.

It was necessary to obtain fertilised eggs at definite periods after pairing had taken place. As mice usually pair during the night, a series of experiments were set up, the object of which was to induce pairing during the hours of daylight. The results of these experiments were published, and the paper is presented as an additional section to this thesis.

OÖGENESIS.

I. A STUDY OF THE CYTOPLASMIC INCLUSIONS
AND NUCLEOLAR EXTRUSIONS.

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1. PREVIOUS WORK.

Kingery (1918) records that in the mouse ovary there is an embryonic proliferation of cells from the germinal epithelium and a second proliferation commencing in the young animal of about one to two days old and continuing almost to sexual maturity. The cells from the first proliferation degenerate and are resorbed, while those of the second form the definitive ova. The development of the oocyte is divided into three stages: Stage A. includes the very early oocytes derived from the germinal epithelium. Stage B. is distinguished by a slight increase in the size of both cell and nucleus. Stage C. is marked by further increase in the size of the oocyte: a follicle wall is usually present. The cell remains in this stage until ready for maturation.

According to Kingery the mitochondria are granular and are situated round the nuclei of the germinal epithelial cells. As the oocytes increase in size the mitochondria come to lie in one end of the cell, forming a crescent-shaped mass; at the beginning of Stage C. they are distributed as granules, uniformly throughout the ooplasm. The mitochondria of the follicle-cells are described as threads, rods and granules. Kingery does not describe yolk-formation.

Lams and Doorme (1908) do not deal with the early stages/

stages of oogenesis of the mouse; consequently the youngest ovum to be described and figured is of considerable size and is surrounded by follicle-cells. The mitochondria are revealed as large granules collected in groups or scattered singly through the ooplasm, and also as fine granules many of which form short chains. In a later ovum which is undergoing maturation in the oviduct, the majority of the mitochondria are figured as collected into fairly compact masses distributed through the ooplasm; the individual granules, however, do not appear to be as large as in the earlier oocytes.

Globules occur at the periphery of the nearly fully formed oocytes; these are faintly brown-black after osmic acid, but appear as clear spaces after corrosive-acetic fixation. They are identified as fat-globules. In ova, undergoing maturation in the oviduct, plastic and deutoplasmic zones are present; the fat-globules and the majority of the mitochondria are situated in the latter. After the entrance of the sperm the mitochondria, although still in preponderance in the deutoplasmic zone, tend to be more evenly distributed in the cytoplasm.

In an egg containing a sperm nucleus a body coloured red by safranin and deep blue by haematoxylin is figured. This body is identified as a "pseudo-chromosome"/

"pseudochromosome" such as is described by Van der Stricht (1904) as giving rise to mitochondria in the egg of the bat. In a slightly more advanced mouse ovum numerous small bodies, similar in staining reactions to the large "pseudochromosome" of the earlier egg are present. Lams and Doorme consider that the "pseudochromosomes" result from a condensation of mitochondria, and that at a later stage they break up to form mitochondria again.

Branca (1925) referring to the ooplasm of the mouse egg, mentions that his observations agree with those of Lams and Doorme. His figures of oocytes in atretic follicles show granules and bodies which, apparently, correspond to the mitochondria and fat-globules respectively of Lams and Doorme.

Kingery (1914), dealing with follicular atresia in the ovary of the mouse, states that the cytoplasm of degenerating eggs stains more deeply than in the normal, and that numerous fat-globules are present. He does not describe Golgi bodies or mitochondria.

From the above account it will be seen that although certain aspects of mouse oogenesis have been described in detail no work has been carried out on the Golgi material or on the details of yolk-formation. Thus Lams and Doorme give a detailed account of oogenesis in the mouse, but owing to the fact that their researches were carried out at a time when work on the Golgi/

Golgi substance had not been greatly developed, there remains room for further inquiry into the form and behaviour of the cytoplasmic inclusions. Kingery's papers, although of more recent date, are concerned chiefly with the growth of the oocyte, the nucleus and related phenomena, and with atresia. Consequently, he does not appear to have employed technique suitable for the demonstration of the Golgi material.

2. MATERIAL AND METHODS.

For the study of the Golgi material and mitochondria, ovaries were fixed according to the methods of Aoyama, Cajal, Da Fano, Mann-Kopsch, Kolatchev and Flemming (without acetic).

The investigation of the nucleolus and nucleolar extrusions and of yolk-formation was carried out on material fixed in Flemming, Carnoy, and corrosive-acetic fixatives. Certain ovaries were treated according to Ciaccio's method for the identification of fats. As a further test for the presence of fats an ovary, fixed in Bouin's micro-formol, was treated with ether and subsequently stained in iron-haematoxylin and counter-stained with eosin. At a later date ovaries were fixed in formol and subsequently stained with Sudan IV according to the method of Kay and Whitehead (1935) for the demonstration of fat.

In all cases the material was dissected out as speedily as possible and immediately placed in the fixing fluid. For the study of the Golgi material and mitochondria, sections were cut 3μ and 5μ in thickness; the other material was cut in sections 5μ and 8μ in thickness.

3. OBSERVATIONS.(a) THE GOLGI MATERIAL AND MITOCHONDRIA.

For the demonstration of the Golgi material Cajal's and Aoyama's method were found to be most satisfactory. In material treated by these methods both Golgi bodies and mitochondria could be identified. Consequently, the following account, unless where otherwise stated, refers to tissue treated according to the technique of Aoyama and of Cajal.

In the early oocytes, which are situated in and below the germinal epithelium and have not yet acquired a follicle wall, a dark mass of material is situated at one pole of the nucleus, while smaller masses of similar appearance are scattered through the ooplasm (fig. 3, Pl. 1.). Dark masses occupy similar positions in preparations treated by the Da Fano, Mann-Kopsch, and Kolatchev methods. Owing to the structure and disposition of this material and to its reaction to silver and osmic techniques, it is identified as the Golgi substance.

The juxta-nuclear Golgi material of the young oocytes consists of rods and granules closely packed together, so that in many cases part of the mass appears as a solid body. This solid appearance, however, is, in all probability, a fixation effect. Most of the smaller/

smaller masses of Golgi material scattered through the ooplasm are closely similar to the larger, while a few seem to consist of single rods.

The mitochondria are revealed as small granules of a golden-brown colour distributed through the ooplasm but are especially numerous round the large mass of Golgi substance. (fig. 3. Pl. 1.).

In oocytes, which have acquired a follicle wall, consisting of a single layer of cells, the Golgi material is clumped at one pole of the nucleus so as to form a juxta-nuclear mass, similar in composition to that of the earlier oocytes (fig. 5, Pl.1. and figs. 10 a and b. Pl. 3). Only a few rods and granules remain outside this mass. On comparing this condition with that of the younger cells described above, it is seen that the majority of the smaller masses of Golgi material have joined the larger, thus forming a dense juxta-nuclear body.

At this stage the mitochondria are scattered through the ooplasm but appear to be most numerous in the vicinity of the nucleus and Golgi substance.

In slightly older oocytes the localised Golgi material begins to separate into the individual rods and granules of which it is composed. These become distributed through the ooplasm (figs. 6 & 7, Pl.1.) but remain more numerous, until a later stage of oogenesis, in the neighbourhood of the ooplasm originally/

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originally occupied by the juxta-nuclear body.

The mitochondria are now fairly evenly distributed throughout the ooplasm except at the periphery, where they are less numerous. In the older oocytes the Golgi bodies are scattered through the ooplasm in the form of large granules or thick rods, while most of the mitochondria are collected into clumps, (fig. 8, Pl. 2.). In late oocytes with a follicle wall of several cell-layers, the mitochondria are gathered into clumps, leaving the intervening ooplasm practically free of these bodies. The Golgi bodies occur distributed throughout the cell and on and around the mitochondrial clumps, (fig. 9, Pl. 2.) In the later oocytes, in mature follicles, this tendency of the Golgi material to collect round the groups of mitochondria is more marked; in many cases the mitochondria are difficult to observe owing to the number of the Golgi bodies around the clumps. In these oocytes the Golgi bodies appear to be more numerous than in the earlier cells.

Additional evidence in favour of the above findings was produced by an examination of the material treated by the Da Fano and osmic techniques. The Da Fano preparations contained oocytes at different growth stages, in which the form and disposition of the Golgi bodies and mitochondria agreed closely with the above account. In Mann-Kopsch and Kolatchev material the mitochondria were not so well shown as by the silver methods/

methods. They demonstrated, however, that while the mitochondria of the early oocytes are scattered through the ooplasm, those of the older cells occur in clumps. The Golgi material was revealed as deeply osmophil masses in close association with the nucleus of the very young cell, but in the later oocytes is distributed as granules or rods occupying similar positions to those of the silver preparations.

In ovaries fixed in Flemming's fixative (Gatenby's modification) and subsequently stained in iron-haematoxylin and light green, the ooplasm of many of the young oocytes, situated below the germinal epithelium, was filled with small granules, while in certain parts of the cell, chiefly at one pole of the nucleus, denser clumps of larger granules or rods occurred. The small granules are undoubtedly mitochondria, while the larger ones, owing to their size and disposition, are identified as the Golgi bodies. The latter, due to the technique employed, are imperfectly preserved.

In young oocytes, with a single follicle layer, masses of darkly stained material occupy similar positions to the Golgi material of the silver and Kolatchev preparations. Mitochondria in the form of fine granules occur distributed through the ooplasm.

In the more advanced oocytes numerous dark granules are scattered through the cell. It was not possible to/

to differentiate with certainty between Golgi bodies and mitochondria; this is due to the clumping of mitochondria and to the imperfect preservation of the Golgi material.

In ovaries treated according to Ciaccio's method for the identification of fats, the fats in the corpora lutea and stroma stain brightly with Sudan III. An examination of the early oocytes revealed bodies in the ooplasm which, although stained with Sudan III, were not of the same shade as the fats mentioned above. These bodies were of various sizes and shapes, and stained orange-brown. A large number of oocytes at this stage of growth were examined and in all cases the orange-brown bodies occupied similar positions to that of the Golgi material of the osmic and silver preparations (fig. 4., Pl.1) Moreover their subsequent behaviour is similar to that of the Golgi bodies, for in the young oocytes, they form a mass at one pole of the nucleus and at a later stage become distributed through the ooplasm. In the older oocytes they are more difficult to distinguish than in the earlier cells. There can be no doubt that these are the Golgi bodies which give a reaction with Ciaccio's technique, but do not stain brightly like the fats of the corpora lutea and stroma.

In silver and osmic preparations the Golgi material of/

of the cells of the germinal epithelium consists of a dark mass of material situated at one pole of the nucleus. The mitochondria occur scattered through the cytoplasm (fig. 2, Pl.1.) In Cajal and Aoyama preparations a dark juxta-nuclear body is situated at one pole of the follicle-cell nuclei, and is identified as the Golgi material (figs. 5,6,7, Pl.1, fig. 8, Pl.2). It appears to consist of rods and granules massed together as in the young oocytes or possibly of a network of filaments. In the older follicles the Golgi substance is similar in appearance (fig. 9, Pl.2). The mitochondria, in the form of rods and granules, occur scattered through the cytoplasm, but in the cells of the young follicles are more numerous in the part of the cell adjoining the oocyte (fig. 5,6,7, Pl.1, Fig. 8, Pl.2.)

It is worthy of note that the Golgi material in follicles consisting of a single layer of cells is situated in the cytoplasm between the nucleus and the surface of the cell bordering on the oocyte. In follicles consisting of several layers of cells, the Golgi material, in the majority of cases, is localised at the pole of the nucleus, situated towards the oocyte. The mitochondria are evenly distributed throughout the cytoplasm. An examination of cells, in late follicles, surrounding the follicular cavity reveal the Golgi substance/

substance of many cells as situated between the nucleus and the surface of the cell, directed towards the cavity; in other cases the position of the Golgi material varies from cell to cell. The probable significance of this phenomenon is discussed later.

In material treated by Ciaccio's method for the identification of fat, masses of material stain orange-brown with Sudan III; they occupy similar positions to the Golgi material of the follicle-cells as shown in silver and osmic preparations.

The Golgi material of the theca-cells is in the form of a mass of closely applied rods and granules, or possibly of a network of filaments, occupying a juxta-nuclear position. The mitochondria are distributed throughout the cell, (fig. 1, Pl.1)

(b) THE NUCLEOLUS AND NUCLEOLAR EXTRUSIONS.

The following investigations on the oocyte nucleolus and nucleolar emissions were carried out chiefly on material fixed in Carnoy, Flemming, and corrosive-acetic fixatives, and subsequently stained in iron-haematoxylin. The basophilia of the nucleoli and nucleolar emissions was determined by preparations fixed in corrosive-acetic and stained in Mann's methyl-blue eosin.

In the early oocytes, situated below the germinal epithelium, one to three nucleoli are usually present; in certain oocytes, however, three to five small nucleoli were observed (fig. 15, Pl.4.) In haematoxylin preparations the nucleoli are deeply chromophil, and as revealed by sections stained in Mann's methyl-blue eosin, are basophil.

In young oocytes, with a single layer of follicle-cells one to three basophil nucleoli are present. In sections stained in iron-haematoxylin the nucleoli are deeply stained homogeneous bodies. (fig. 16, Pl.4.)

The nuclei of the slightly older oocytes (with about 2 layers of follicle cells) contain numerous small homogeneous basophil bodies, many of which are in contact with the single nucleolus, while others are scattered through the nucleoplasm, (fig. 17, Pl.4.)

The/

The position and staining reactions of these bodies point to their nucleolar origin. In certain oocytes, a second nucleolus was observed; in most cases the latter contained small vacuoles (fig. 18, Pl.5.) Both types of nucleoli and the nucleolar emissions are basophil.

The older oocytes, surrounded by several layers of follicle-cells, usually contain a single deeply stained nucleolus; in certain oocytes, however, a second vacuolated nucleolus was observed. The nucleolar emissions are stained deeply by iron-haemotoxylin and, as described for the previous stage, are basophil. The nucleoli appear to be more faintly basophil than in the young oocytes (fig. 19, Pl.5).

In the late oocytes, situated in nearly mature follicles, the nucleolus has lost its spherical shape and, in many cases, appears to be giving rise to numerous nucleolar buds or emissions (fig. 20, Pl.5.) In some cases the central part of the nucleolus, stained more faintly than the periphery. Both the nucleolus and the nucleolar emissions are basophil. A second nucleolus was present in certain of the late oocyte nuclei examined; both nucleoli stained in the usual manner.

The presence in the older oocytes of nucleolar buds or emissions, close to and in contact with, the inside of the nuclear membrane suggests that these bodies are extruded/

extruded to the ooplasm. An examination of the late oocytes, surrounded by several layers of follicle-cells, produced further evidence in favour of this view; for numerous small bodies occur on the outside of the nuclear membrane and scattered through the ooplasm in the vicinity of the nucleus and towards the periphery of the cell (fig. 19, 20, Pl.5.) These bodies are closely similar in shape and staining properties to the nucleolar emissions situated in the nucleoplasm; in size they resemble the smaller nucleolar buds. They are identified as nucleolar extrusions. The extrusions were particularly well shown in preparations fixed in Carnoy's and in corrosive-acetic.

In no case were nucleolar extrusions observed passing through the nuclear membrane. In a few oocytes the nuclear membrane seemed to be pushed outward by the presence of emissions on its inner surface. This appearance was probably produced during fixation or the subsequent manipulation of the sections.

Owing to the presence of nucleolar buds in the nucleoplasm and to the occurrence of the same type of structure on the outside of the nuclear membrane, there appears to be little doubt that nucleolar material is extruded to the ooplasm. The nucleolar extrusions are, in all probability, passed through the nuclear membrane in solution and become condensed on reaching the ooplasm.

In/

In some of the late oocytes, in fully formed follicles, many of the nucleolar extrusions are more lightly stained than those of the younger oocytes, while numerous small faintly stained granules occur scattered through the ooplasm. (fig. 20, Pl.5). The appearance and position of these granules suggests that they may have been formed by the fragmentation of the less deeply stained nucleolar extrusions. No direct evidence in favour of this view was produced.

In some cases, stains but lightly, thus giving to the bodies a vacuolated appearance (fig. 1, Pl.4, fig. 18, Pl.3.)

In order to determine the nature of the cytoplasmic bodies, ovaries were treated according to Clauvic's method (-----) of fat, and according to the technique of Kay and Whithead. The fat present in the corpora lutea and in the serum was deeply stained by Sudan III, and by Sudan IV, while the cytoplasmic bodies gave a negative reaction. Consequently it is evident that these bodies are non-fatty. As a further test sections from an ovary fixed in Bouin's picro-formal were treated with ether in order to extract any fat which might be present; these sections were subsequently stained in iron-haematein and counter-stained in eosin. An examination of this material showed that the cytoplasmic bodies stained with

(c) YOLK-FORMATION.

In young oocytes, with about two layers of follicle-cells, a number of bodies make their appearance in the ooplasm; they are situated, in the majority of oocytes, towards the periphery but may also occur in the deeper cytoplasm. These bodies are roughly spherical or somewhat egg-shaped; they stain deeply with iron-haematoxylin except the central portion, which, in many cases, stains but lightly, thus giving to the bodies a vacuolated appearance (fig. 17, Pl.4, fig. 18, Pl.5.)

In order to determine the nature of the cytoplasmic bodies, ovaries were treated according to Ciaccio's method for the identification of fats, and according to the technique of Kay and Whitehead. The fat present in the corpora lutea and in the stroma was deeply stained by Sudan III, and by Sudan IV, while the cytoplasmic bodies gave a negative reaction. Consequently it is evident that these bodies are non-fatty. As a further test sections from an ovary fixed in Bouin's picro-formal were treated with ether in order to extract any fat which might be present; these sections were subsequently stained in iron-haematoxylin and counter-stained in eosin. An examination of this material showed that the cytoplasmic bodies stained with eosin/

eosin and were unaffected by the ether. A further proof of the non-fatty nature of these bodies was revealed by the fact that they did not blacken in ovaries fixed by osmic methods. As the result of these tests there can be no doubt as to the non-fatty nature of the cytoplasmic bodies; consequently, they are identified as protein yolk-spheres.

The method of yolk-formation could not be determined with certainty. In the young oocytes, before the nucleolar extrusions make their appearance in the ooplasm, a few yolk-globules are present; in the late oocytes, the yolk-globules are slightly more numerous and in the majority of cases they have increased in size. It seems likely that the nucleolar extrusions fragment into granules, and that the latter become dissolved in the ooplasm, their substance being added to the yolk-spheres already present. This matter is discussed further on page 39 . The amount of yolk present in all stages of the growth of the oocyte is scanty; even the late oocytes contain few more yolk-spheres than are present in the young cells (fig. 17, Pl.4, fig. 18, 19, 20, Pl.5).

A preliminary examination of sections of the upper parts of oviducts fixed in Bouin's fixative, showed that yolk-globules are present in fertilised eggs/

(3) ATRETIC FOLLICLES.

eggs and in cells of the two cell stage. In the two cell stage they appear to be fairly evenly distributed between the first two blastomeres (fig. 21, Pl.5).

the description of the cytoplasmic inclusions. For a summary of previous work on atretia see Branta (1928).

An examination of oocytes, which, although situated in atretic follicles had not yet undergone degenerative fragmentation, reveal the majority of the mitochondria (Cajal preparation) as clumped together, forming large masses while a few occurred scattered through the cell. The Golgi bodies are distributed through the cytoplasm in small groups or are closely applied to the mitochondrial masses.

Certain of the Fleming's material contained a greater number of atretic follicles than were present in the salivary preparations; For this reason and also because the corresponding nuclear changes could be followed, this enabling the stages of degeneration to be determined, the behaviour of the cytoplasmic inclusions was carefully studied in Fleming material. A comparison with the other preparations enabled the Golgi bodies and mitochondria to be identified.

In eggs containing the spindle of the first division the mitochondria occur in clumps and are also scattered singly through the cell. The Golgi bodies are situated chiefly towards the pole opposite the spindle, (fig. 22, Pl.5)

(d) ATRETIC FOLLICLES.

As atresia in the mouse has been described by several workers the following account is confined to the description of the cytoplasmic inclusions. For a summary of previous work on atresia see Branca (1925).

An examination of oocytes, which, although situated in atretic follicles had not yet undergone degenerative fragmentation, reveal the majority of the mitochondria (Cajal preparation) as clumped together, forming large masses while a few occurred scattered through the cell. The Golgi bodies are distributed through the cytoplasm in small groups or are closely applied to the mitochondrial masses.

Certain of the Flemming's material contained a greater number of atretic follicles than were present in the silver preparations; For this reason and also because the corresponding nuclear changes could be followed, thus enabling the stages of degeneration to be determined, the behaviour of the cytoplasmic inclusions was carefully studied in Flemming material. A comparison with the other preparations enabled the Golgi bodies and mitochondria to be identified.

In eggs containing the spindle of the first division the mitochondria occur in clumps and are also scattered singly through the cell. The Golgi bodies are situated chiefly towards the pole opposite the spindle, (fig. 11, Pl.3)

At a later stage the mitochondrial clumps are larger and denser, and the Golgi bodies are, for the most part, situated towards one pole of the egg. (fig. 12, Pl.3).

In eggs which have fragmented into several pieces the majority of the mitochondria are still clumped and the Golgi bodies distributed unevenly through the cytoplasm. Large vacuoles may be present (fig. 13, Pl.4).

The number and disposition of the yolk-globules in the early stages of atresia appear to be the same as in the normal oocytes. In the later stages, however, yolk could not be identified. During atresia the cytoplasm becomes filled with numerous fat-globules, and in some cases "crystalloid bodies" were also observed.

In material treated according to Ciaccio's technique irregularly-shaped granular masses were observed in the cytoplasm, these stained faintly with Sudan III and corresponded in position to the masses of mitochondria and Golgi elements revealed by silver and osmic methods. In most cases fat-globules were more numerous in the vicinity of these granules than in other parts of the cell. (fig. 14, Pl.4). In ovaries treated according to the method of Kay and Whitehead the degenerating/

degenerating oocytes contained numerous globules which stained with Sudan IV. In unsegmented eggs the globules are small and may not be very numerous; in eggs which have undergone fragmentation the fat-globules are larger and more numerous. They may be in clumps or distributed throughout the cytoplasm.

has been the subject of a certain amount of disagreement, especially before discussing the present findings it is necessary briefly to refer to certain papers on oocyte maturation. The first observations on the Golgi apparatus in the eggs of mammals was carried out by Sjvall (1906) working on the ovum of *Gavia*, who states that the Golgi material of the young oocyte is in the form of a hollow sphere situated at one pole of the nucleus. Later, the sphere breaks up and the fragments pass to the periphery. Sjvall (1907) and Sjvall's findings with those of Sjvall.

According to de Hertoghe (1913) the Golgi material of the early oocyte of the guinea-pig and rabbit is in the form of a network occupying a juxta-nuclear position. The Golgi elements of later oocytes, in primary follicles, are figured as scattered throughout the nucleus in the form of a loose mesh-work or collection of threads united by short extensions. In the older egg the Golgi material occurs as a loose network of threads.

4. DISCUSSION.

The above account of the disposition and behaviour of the juxta-nuclear Golgi substance appears to agree fairly closely with the findings of other workers on the Golgi material of the mammalian ovum. The actual structure of the localised Golgi material of the oocyte has been the subject of a certain amount of disagreement, consequently before discussing the present findings it is necessary briefly to refer to certain papers on mammalian oogenesis. The first observations on the Golgi substance in the eggs of mammals was carried out by Sjövall (1906) working on the ovum of *Cavia*, who states that the Golgi material of the young oocyte is in the form of a hollow sphere situated at one pole of the nucleus. Later, the sphere breaks up and the fragments pass to the periphery. Weigl (Nihoul, (1927)) and Kulesch's findings (1914) agree with those of Sjövall.

According to Rio Hortega (1913) the Golgi material of the early oocyte of the guinea-pig and rabbit is in the form of a network occupying a juxta-nuclear position. The Golgi elements of later oocytes, in primary follicles, are figured as scattered through the ooplasm in the form of a loose mesh-work or collections of threads united by short extensions. In the older eggs the Golgi material occurs as a loose network of/

of thick threads situated towards the periphery so that a clear space is left surrounding the nucleus.

Gatenby and Woodger, (1920) believe that the mammalian Golgi material "consists of numerous semi-lunar plates or rods and not of branched straight bodies as drawn by Hortega"; the appearance of branched rods, they state, is possibly due to distortion caused by formalin fixation.

Catteneo (1914) states that the Golgi material of the young oocyte of the bat, guinea-pig, and rabbit is in the form of a network situated at one pole of the nucleus. In the older oocytes the structure increases in size, breaks up, and passes to the periphery, where it forms a sort of fenestrated membrane in the neighbourhood of the pellucid zone.

Henneguy (1926), in a short note, states that the Golgi material of the young oocyte of the guinea-pig, occurs as "quelques amas irréguliers de petits cordons granuleux". They are disposed without order in the ooplasm, but in the late oocyte, surrounded by follicles with a follicular cavity, they are situated at the periphery.

Nihoul (1927) believes that the Golgi substance of the young oocyte of the rabbit is in the form of grains of batonnets forming a compact mass at one pole of the nucleus. Later, the localised Golgi material fragments into several masses which pass to the periphery/

periphery of the cell. With the growth of the egg it is probable that the amount of Golgi substance increases. In silver preparations the Golgi material of eggs at this stage is constituted "par une série de travées sans structure, ou présentant une structure finement granuleuse, et anastomosée". A tangential section of an egg which has just reached this stage gives the impression of a fenestrated membrane similar to that described by Cattaneo. In sections treated according to Weigl's method the Golgi material seems to be formed of filaments which appear as closely entangled and compact masses. The filaments are sometimes curved and give the impression of vesicles of which the wall is strongly coloured and the contents uncoloured. Nihoul believes that the appearance of the Golgi material in Silver preparations is due to precipitation on and between the filament.

The present findings for the mouse agree with those mentioned above in that the Golgi material of the young oocyte is at first in the localised condition and later breaks up and becomes scattered through the ooplasm. In the mouse ovum, however, the Golgi bodies are fairly evenly distributed throughout the cell; furthermore the dispersed Golgi elements appear as rods and granules and do not form a loose mesh-work or masses of entangled filaments, as described for the bat, guinea-pig/

guinea-pig and rabbit.

Since the publication of the writer's findings on the oocyte of the mouse, two further contributions have appeared on mammalian oogenesis. In one of these Aykroyd (1938) gives an account of the cytoplasmic inclusions of the human oocyte. The Golgi material of the young oocyte is at first juxta-nuclear and is figured as consisting of individual elements. Later, the Golgi bodies occupy a peri-nuclear position. In older eggs a retispersion takes place and the Golgi material, for the second time, occupies a juxta-nuclear position. Finally, the elements become dispersed and the majority come to lie under the zona pellucida. The second contribution referred to above is a paper by Beams and King (1938) on the cytoplasmic inclusions of the egg of the guinea-pig. In reviewing the literature on the Golgi material of the mammalian egg, these authors state that "Gresson seems to have made the most complete study of this subject in developing eggs of the mouse, and it will be seen that our results from the guinea-pig corroborate his to a great extent". Thus, in the young oocyte the Golgi material occupies a juxta-nuclear position surrounding the idiosome. With the growth of the oocyte it breaks up and is dispersed "in the form of irregular masses, rods and granules". Later it becomes broken up into small bodies or granules.

Beams/

Beams and King state that it is difficult to determine the structure of the localised Golgi material. Its appearance varies slightly with the degree of osmic acid impregnation, and also after staining with haematoxylin - "it appears to be in the form of rodlets or a loose net-work which surrounds and encloses the idiosome".

It is of interest to note that the localised Golgi material of the early oocytes of certain vertebrates, other than mammals, has been described as consisting of collections of individual Golgi elements. Thus Brambell (1926) has shown that the Golgi material of the young oocyte of the fowl consists of rods and granules surrounding the centrosphere. Bhattacharya and Lal (1929) have figured the Golgi elements in the young oocyte of the tortoise, *Kachuga*, as spherical or granular bodies forming a fairly compact mass beside the nucleus. Nath (1931) states that, in unstained Champy, preparations, the Golgi material of the young oocytes of *Rana tigrina* is present as granules occupying a juxta-nuclear position. In Da Fano and Kolatchev material "they tend to appear as one compact body". Later, they increase in size and finally become distributed throughout the ooplasm.

The writer believes that the juxta-nuclear Golgi material of the young oocyte of the mouse consists of individual elements which have come together to form a compact/

compact mass. This agrees with Nihoul's description of the young oocyte of the rabbit, with Aykroyd, for the human oocyte, and with the findings on the eggs of the other vertebrates cited above. Further, Beams and King believe that the localised Golgi material of the egg of the guinea-pig may be in the form of rodlets. The recent work of Aykroyd, and of Beams and King corroborate the writer's findings that the dispersed Golgi material becomes broken up into individual bodies.

As previously stated Lams and Doorme describe two types of mitochondria in the oocyte of the mouse - - large granules collected into groups or scattered singly through the cell, and fine granules which in some cases were arranged in short chains. In the opinion of the present writer some, at least, of the large granules of Lams and Doorme are Golgi bodies which, owing to the methods employed, were confused with the mitochondria. It has been shown, in the present paper, that the Golgi elements in the diffuse state tend to collect on the clumps of mitochondria and that imperfectly preserved Golgi bodies are present in certain of the Flemming preparations. It is evident, therefore, that without the aid of modern osmic and silver methods, suitable for the demonstration of the Golgi material, it would be impossible to distinguish with certainty between the two types of cytoplasmic inclusions.

The/

The mitochondria of the early oocyte are more numerous in the neighbourhood of the localised Golgi material; this agrees with Kingery's observation (1918) that as the early oocyte increases in size the mitochondria come to lie at one end of the cell. The present findings do not agree with Kingery's statement that the mitochondria form a crescent-shaped mass. According to Kingery the mitochondria later become evenly scattered throughout the cell; the present writer, however, finds that they are less numerous at the periphery, and that in the older oocytes they become collected into clumps.

According to Aykroyd (1938) the mitochondria of the early human oocyte are massed at one side of the nucleus; they then become dispersed forming a ring around the nucleus. Finally they become evenly distributed throughout the central ooplasm. The mitochondria of the egg of the guinea-pig, as recorded by Beams and King (1938), are scattered through the cytoplasm.

In view of previous work it is of interest to note that the Golgi material stained faintly with Sudan III. Bowen (1928) has pointed out that, although it is impossible to arrive at any definite opinion as to the chemical nature of the Golgi substance, Ciaccio (1910) found that the area of the Golgi material of the testis of the mouse stained with Sudan III, while Karpova (1925) and/

and Parat and Painleve (1926) attained positive results with the male germ-cells of *Helix* and Weiner (Bowen, 1928) with a modification of Ciaccio's technique, with the cells of the intestinal epithelium of the mouse.

Henneguy (1926) states that in the young follicle of the guinea-pig, when it consists of a single layer of cells, the Golgi material is situated between the nucleus and the surface of the cell in contact with the oocyte. In follicles in which the granulosa consists of several layers the situation of the Golgi material varies from cell to cell. In the cells of the discus proligerus the Golgi material is situated in the part of the cell directed towards the follicular cavity.

Henneguy believes that the follicle-cells which surround the young oocyte secrete a substance which is absorbed by the latter, and that with the appearance of the follicular liquid, which is the product of the cells, an inversion in the situation of the localised Golgi substance takes place.

The position of the Golgi material in the follicle-cells of the mouse, described in the present contribution, is closely similar to that of the Golgi material in the follicle-cells of the guinea-pig previously recorded by Henneguy. In the opinion of the writer the situation of the Golgi material in the young follicle of the mouse strongly suggests that it takes part in the formation/

formation of a secretion which is utilised by the growing oocyte. In appearance it closely resembles the polarised Golgi substance of glands-cells; the latter, however, is so well known that further discussion is not required. The inversion in the position of many of the Golgi bodies in older follicles would seem to support Henneguy's suggestion that the Golgi material plays some part in the formation of the follicular liquid.

As the mitochondria of the young follicle-cells are more numerous in the part of the cell next to the oocyte, it is reasonable to suppose that the mitochondria, as well as the Golgi material, may take part in the formation of a secretion which is absorbed in the oocyte.

The infiltration of Golgi material from the follicle-cells into the oocyte of certain vertebrates has been recorded. Bhattacharya (1931) has observed this process in the egg of the squirrel, rabbit and rat. The present writer finds no evidence that Golgi material infiltrates into the oocyte of the mouse. In support of the present findings Aykroyd (1938), for the human oocyte, and Beams and King (1938), for the egg of the guinea-pig, were unable to detect the passage of Golgi substance from the follicle-cells.

Attention has already been directed to the occurrence, in degenerating ova, of granules of varying size, which

The number of nucleoli observed during the present investigations agrees with Kingery's findings (1918); the occurrence of nucleolar extrusions, however, does not appear to have been previously recorded for the mouse ovum. It is of interest to note that although Lams and Doorme (1908) do not mention nucleolar emissions they figure what seem to be nucleolar buds in an oocyte nucleus.

The occurrence of nucleolar emissions on the inside of the nuclear membrane, and of closely similar bodies outside the nuclear membrane and scattered through the ooplasm, offers strong evidence in favour of the view that nucleolar material is extruded from the nucleus. It is highly probable that this material is passed through the nuclear membrane in solution and is condensed into granular form on reaching the ooplasm. This view of nucleolar extrusion has been put forward by Harvey for *Carcinus* (1929) and for *Antedon* (1931 b). The presence of small granules in the ooplasm suggests that the nucleolar extrusions finally fragment in a somewhat similar manner to that described by the writer (1931) for *Periplaneta*.

The yolk-globules of the mouse oocyte are not numerous; they appear chiefly towards the periphery of the oocyte, but a few may be situated in the neighbourhood of the nucleus. They were not observed to arise
in/

in relation to the cytoplasmic inclusions.

Harvey (1929 and 1931 a) has stated his belief that protein yolk arises under the influence of the mitochondria and Golgi bodies, from material derived from the plasmosome, ground cytoplasm, and from external sources. If this be true it is probable that the mitochondria, or Golgi elements, or both, play a part in the formation of the scanty yolk of the mouse ovum. Material derived from the nucleolar extrusions may be added to the yolk-globules, as suggested by Harvey (1929) for the protein yolk of *Carcinus*. Owing to the large number of nucleolar extrusions present, and to the scanty amount of yolk, it is probable that much of the nucleolar material remains dissolved in the ooplasm, possibly in the form of nutriment which is utilized at a later stage.

Beams and King (1938) point out that while the egg of the guinea-pig is not suitable for the study of yolk-formation, it affords no evidence that the Golgi material and mitochondria play a direct part in the synthesis of yolk "beyond that as integral parts of the cell system as a whole". They state that they wish to make it clear that they do not deny that the Golgi material or mitochondria may function directly in yolk-formation in other types of eggs.

Lams and Doorme (1908) state that fat-globules are present/

present at the periphery of the mouse oocyte and that they are faintly brown-black after osmic acid and appear as clear spaces after corrosive-acetic fixation. The present investigation shows that these globules, as demonstrated by Ciaccio's method, by the method of Kay and Whitehead and by the action of ether, acetic and osmic acid, are non-fatty in nature. The only ooplasmic fat-globules present are those of the oocytes situated in atretic follicles. These fats stain in a similar manner to those of the corpora lutea and stroma cells; the latter have been described by Deanesly (1930 a, and 1930 b).

Fat-globules in degenerating eggs of the mouse have previously been recorded by Kingery (1914) and by Branca (1925). The "crystalloid bodies" described by Kingery were observed during the present investigations.

occur in close association with the mitochondrial clumps and also scattered through the cytoplasm.

4. The mitochondria of the young oocytes occur scattered through the cytoplasm but are more numerous

in the vicinity of the nucleus and Golgi material.

Later, the majority of the mitochondria become collected into clumps.

5. The Golgi material of young follicles is situated between the follicle-cell nucleus and the pole of the cell directed towards the oocyte; in follicles consisting/

5. SUMMARY.

1. The Golgi substance of the germinal epithelium consists of a mass of material situated at one pole of the nucleus. The mitochondria occur scattered throughout the cytoplasm.
2. The Golgi material of the very early oocyte consists of rods and granules clumped together to form a large mass at one pole of the nucleus; smaller masses of Golgi material may also be present.
3. In the young oocyte, surrounded by a follicle wall, a single juxta-nuclear mass of Golgi material is present; at a slightly later stage the individual Golgi elements become distributed throughout the ooplasm. In the later oocytes the Golgi elements occur in close association with the mitochondrial clumps and also scattered through the ooplasm.
4. The mitochondria of the young oocytes occur scattered through the ooplasm, but are more numerous in the vicinity of the nucleus and Golgi material. Later, the majority of the mitochondria become collected into clumps.
5. The Golgi material of young follicles is situated between the follicle-cell nucleus and the pole of the cell directed towards the oocyte; in follicles consisting/

consisting of several layers the position of the Golgi material varies, while in fully formed follicles the Golgi material of many of the cells surrounding the follicular cavity is directed towards the cavity. The mitochondria of the follicle-cells occur scattered through the cytoplasm but are more numerous towards the pole of the cell adjoining the oocyte.

6. The number of nucleoli present in the early oocyte varies from one to five; the majority of the older oocytes contain a single nucleolus but two may be present. Extrusion into the ooplasm of nucleolar material takes place. The nucleoli and the nucleolar extrusions are basophil.
7. Fatty yolk is not present in the mouse ovum. It is suggested that the Golgi elements and mitochondria play a part in yolk-formation, and that some of the granules formed by the fragmentation of the nucleolar extrusions are added to the yolk-globules already present.
8. In degenerating eggs the mitochondria are clumped; the Golgi bodies occur in small groups or are closely applied to the mitochondrial clumps. In eggs which have undergone fragmentation the Golgi bodies/

bodies occur in groups, while the majority of the mitochondria are clumped. In material treated by Ciaccio's method for the identification of fats, appearances suggest that the Golgi elements, and possibly the mitochondria, give rise to fat. Yolk-globules could not be distinguished in the late stages of degenerating eggs.

DESCRIPTION OF PLATES 1 - 5.Lettering.

F., fat-globules; F.C., follicle-cells; G., localised Golgi material; Gr., granules formed by fragmentation of nucleolar extrusions; G.E. Golgi element; G.M., Golgi bodies and mitochondria; M., mitochondria; M.C., mitochondrial clumps; N., nucleus; Nu., nucleolus; Nu.B., nucleolar bud; Nu.E., nucleolar extrusion; Oo., Ooplasm; V., vacuole; Y., yolk.

PLATE 1.

Photographs of original drawings.

- Fig.1. - Theca-cells showing Golgi material and mitochondria. Cajal.
 Fig.2. - Germinal Epithelium showing Golgi material and mitochondria. Cajal.
 Fig.3. - Early oocytes; follicle wall not formed. Cajal.
 Fig.4. - Oocyte at same stage as fig.3. Ciaccio.
 Fig.5. - Oocyte with single layer of follicle-cells. Cajal.
 Fig.6. - Slightly later stage showing Golgi elements breaking away from juxta-nuclear clump. Cajal.
 Fig.7. - Later stage; most of the Golgi elements have become distributed throughout the ooplasm. Cajal.

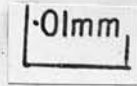
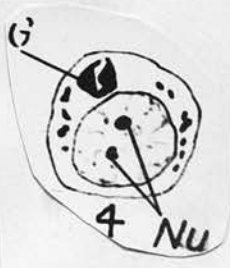
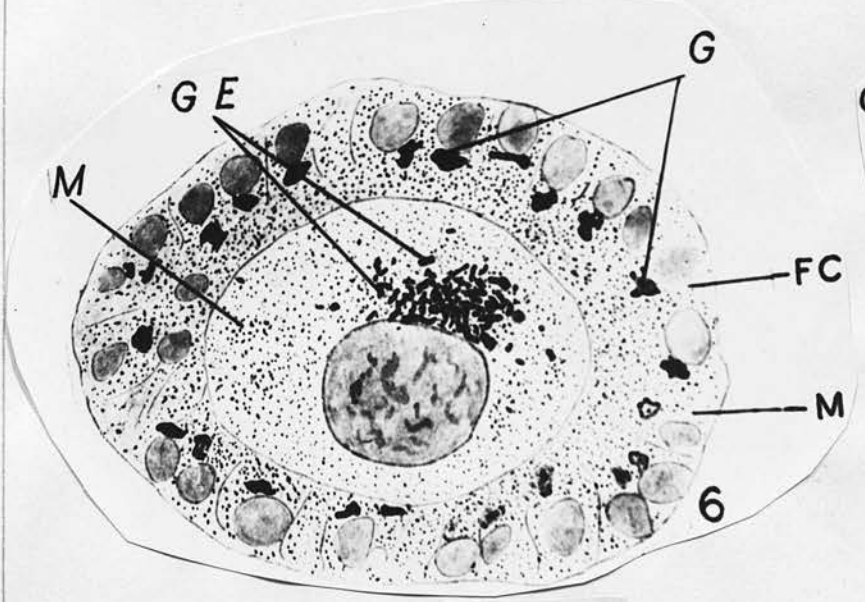
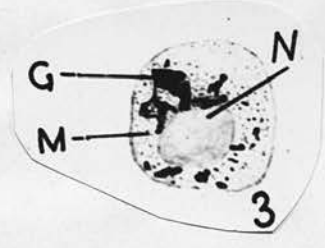
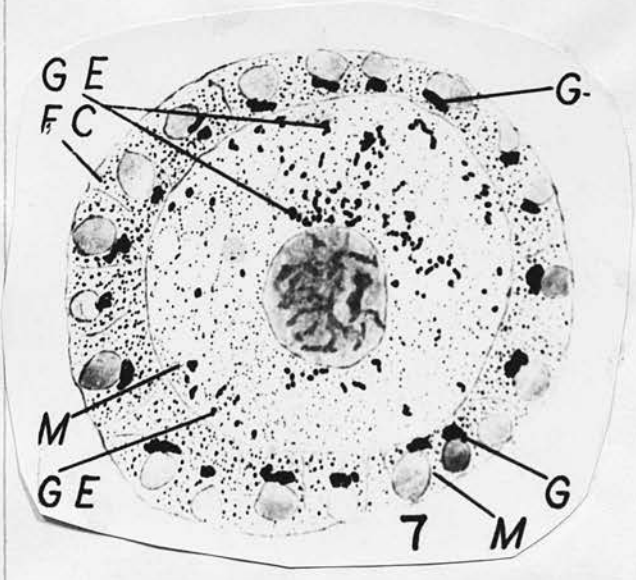
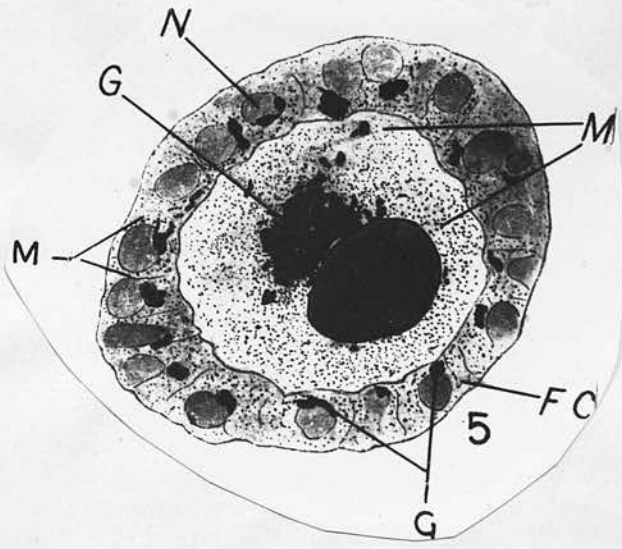


PLATE 1.

PLATE 2.

Fig.8. - Older oocyte with single layer of follicle-cells; the majority of the mitochondria are collected into clumps; the Golgi elements are distributed throughout the ooplasm. Cajal.

Fig.9. - Late oocyte; Golgi bodies and mitochondria clumped in ooplasm; the position of the Golgi material of the follicle-cells varies from cell to cell. Cajal.

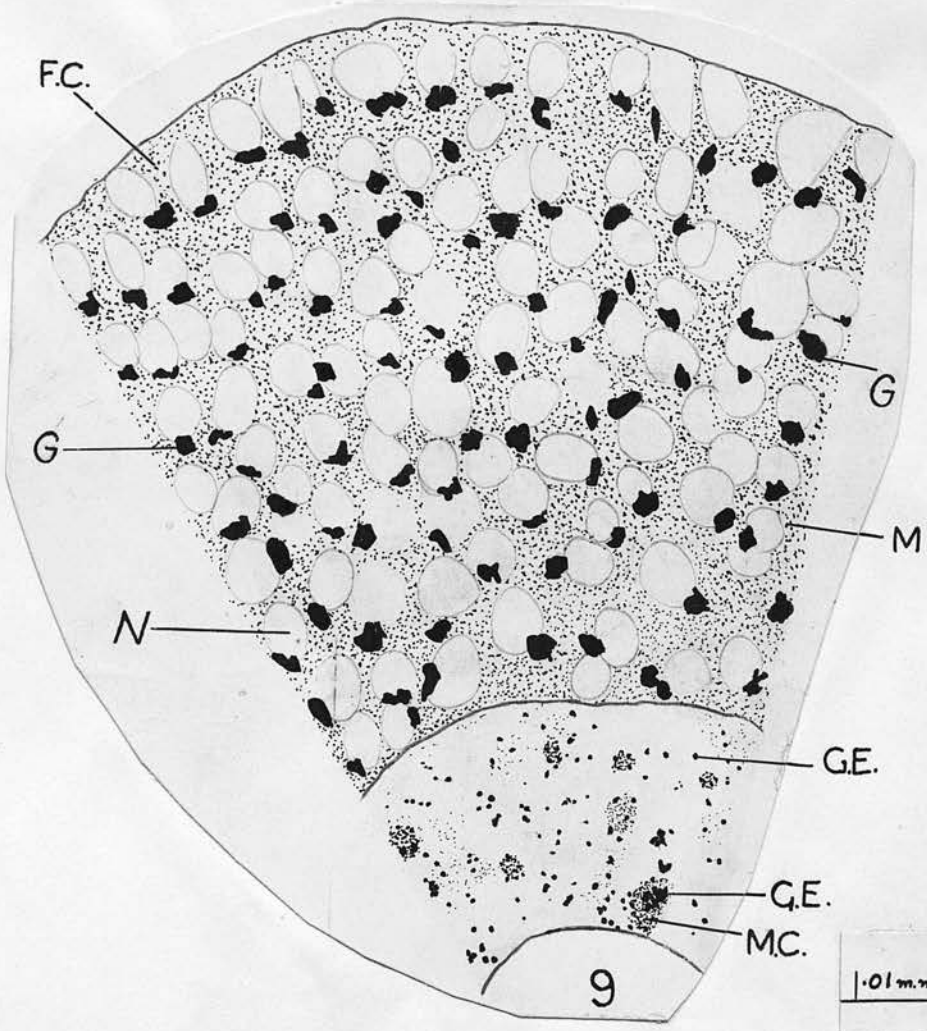
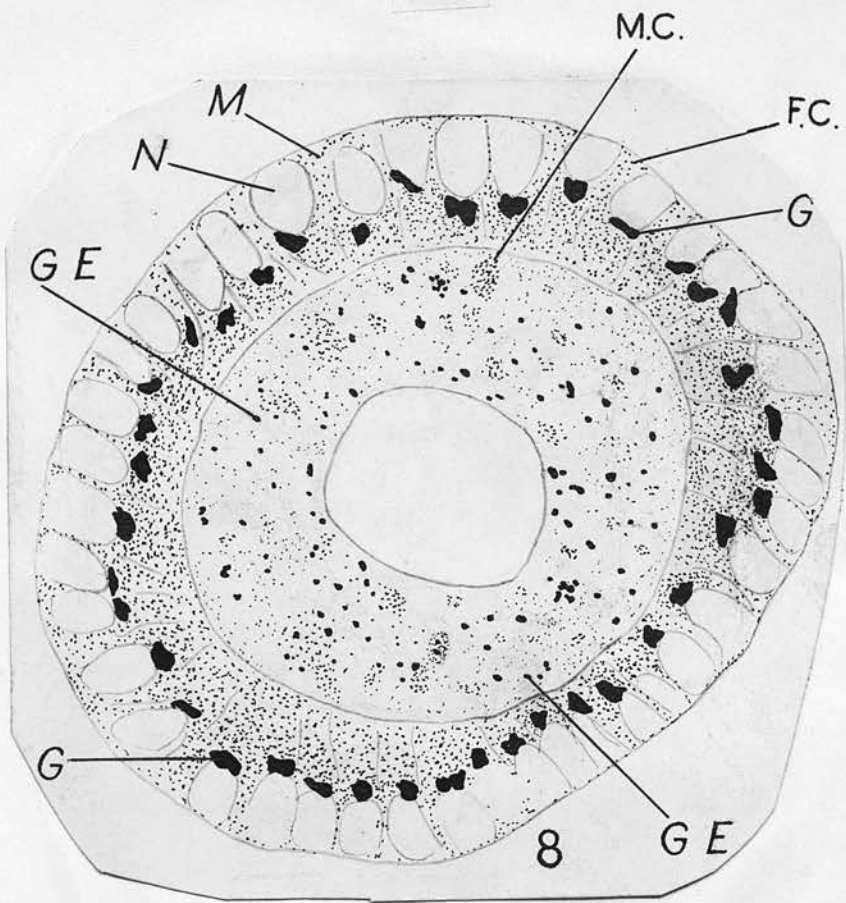


PLATE 3.

Fig.10 a and b - Young oocytes to show Golgi material in localised condition. Aoyama.

Fig.11.- Dividing oocyte from atretic follicle showing arrangement of Golgi elements and mitochondria. Flemming.

Fig.12.- Later stage of degeneration. Cajal.

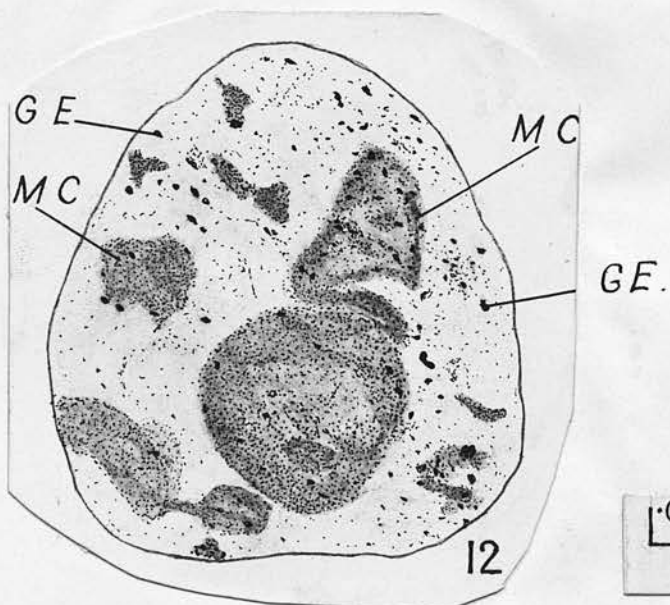
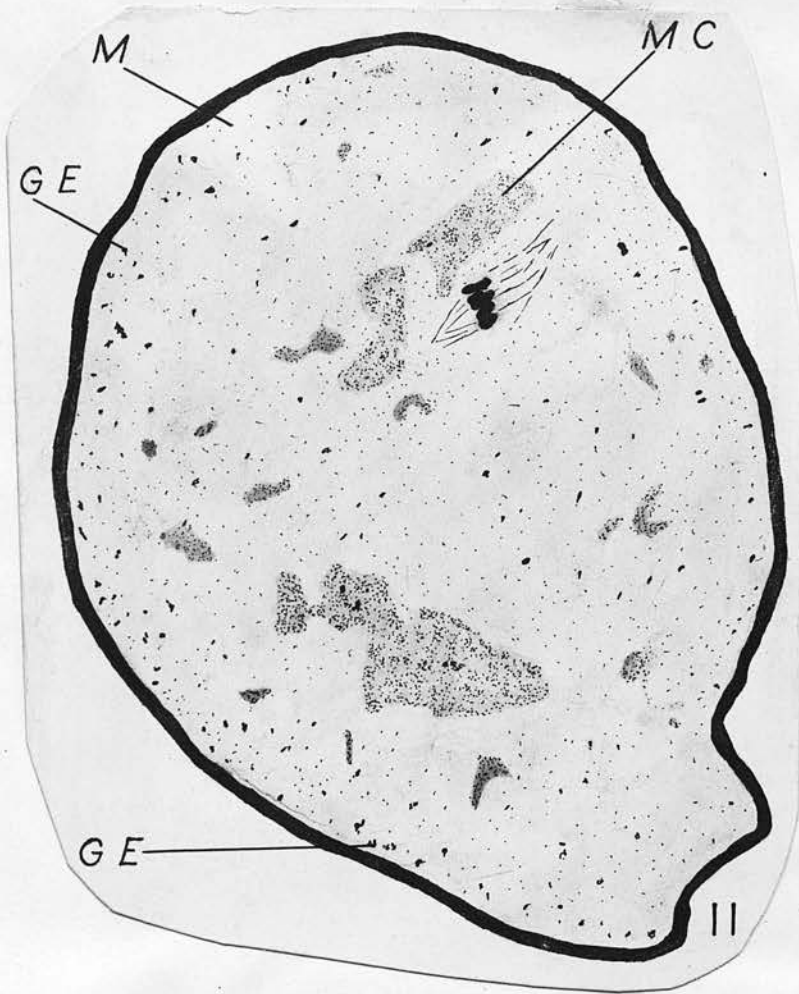
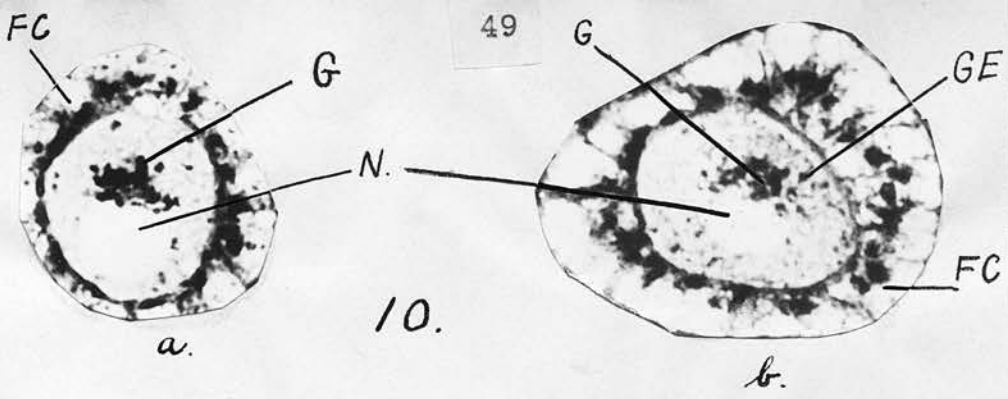
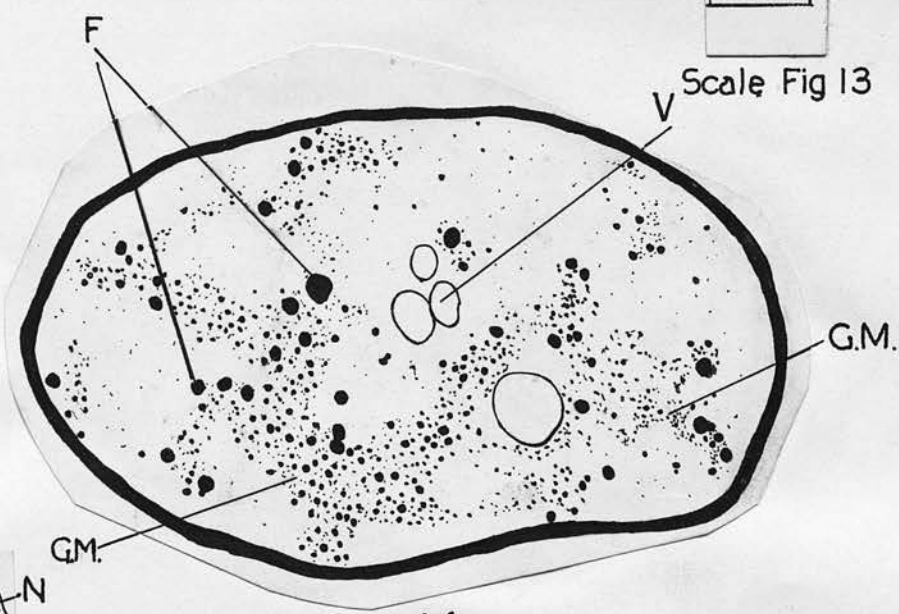
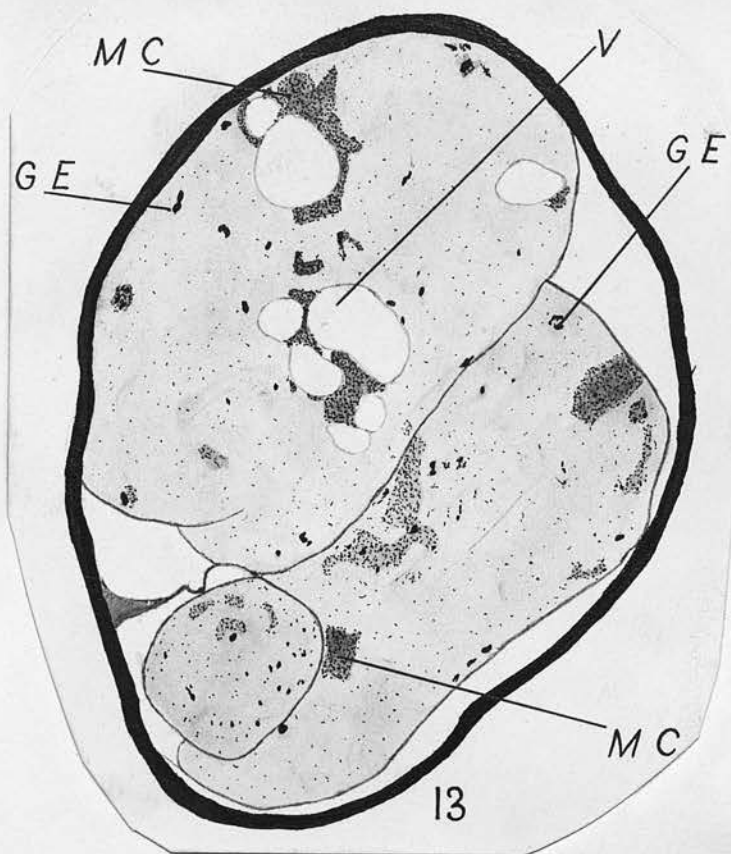
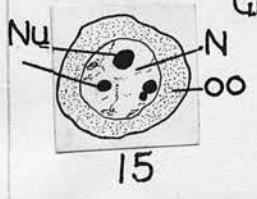


PLATE 4.

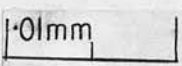
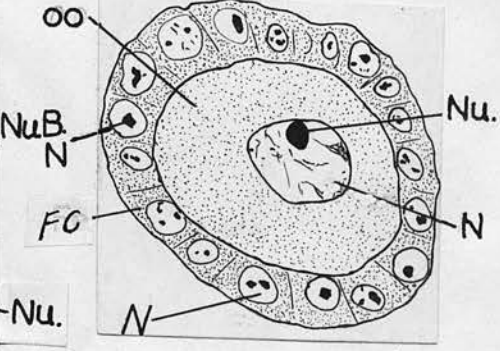
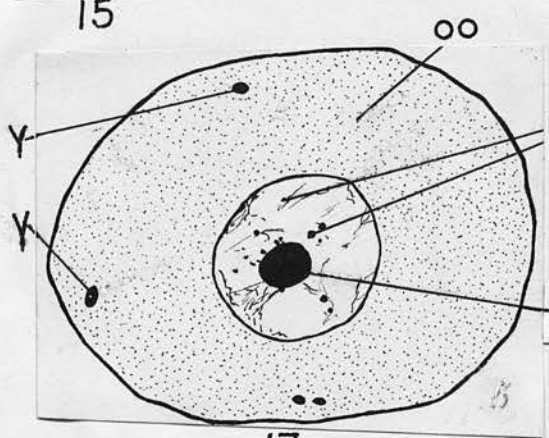
- Fig.13.- Egg from atretic follicle which has fragmented into several pieces. Flemming.
- Fig.14.- Oocyte from atretic follicle. Ciaccio.
- Fig.15.- Early oocyte situated below germinal epithelium. Carnoy.
- Fig.16.- Early oocyte with single layer of follicle-cells. Carnoy.
- Fig.17.- Oocyte surrounded by two layers of follicle-cells; showing nucleolar buds in nucleoplasm and yolk in cytoplasm. Carnoy.



Scale Fig 13



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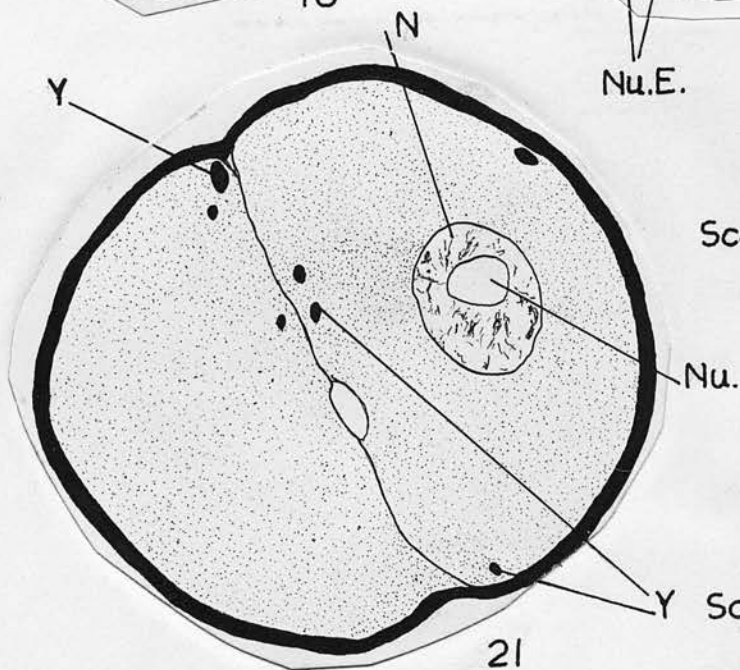
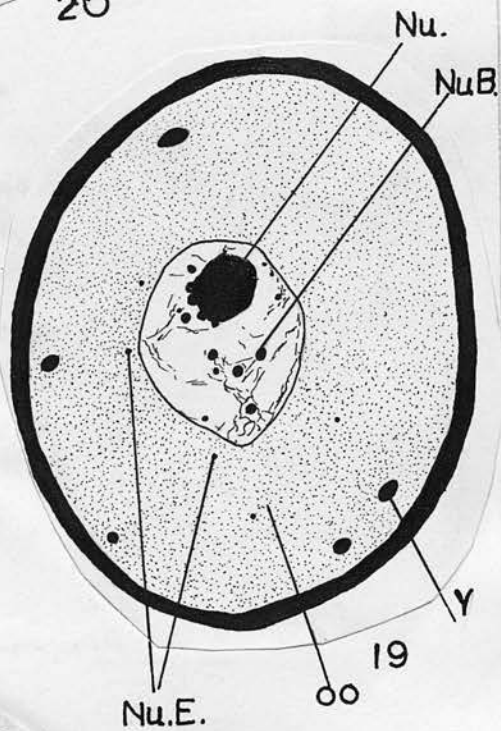
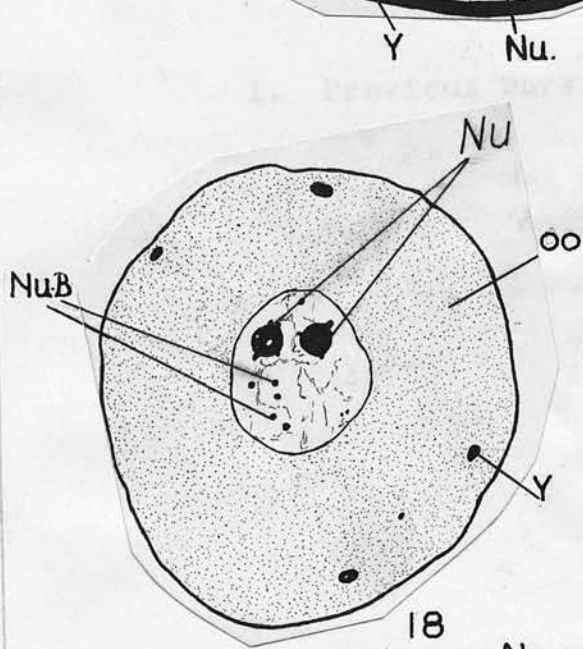
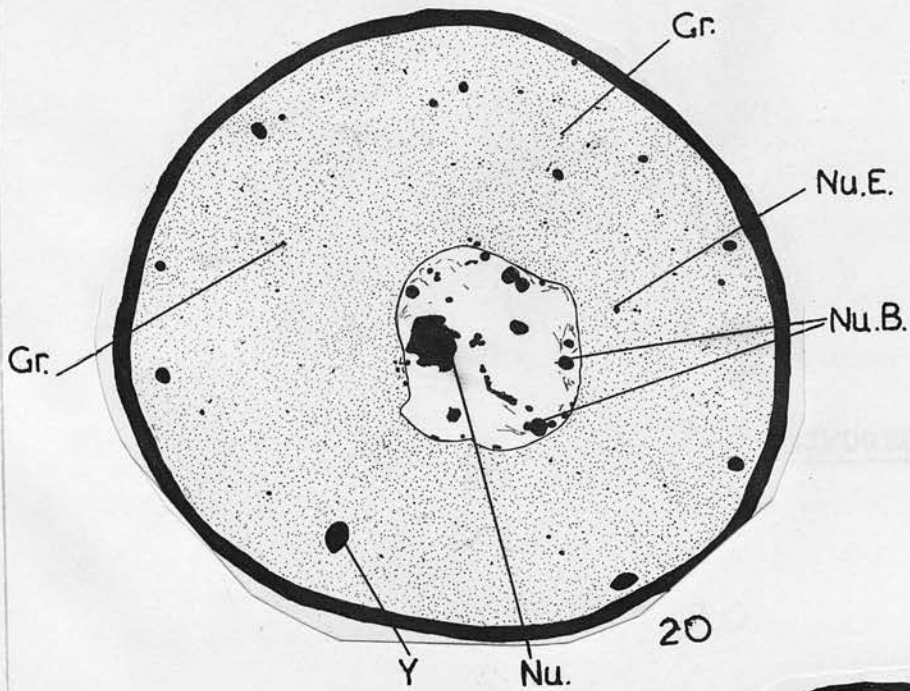


Scale Figs. 14-17



PLATE 5.

- Fig.18.- Same stage as Fig.17; two nucleoli present. Carnoy.
- Fig.19.- Late oocyte; nucleolar extrusions in cytoplasm. Carnoy.
- Fig.20.- Oocyte in nearly mature follicle; numerous granules and nucleolar extrusions scattered through the cytoplasm. Carnoy.
- Fig.21.- Two cell stage. Bouin.



0.01mm

Scale Figs. 18-20

0.01mm

Scale Fig. 21.

I. PREVIOUS WORK.

The investigations recorded in this section were undertaken in the hope that the study of oocytes which had been centrifuged in a driven ultra-centrifuge

OÖGENESIS.

II. A CYTOLOGICAL STUDY OF CENTRIFUGED OÖCYTES.

	<u>Page</u>
1. Previous Work.	55
2. Material and Methods.	59
3. Observations.	62
4. Discussion.	68
5. Summary.	75

of the young oocyte of the guinea-pig, situated in the germinal epithelium, possesses a well-defined yolk-nucleus in the form of a diplocaulic nucleus. Later, the yolk-nucleus breaks up and is dispersed in the form of irregular masses, rods, and granules. Just before the appearance of the follicular cavity there is a slight concentration

1. PREVIOUS WORK.

The investigations recorded in this section were undertaken in the hope that the study of oocytes which had been centrifuged, in the air-driven ultra-centrifuge, would throw further light on the cytoplasmic inclusions of the egg of the mouse. As is now well-known, material so treated has proved to be of special value for the study of the inclusions and components of the cytoplasm in other cells.

Apart from a contribution by Beams and King (1938) on the ovary of the guinea-pig, and the work of the present writer on the ovum of the mouse, there does not appear to be any published work on the ultra-centrifuged mammalian ovum.

Beams and King (1938) have shown that the Golgi material of the young oocyte of the guinea-pig, situated in the germinal epithelium, possesses a "more or less concentrated type of Golgi apparatus". As the oocyte passes into the stroma of the cortex "a well defined yolk-nucleus in the form of a diplosome-idiosome-Golgi apparatus complex makes its appearance in a juxta-nuclear position." Later, the "yolk-nucleus" breaks up and is dispersed in the form of irregular masses, rods, and granules. Just before the appearance of the follicular cavity there is a slight concentration of/
of/

of Golgi material at the periphery of the cell. The mitochondria are granular and are fairly evenly distributed throughout the cytoplasm. It will be seen from the above account that the distribution of the cytoplasmic inclusions during the growth of the egg of the guinea-pig is similar to that of the mouse, except that in the mouse there is no concentration of Golgi material at the periphery, and that in the older oocytes the mitochondria tend to clump.

Beams and King (1938) have centrifuged the ovaries of the guinea-pig at 150,000 times gravity for thirty minutes. In the centrifuged egg the cytoplasmic inclusions and components are stratified, according to their decreasing specific gravity, in the following order: mitochondria in the centrifugal half of the cell; the nucleus; material of the "yolk-nucleus" in the middle or in the centripetal half of about 75 per cent. of the eggs, in the other 25 per cent. this material may be displaced among the mitochondria; yolk-granules when present; a layer of clear cytoplasm; fat-globules, when present, at the centripetal pole. The authors are unable to state whether the variation in the distribution of the material of the "yolk-nucleus" is due to difference in specific gravity within the cell, or to mechanical reasons. The nucleus is usually stratified with the nucleolus and chromatin at/

at the centrifugal pole; it may or may not show marked displacement.

Beams, King, and Risley (1934) have ultra-centrifuged the eggs of Rana aurora, and have found that the contents are stratified into three layers: a centripetal layer of fat; a middle layer of clear protoplasm; a centrifugal layer of yolk containing pigment granules. Beams and King (1936) have obtained similar results for the fertilized eggs of Ascaris suum. These authors did not determine whether or not the cytoplasmic inclusions of the egg of Rana and of Ascaris were stratified.

Norminton (1937) ultra-centrifuged the oocytes of Lumbricus terrestris for four minutes at a force of about 40,000 times gravity. The egg was stratified into four layers: fat-globules at the centripetal pole; Golgi bodies and the nucleus; mitochondria; a layer of non-granular substance at the centrifugal end of the cell.

Singh (1938) ultra-centrifuged the oocytes of the Pigeon for fifteen to twenty minutes at pressures varying from 45 to 75 lb. per square inch. The cytoplasmic inclusions and components are arranged in the following layers: fat and cholesterol at the centripetal end of the cell; a layer of granular Golgi elements; a layer of yolk; a layer of mitochondria at the centrifugal end of the cell. Singh states that the nucleus is sometimes in the Golgi layer and sometimes centrifugal to it.

"This depends to some extent on the pressure of centrifuging/

centrifuging, and on the state of the oocyte". In very young oocytes the nucleus is displaced but little.

It will be seen, from the above account, that fat is the lightest substance in the cell, and always occupies the centripetal pole. There is, however, a certain amount of variation in the disposition of the other layers. In the pigeon and in Lumbricus the fat is followed by the Golgi layer, while in the guinea-pig a layer of clear cytoplasm and a layer of yolk intervenes between the fat and the Golgi elements. In the pigeon the yolk is stratified centrifugally to the Golgi layer. In the guinea-pig and in the pigeon the mitochondria are situated in the centrifugal end of the cell, while in Lumbricus a layer of non-granular substance occurs at the centrifugal pole.

The centrifugal ovaries were fixed in formalin, and subsequently stained with Sudan IV according to the method of Kay and Whithead (1935) for the demonstration of fat.

Uncentrifuged ovaries were fixed and used as control material.

2. MATERIAL AND METHODS.

The ultra-centrifuge used was one employed by Professor Gatenby and his students, and is similar to that described by Beams, Weed, and Pickles (1934).

The ovaries were dissected out, placed in saline solution in the rotor of the centrifuge, and immediately centrifuged. The centrifuge was run at a pressure of 50 lb. per square inch for twelve to fourteen minutes: this gives a force of about 130,000 times gravity. After centrifuging, the ovaries were removed from the rotor, as speedily as possible, and placed in the fixing fluid. It was found that material fixed according to the method of Mann-Kopsch gave very good results. Some of the centrifuged ovaries were fixed in formol, and subsequently stained with Sudan IV according to the method of Kay and Whitehead (1935) for the demonstration of fat.

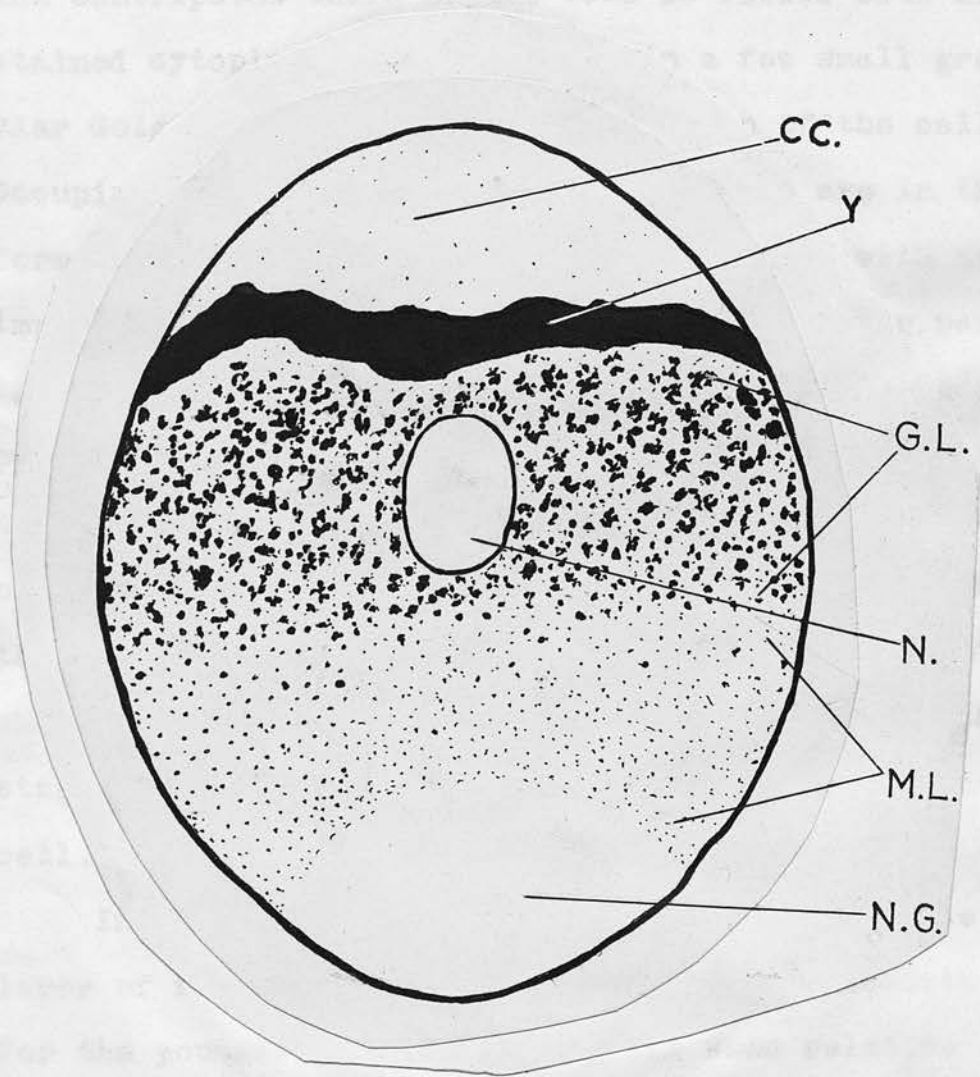
Uncentrifuged ovaries were fixed and used as control material.

TEXT-FIGURE 1.

Generalised diagram of centrifuged oocyte with three to four layers of follicle-cells.

Lettering.

C.C., lightly stained centripetal layer with a few small granular Golgi elements; G.L., Golgi layer; M.L., mitochondrial layer; N., nucleus; N.G., centrifugal non-granular layer; Y., yolk.



TEXT-FIGURE 1.

3. OBSERVATIONS.

In the very young oocytes the cytoplasmic inclusions and components are stratified into three layers. The centripetal third of the cell is filled with lightly stained cytoplasm which may contain a few small granular Golgi elements. The middle region of the cell is occupied by a layer of Golgi bodies which are in the form of curved rods, small spherical bodies with deeply impregnated rims, and of granules. The greater part of the nucleus lies in the Golgi layer, its centripetal pole, however, is free of Golgi elements and lies in the lightly stained cytoplasm of the centripetal end of the cell. The region between the Golgi layer and the centrifugal pole is filled with granular mitochondria. Each of the three layers occupies, at this stage, approximately one-third of the volume of the cell.

In many of the oocytes surrounded by a single layer of follicle-cells, the three layers, described for the younger oocytes, occupy the same relative positions; there is, however, variation in the situation of the nucleus. In many cases the centripetal pole of the nucleus is free of Golgi material (fig. 22, Pl. 6), while in other oocytes the nucleus is completely surrounded by Golgi elements. In a few eggs a small area of non-granular cytoplasm is present at the centrifugal pole/

pole of the cell.

In oocytes with about two layers of follicle-cells the nucleus usually lies in the Golgi layer. Non-granular cytoplasm occupies the centrifugal pole of the cell. In some of the eggs, at this stage of development, the centripetal end of the nucleus lies in the clear cytoplasm, while in a few a narrow layer of deeply stained material is situated centripetally to the Golgi layer. The narrow layer is identified as yolk (fig.23, Pl.6).

In larger oocytes (three to four layers of follicle-cells) the centripetal end of the cell is filled with lightly stained cytoplasm, which usually contains a few small Golgi elements. The Golgi layer, as in the earlier stages, occupies the middle part of the cell; it tends, however, to be differentiated into two regions. In the centripetal region of the Golgi layer mitochondria are few or absent, while in the centrifugal part mitochondria are numerous. Non-granular cytoplasm still occupies the centrifugal end of the cell, and the layer of mitochondria lies between this area and the Golgi material. Yolk is situated between the Golgi layer and the centripetal cytoplasm (figs. 24, 25 and 26, Pl.6; Text-fig. 1). The position of the nucleus varies slightly. In the majority of cases it is completely surrounded by the Golgi elements, but in a few instances/

instances the centrifugal pole lies in the mitochondrial layer.

In oocytes with more than four layers of follicle-cells, non granular cytoplasm is absent from the centrifugal end of the cell, so that mitochondria extend from the Golgi layer to the centrifugal pole.

In the mature oocyte the centripetal end of the cell is occupied by lightly stained cytoplasm, which may contain a few small Golgi elements. This is followed by a thin layer of yolk. The Golgi material occupies the middle region, but the division of the Golgi layer is more marked than in the preceding stage. The Golgi elements are closely packed together to form a dense layer next to the yolk. The centrifugal part of the layer contains fewer Golgi bodies, while mitochondria are numerous. The mitochondrial layer occupies, approximately, the centrifugal third of the cell (figs. 27 and 28, Pl.7). In some of the cells examined a few Golgi elements were identified in the mitochondrial layer. There is, therefore, a more gradual transition between the mitochondrial and Golgi layers than in the younger eggs. The nucleus of the mature oocyte is always situated in the layer of mitochondria, and is usually in the extreme centrifugal end of the cell. The nucleolus, or nucleoli, are always close to, or at the/

the centrifugal pole of the nucleus. In many of these oocytes the nucleus is dumb-bell shaped; a large single nucleolus lies against the nuclear membrane at the centrifugal pole (fig. 31, Pl.7). In other cases a large nucleolus occupies a similar position, but the nucleus is very irregular in outline, and the periphery is frequently drawn out into processes (fig. 30, Pl.7). In a few of the mature oocytes the nucleolus is thrown out of the nucleus and is present at the centrifugal pole of the cell. In these cases the nucleus is situated, in the mitochondrial layer, centripetally to the nucleolus.

Centrifuged ovaries were fixed in formol and subsequently stained with Sudan IV. Fat was not present in the oocytes, although fat-globules in other parts of the ovary gave a positive reaction. An examination of uncentrifuged control material failed to reveal the presence, in oocytes, of globules or granules which stained with Sudan IV.

Owing to the stratification of the cytoplasmic inclusions centrifuged oocytes are favourable material in which to study the Golgi elements and mitochondria. In the young oocytes, at the stage when the Golgi material of the normal oocyte is localized, the Golgi elements are in the form of curved rods, granules, and irregularly shaped bodies. Some of the irregularly shaped/

shaped bodies have a deeply impregnated rim, so that they appear to be somewhat ring-shaped. In the mature oocytes the Golgi bodies are smaller, and are present chiefly as granules and short rods; a few ring-shaped bodies may, however, be present. The mitochondria are granular throughout all stages of the growth of the oocyte. In the older eggs the granules are more numerous, and tend, for the most part, to be larger than in the young oocytes.

The examination of preparations of uncentrifuged control material confirmed previous findings that the Golgi elements are, like those of the centrifuged material, in the form of rods, irregular shaped bodies, and granules. Many of the Golgi bodies of the older oocytes are larger than those of the centrifuged eggs; this appears to be due to two or more elements coming together and forming a clump.

A number of follicles with degenerating oocytes were observed in sections of the centrifuged ovaries. Two of these follicles contained abnormal eggs which were unsegmented. The cytoplasm contained numerous fat-globules of various sizes. Osmiophilic granules were present scattered amongst the fat-droplets; these granules are probably Golgi elements. Mitochondria were not identified with certainty. Some of the globules and granules were present in groups, but were not/

not stratified. Certain follicles contained eggs which had fragmented to form several bodies. In most of these bodies the cytoplasm was filled with fat-droplets, while Golgi elements were few or absent. As in the earlier stages of degeneration, the cytoplasmic components were not stratified. Atretic follicles were not identified in the centrifuged material fixed in formol and stained with Sudan IV. Degenerating oocytes in uncentrifuged control material contained numerous fat globules which were brightly stained with Sudan IV.

An examination of centrifuged ovaries showed that there was considerable variation in the distribution of the cytoplasmic inclusions of the follicle-cells. In some of the centrifuged follicles the Golgi elements and mitochondria were not appreciably moved from their normal position, while in others the Golgi material lies between the nucleus and the centripetal end of the cell, or is situated laterally to the nucleus. In these cells the mitochondria occupy the centrifugal end of the cell. The variation in the position of the cytoplasmic inclusions indicates that the force necessary to bring about stratification of the oocyte is not always sufficient to produce a corresponding change in the follicle-cells.

Daniels (1936), describing ultra-centrifugal preparations, stated that Golgi bodies, in the form of small granules and short rods, are present among the fat-globules at the extreme centripetal pole while/

4. DISCUSSION.

The present account of the ultra-centrifuged oocyte of the mouse agrees in general with that of Beams and King (1938) for the guinea-pig; there are, however, certain differences. In the eggs of the earthworm, pigeon, and guinea-pig, fat is the lightest substance and occupies the centripetal pole. It is remarkable that fat is absent from the ovarian egg of the mouse; the present findings, as the result of tests with Sudan IV, confirm earlier observations. In the egg of the mouse and of the guinea-pig a layer of yolk is situated between the Golgi layer and the clear centripetal cytoplasm. Singh (1938) has shown that in the egg of the pigeon yolk is stratified between the mitochondria and the Golgi material. He claims that the yolk is mitochondrial in origin, and states that this would explain the stratification of the yolk close to the layer of mitochondria.

In the oocyte of the mouse a few small granular Golgi bodies usually lie in the clear cytoplasm in the centripetal end of the cell. A similar condition has not been recorded for the egg of the earthworm, pigeon, or guinea-pig. Daniels (1938), describing ultra-centrifuged gregarines, stated that Golgi bodies, in the form of small granules and short rods, are present among the fat-globules at the extreme centripetal pole, while/

while irregular ring-like Golgi elements are situated centrifugally to the granules and rods.

Beams and King found that the "yolk nucleus" (Golgi) material in about 75 per cent. of the guinea-pig ova examined was situated near the middle or in the centripetal half of the cell. In the remaining 25 per cent. it is found amongst the mitochondria. It is of interest that in some of the mature eggs of the mouse Golgi elements were identified in the mitochondrial layer, and, further, that in the older cells the Golgi layer is differentiated into two parts. This division of the layer of Golgi material into two regions would appear to be due in part to changes in the specific gravity of some of the mitochondria. In the young oocytes a few granular mitochondria can usually be identified scattered amongst the Golgi elements. In the older oocytes some of the Golgi material forms a narrow band centrifugal to the layer of yolk; a few mitochondria may be present in this region. In the centrifugal part of the Golgi layer granular mitochondria are numerous. It is evident, therefore, that many of the granules are less dense than in the younger oocytes.

The presence of a non-granular layer at the centrifugal pole of oocytes of a certain age, and its absence from/

from the young and from the mature oocytes may be due to changes in viscosity correlated with the growth of the egg. A centrifugal non-granular layer is not present in the egg of the guinea-pig, but is described by Normington (1937) for the oocyte of the earthworm. In osmic and silver preparations this region is composed of highly refringent globular masses. Normington concludes that as this substance is not visible in control material, it must be a fluid present in the form of minute droplets which are heavier than the cytoplasm and, when centrifuged, collect at the centrifugal pole. The appearance of droplets of a similar nature, at a certain stage in the growth of the mouse oocyte, may account for the centrifugal non-granular layer. If such droplets are present they must disappear, or undergo a change in composition, at a later stage.

The nucleus of the young oocyte of the mouse, in centrifuged material, is either completely surrounded by Golgi material or lies with its centripetal pole in the clear cytoplasm. At this stage the nucleus is not displaced to any great extent from its normal position; its specific gravity, therefore, differs but little from that of the surrounding cytoplasm. With the growth of the oocyte the nucleus becomes relatively increasingly heavy, so that in the mature egg it lies in the mitochondrial layer at, or close to, the centrifugal pole of/

of the cell. The nucleolus, or nucleoli, are always the heaviest structures in the nucleus. In the mature oocyte the nucleus is often dumb-bell shaped, or else is very irregular in outline. In these cases the appearance of the nucleus suggests that it is thrown towards the centrifugal pole by the weight of the nucleolus. Further evidence in support of this view is afforded by the examination of eggs in which the nucleolus is thrown out of the nucleus. The nucleolus is always at the centrifugal pole, while the nucleus is situated more centripetally. It would appear that the position of the nucleus, in the centrifuged mature oocyte of the mouse, is largely determined by the nucleolus, and that the nucleolus increases in density with the growth of the egg. It is of interest that Singh figures the nucleus of the oocyte of the pigeon as being somewhat dumb-bell shaped and with chromatin at its centrifugal pole.

Beams and King (1935), working on centrifuged mammalian spinal ganglion cells, found that the nucleus is often elongate and sometimes dumb-bell shaped. This, they state, suggests that some of the nuclear contents are slightly heavier than others. They found that the nucleus is stratified into three layers of which the nucleolar material is the heaviest. They point out that the parts of the nucleus do not appear to differ greatly in/

in density from the cytoplasm, and that the different positions of the nucleus in centrifuged cells "are probably explained by the assumption that the specific gravity of the nucleus does not differ from cell to cell in the same degree as does that of the cytoplasm".

Beams and King (1938) found that the nucleus of the guinea-pig egg may or may not show marked displacement. The nucleolus and chromatin are situated at the centrifugal pole.

The present observations on the ultra-centrifuged oocyte add further evidence in support of the writer's view that the localised Golgi material of the mouse egg is not in the form of a network, but consists of rods and granules clumped together to form a juxta-nuclear mass. In the young oocyte the elements of the Golgi layer consist of rods, irregularly shaped bodies, and granules. It is reasonable to suppose that if the localised Golgi material be in the form of a network, then it might, sometimes, be displaced as a whole, or if broken up, by the centrifugal force, some of the strands of the network would be present as filaments. That the Golgi bodies of the young oocytes are always in the form of rods, irregularly shaped bodies, and of granules, and that long filaments have not been identified argues against the existence of a network. As the granules are, at first, not numerous, it is possible that/

that the localized Golgi material consists of rods and irregularly shaped bodies only, and that, in both the normal and the centrifuged oocyte, the granules are broken from the rods and irregular bodies during dispersal. The absence of large rods from the Golgi layer of the mature oocyte shows that fragmentation of the Golgi elements does take place. It is of interest that Daniels (1938), working on gregarines, has seen small black granules attached to irregular ring-like Golgi elements, and believes that the granules are broken off to form the granular Golgi bodies which, in the centrifuged gregarine, lie centripetally to the larger Golgi elements.

Yolk-globules or nucleolar extrusions were not identified in the centrifuged eggs. A layer of deeply stained material, centripetal to the Golgi layer, is present in the older oocytes. As this layer could not be identified with any of the other components of the cell, and as globules were not present in other parts of the egg, it is concluded that it consists of yolk. If this be correct, the globules, due to the centrifugal force, must have broken down and run together. If, as has been suggested the nucleolar extrusions supply some of the material for yolk-formation, it is probable that the extrusions are stratified together with the yolk. The examination of the centrifuged material has not produced evidence to show that the Golgi elements, or the mitochondria, play a direct part in the formation/

formation of yolk.

The study of centrifuged ovaries adds further evidence in support of the view that the Golgi bodies of degenerating eggs give rise to fat. In eggs which had fragmented into several pieces Golgi elements are few or absent, while fat-globules are more numerous than in the early stages of atresia. It is worthy of note that the cytoplasmic inclusions and components were not visibly affected by the ultra-centrifuge. It is evident that the viscosity of the degenerating ovum differs considerably from that of the normal egg, and that a much higher force is necessary to stratify the cytoplasmic inclusions and components.

present at the centrifugal pole.

4. The mature oocyte is stratified into four layers: a lightly stained centripetal layer; a layer of yolk; the Golgi layer; a centrifugal layer of mitochondria. The Golgi layer is now divided into two parts; the centrifugal part contains numerous mitochondria amongst the Golgi elements.

5. The nucleolar material is the heaviest substance in the nucleus; it increases in density with the growth of the egg. In the mature oocyte the nucleus lies in the mitochondrial layer; the nucleus is thrown towards the centrifugal pole by the weight of the nucleolus.

5. SUMMARY.

1. The ovaries of the mouse were ultra-centrifuged at a force of about 130,000 times gravity.
2. The young oocyte is stratified into three layers: lightly stained cytoplasm, usually containing a few small granular Golgi elements, in the centripetal end of the cell; a middle layer of Golgi elements containing the nucleus; a centrifugal layer of mitochondria.
3. In the larger oocytes a thin layer of yolk is present between the Golgi layer and the centripetal pole, while a layer of non-granular substance is present at the centrifugal pole.
4. The mature oocyte is stratified into four layers: a lightly stained centripetal layer; a layer of yolk; the Golgi layer; a centrifugal layer of mitochondria. The Golgi layer is now divided into two parts; the centrifugal part contains numerous mitochondria amongst the Golgi elements.
5. The nucleolar material is the heaviest substance in the nucleus; it increases in density with the growth of the egg. In the mature oocyte the nucleus lies in the mitochondrial layer; the nucleus is thrown towards the centrifugal pole by the weight of the nucleolus.

6. The Golgi elements are in the form of granules, rods, and of irregularly shaped bodies. The localised Golgi material of the mouse egg is not in the form of a network.
 7. Fat was not identified in the normal ovarian eggs, but is present in degenerating ova. The Golgi elements give rise to the fat of degenerating eggs.
 8. The inclusions and components of the follicle-cells were not always affected by the force necessary to bring about stratification of the oocyte. The degenerating eggs were not stratified.
-

DESCRIPTION OF PLATES 6 and 7.LETTERING.

C.C., lightly stained centripetal cytoplasm; F., fat-globules; G.L., Golgi layer; M.L., layer of mitochondria; N., nucleus; N.G., centrifugal non-granular layer; Nu., nucleolus; Y., yolk.

PLATE 6.

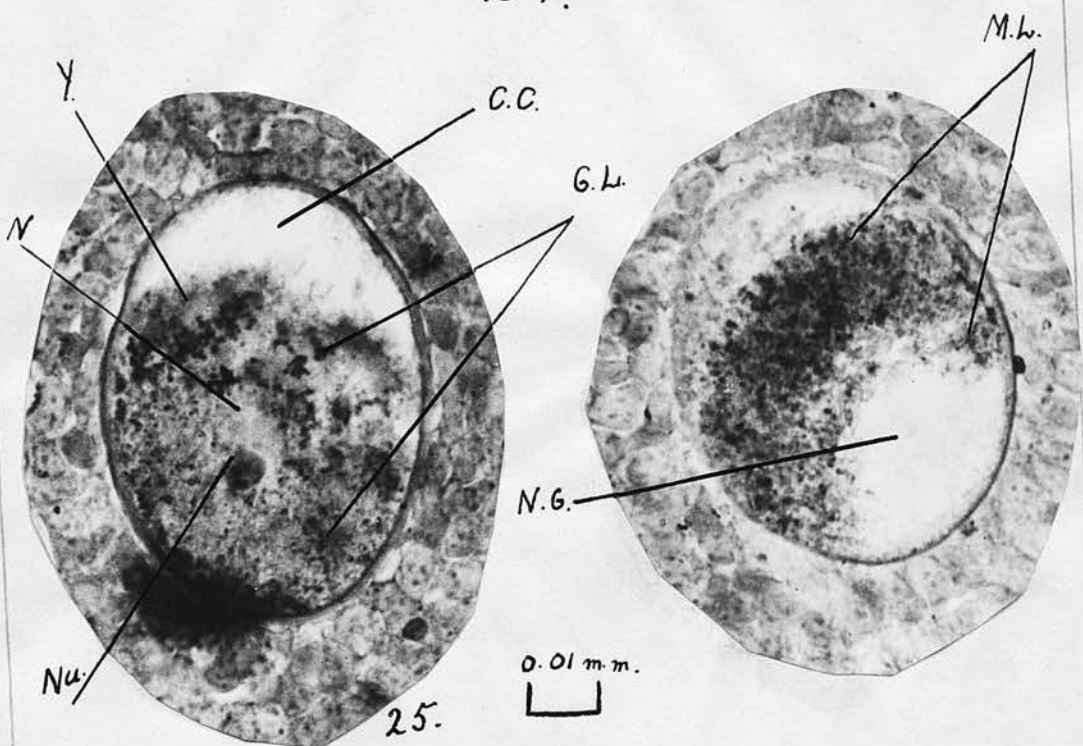
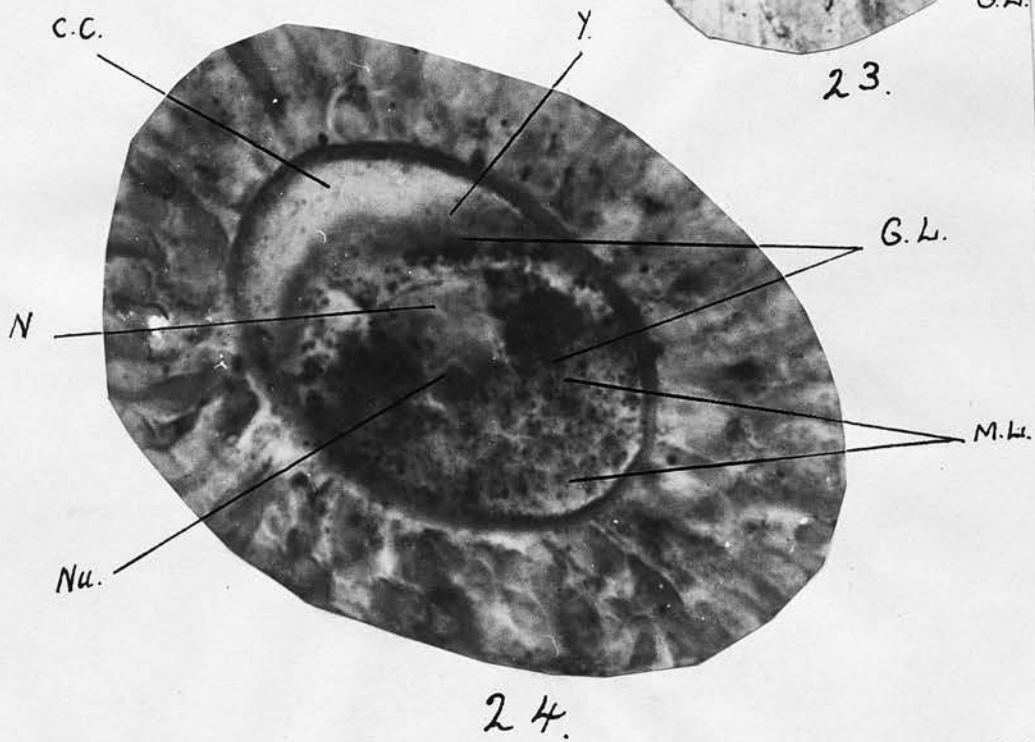
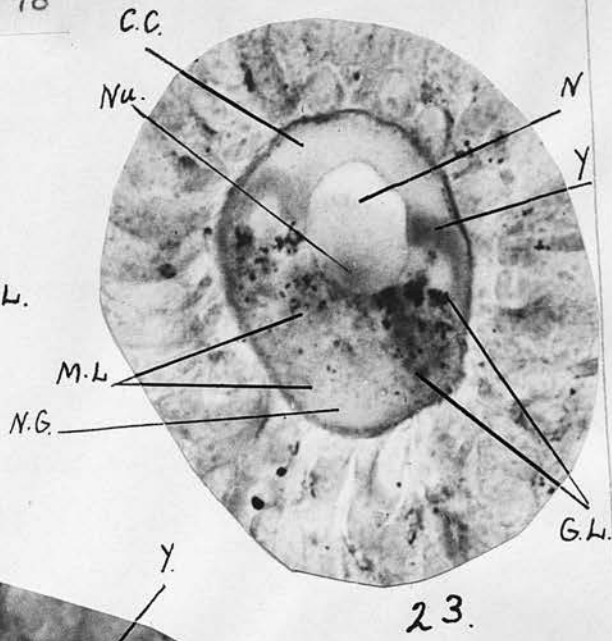
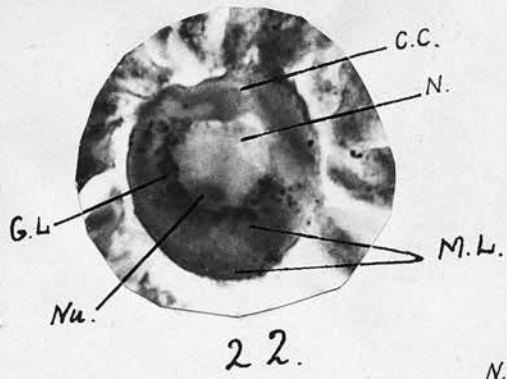
Photomicrographs. All figures from osmic preparations.

Fig.22.- Centrifuged young oocyte.

Fig.23.- Centrifuged oocyte. Later stage than fig.22. Layer of yolk and small non-granular area at centrifugal pole.

Fig.24.- Centrifuged. Oocyte with 3-4 layers of follicle-cells. The centrifugal pole of the cell is not shown.

Figs.25 and 26.- Two sections through the same egg. Centrifuged. Centrifugal non-granular layer shown.




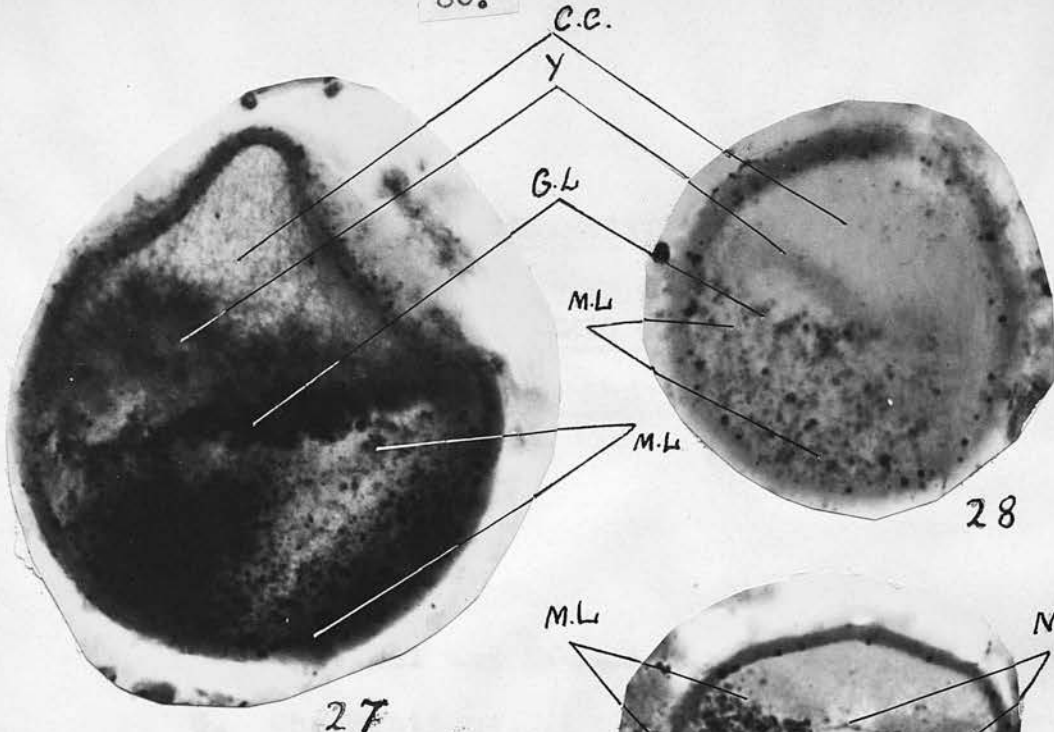
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PLATE 7.

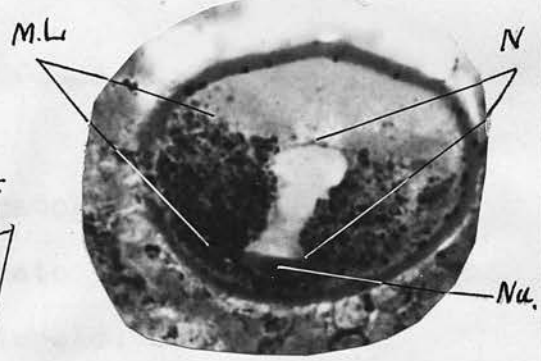
Photomicrographs. Fig. 29 from preparation fixed in formol and stained in Sudan IV. All other figures from osmic preparations.

- Fig.27 and 28.- Centrifuged mature oocytes. Nuclei not shown.
- Fig.29.- Uncentrifuged atretic follicle. Egg has undergone fragmentation. Fat-globules shown.
- Fig.30.- Centrifuged mature oocyte. Nucleus very irregular in outline; nucleolus at centrifugal pole.
- Fig.31.- Centrifuged mature oocyte. Nucleus dumb-bell shaped; nucleolus at centrifugal pole.
-

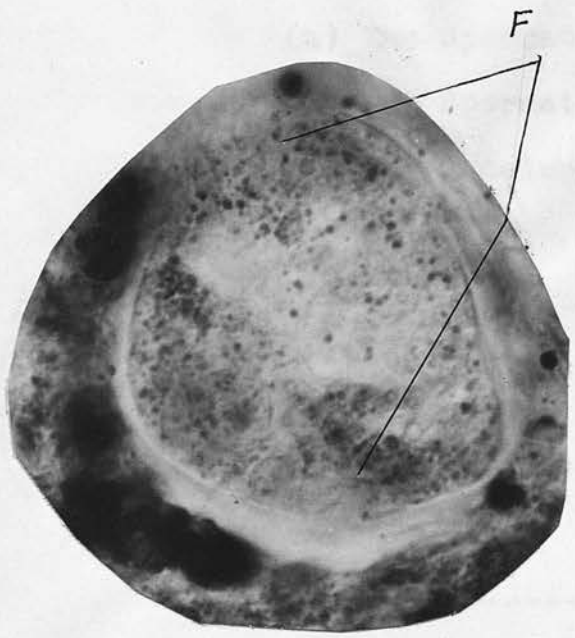


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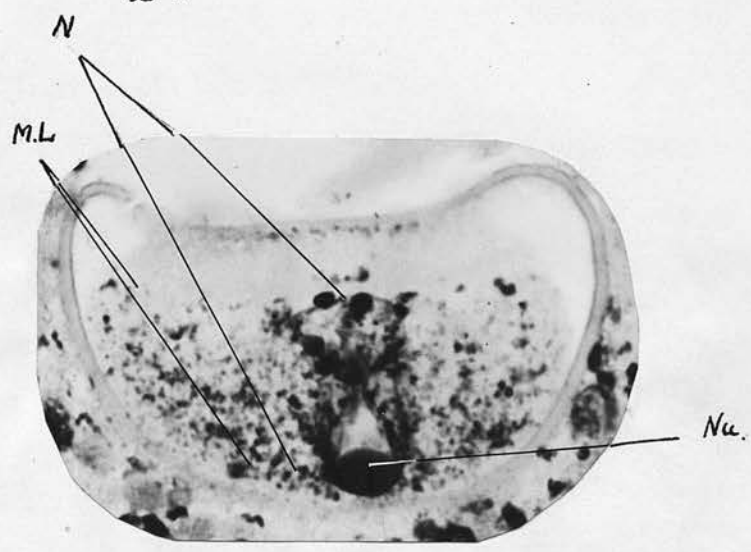


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30.

SPERMATOGENESIS.A STUDY OF THE CYTOPLASMIC INCLUSIONS DURING
SPERMATOGENESIS AND SPERMATELEOSIS.

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Although a large number of papers have been published on the spermatogenesis of mammals, very little work has been carried out with the aid of techniques for the demonstration of the Golgi substance. Previous contributions on the cytoplasmic inclusions of

1. PREVIOUS WORK.

As observations were carried out by the writer on the mitochondria and Golgi material of the sperm middle-piece, as seen in the fertilised egg of the mouse, it was considered desirable that these bodies be traced through the stages of spermatogenesis and spermateleosis.

So far as the writer is aware a comprehensive study of the mitochondria and Golgi material of the male germ-cells of the mouse has not previously been published. The present contribution, therefore presents, for the first time, an account of these bodies during all stages of the spermatogenesis and spermateleosis of the mouse. Taken together with the earlier work on oogenesis and with the investigation, presently to be described, of the cytoplasmic bodies during maturation, fertilisation, and the first cleavage division, it forms part of a comprehensive study of the cytoplasmic inclusions of the germ-cells and fertilised egg of the mouse.

Although a large number of papers have been published on the spermatogenesis of mammals, very little work has been carried out with the aid of techniques for the demonstration of the Golgi substance. Previous contributions on the cytoplasmic inclusions of/

of the germ-cells of mammals will be discussed later, in so far as they have a bearing on the present findings. Mention of other works on spermatogenesis will be found in the papers cited.

For the study of the mitochondria, material fixed in Flemming (without azotic), and treated according to the method of Chassy-Kull was found to be satisfactory for the demonstration of the Golgi substance, material was prepared according to the methods of Mann-Rogee, Kolatsher, and Aoyama. The Kolatsher and Aoyama preparations gave the best results. The mitochondria were also visible in Aoyama sections, and the Golgi bodies, particularly those of the resting spermatocyte and of the young spermatids, were shown in some of the Chassy-Kull preparations. Material was fixed in formal and the sections subsequently stained in Sudan IV according to the method of Kay and Hatched (1936) for the demonstration of fat.

2. MATERIAL AND METHODS.

Testes were dissected out and small pieces immediately placed in the fixing fluid. Sections were subsequently cut at 5μ and at 8μ in thickness.

For the study of the mitochondria, material fixed in Flemming (without acetic), and treated according to the method of Champy-Kull was found to be satisfactory. For the demonstration of the Golgi substance, material was prepared according to the methods of Mann-Kopsch, Kolatchev, and Aoyama. The Kolatchev and Aoyama preparations gave the best results. The mitochondria were also visible in Aoyama sections, and the Golgi bodies, particularly those of the resting spermatocytes and of the young spermatids, were shown in some of the Champy-Kull preparations. Material was fixed in formol and the sections subsequently stained in Sudan IV according to the method of Kay and Whitehead (1935) for the demonstration of fat.

3. OBSERVATIONS.

(a) THE SPERMATOGONIA.

The mitochondria of the spermatogonia are large and spherical. They are scattered through the cytoplasm, but are most numerous in the neighbourhood of the nucleus; they are practically absent from the extreme periphery of the cell. The Golgi material is in the form of a flattened mass in close contact with one pole of the nucleus. It is probable that it is made up of individual elements closely packed together (fig. 37, Pl. 8). Spermatogonia in division were not observed.

(b) THE SPERMATOCYTES.

The mitochondria of the resting primary spermatocytes are spherical. They are situated in small clumps more or less closely surrounding the nucleus; a few, however, may be scattered singly in the cytoplasm (fig. 32, Pl. 8). Prior to division they become distributed through the cell (fig. 33, Pl. 8), and during the prophase appear to be smaller and more numerous than in the resting stage (fig. 42, Pl. 8). They remain scattered through the cytoplasm during the subsequent phases of nuclear division, but in the metaphase show a slight tendency to form small clumps in the vicinity of the spindle (figs. 34, 35, and 36, Pl. 8). With the division of the cell they are distributed in approximately equal numbers to the second spermatocytes.

The Golgi material is in the localised condition, and is greater in amount than in the spermatogonia. It is well shown in Aoyama and in Kolatchev preparations and is present in some of the Champy-Kull sections. When it is not visible in Champy-Kull material the idiosome or archoplasm is frequently shown as an area which stains more deeply than the surrounding cytoplasm. In some of the younger primary spermatocytes the Golgi material is situated some distance from the nucleus, but/

but later it is in close contact with the nuclear membrane, when it often appears as a homogeneous, deeply impregnated mass (fig. 38, Pl. 8). Examination of a large number of sections indicates that the Golgi mass is made up of individual elements, probably in the form of rods, threads and granules, which closely invest the archoplasm (Figs. 39 and 40, Pl. 8). In some cases ring-shaped elements are situated at the periphery of the Golgi mass (fig. 41, Pl. 8).

In the prophase of the division of the primary spermatocytes the Golgi material forms a more compact deeply impregnated mass situated close to the nuclear membrane (fig. 42, Pl. 8). In sections containing metaphase figures the Golgi material is shown as a clump of rods and granules lying close to the spindle in the equatorial region. The elements are more widely separated than in the resting condition or during the prophase (figs. 43 and 44, Pl. 9). During the anaphase and early telophase the Golgi material is still situated in the equatorial region (fig. 45, Pl. 9). Cells in the late telophase were not identified, but there is little doubt that the Golgi substance, owing to its equatorial position during the phases of division, is divided passively into two with the division of the cytoplasm. It should be pointed out that division figures showing Golgi material were present in the

Aoyama/

Aoyama material only. Consequently, it was not possible to examine the Golgi material, during the stages of cell division, in the osmic preparations.

The behaviour of the mitochondria and Golgi material during the division of the second spermatocytes appears to be similar to that of the first division.

Increase in size and number.

The Golgi material of the young spermatid is in the form of a deeply impregnated zone closely applied to the anterior pole of the nucleus (Fig. 43, Pl. 5). At this stage, and during the formation and early growth of the acrosome, the Golgi material is visible in the Champy-Kulikov and Aoyama and Kolatchev preparations. Although the localized Golgi material of the spermatid often appears to have the structure of a network, careful examination indicates that, in reality, it consists of rod-shaped elements which closely invest the acrosome. Owing to the denseness of the localized Golgi material, surrounding the nucleus, it was not possible to identify pro-acrosomic granules or to follow the very early stages of the formation of the acrosome, nor was this possible in preparations in which the Golgi substance was not impregnated.

The Golgi material becomes somewhat flattened over the anterior pole of the nucleus, and, later, moves away from the nuclear membrane, revealing a clear unstained area, or vacuole, which is closely applied to the anterior/

(c) SPERMATELEOSIS.

Granular mitochondria are present in the young spermatids. They are, at first, smaller and less numerous than those of the spermatocytes, but later increase in size and number.

The Golgi material of the young spermatid is in the form of a deeply impregnated mass closely applied to the anterior pole of the nucleus (fig. 46, Pl. 9). At this stage, and during the formation and early growth of the acrosome, the Golgi material is visible in the Champy-Kull as well as in the Aoyama and Kolatchev preparations. Although the localised Golgi material of the spermatid often appears to have the structure of a net-work, careful examination indicates that, in reality, it consists of rod-shaped elements which closely invest the archoplasm. Owing to the denseness of the localised Golgi material, surrounding the idiosome, it was not possible to identify pro-acrosomic granules or to follow the very early stages of the formation of the acrosome, nor was this possible in preparations in which the Golgi substance was not impregnated.

The Golgi material becomes somewhat flattened over the anterior pole of the nucleus, and, later, moves away from the nuclear membrane, revealing a clear unstained area, or vacuole, which is closely applied to the anterior/

anterior pole of the nucleus so that the nuclear membrane is slightly depressed. In Champy-Kull preparations the clear area is seen to contain a large deeply stained granule in contact with the nuclear membrane (fig. 47, Pl. 9). The granule is the developing acrosome. The vacuole and the acrosome increase in size so that the Golgi material is pushed further away from the nucleus (fig. 48, Pl. 9), becoming free and migrating towards the posterior part of the cell. At the same time the vacuole and the acrosome spread out over the anterior pole of the nucleus which regains its original shape (fig. 49, Pl. 9). The Golgi material, which is now in the vicinity of the posterior pole of the nucleus, is very compact and divides into a small and a larger part. The axial filament is now present and the proximal and distal centrioles can sometimes be identified in Champy-Kull sections (figs. 49 and 50, Pl. 9). After the Golgi material has reached the neighbourhood of the posterior pole of the nucleus, the cell begins to elongate, and the mitochondria increase in size and often appear as spherical bodies with a deeply stained cortex. In some sections a body which stains more deeply than the surrounding cytoplasm is shown in contact with the acrosome vacuole. This is part of the archoplasm (fig. 50, Pl. 9). With the further elongation of the cell the small spherical/

spherical piece of Golgi material separates from the main mass and moves towards the region of the centrioles, becoming smaller and more compact as it does so. The residual Golgi material moves towards the posterior part of the cell and becomes less deeply impregnated. It surrounds a material which stains more deeply than the cytoplasm, and is part of the archoplasm. The mitochondria are spherical but stain homogeneously and are distributed throughout the cytoplasm (figs. 51 and 52, Pl. 9). While these changes have been taking place the acrosome continues its development, increasing in size at the expense of the outer clear area (fig. 51, Pl. 9), which it soon fills completely. The Golgi body, which has previously separated from the main mass, is now in close proximity to the centrioles (fig. 52, Pl. 9).

The nucleus rapidly elongates and the head of the sperm assumes its characteristic form. At this stage the acrosome is lightly stained, and, with the technique used during the present investigation, is difficult to differentiate from the rest of the head region (fig. 53, Pl. 9).

After the differentiation of the head region, a deeply impregnated granule is visible lying in the region of the centrioles immediately posterior to the nucleus. This granule is present in Champy-Kull, Aoyama, and in Kolatchev preparations, and is identified as the Golgi substance which, at an earlier stage, is/

is budded off from the main mass of Golgi material and migrates to the posterior pole of the nucleus (figs. 54, 55 and 56, Pl. 10). Its close proximity to the centrioles makes the certain identification of the latter very difficult. The residual Golgi material still surrounds the archoplasm but appears to be breaking up into smaller pieces (fig. 58, Pl. 10).

The majority of the mitochondria soon collect into large clumps situated in the vicinity of the axial filament. At this stage the residual Golgi material is not shown in the Champy-Kull sections. Vacuoles make their appearance in the cytoplasm (fig. 54, Pl. 10).

After the mitochondria become clumped they move closer to the axial filament, and finally become arranged around the filament to form the mitochondrial sheath of the middle-piece of the sperm. At first the mitochondria are arranged loosely around the axial filament (fig. 55, Pl. 10), but later become more compact (fig. 56, Pl. 10). A few mitochondria remain in small clumps and scattered singly in the residual cytoplasm. The Golgi material of the residual cytoplasm has broken up and is becoming scattered. Examination of Kolatchev preparations of late spermatids shows that the residual cytoplasm contains a large number of deeply impregnated globules and granules/

granules, which is identified as fat. A small number of vacuoles are also present (fig. 57, Pl. 10). That the globules and granules are of a fatty nature was confirmed by an examination of sections prepared according to the method of Kay and Whitehead (1935) for the identification of fat. The spermatocytes and early spermatids do not contain any material which stains with Sudan IV, while brightly coloured globules and granules are present in the residual cytoplasm of the late spermatids.

As has already been pointed out, the deeply impregnated granule situated in the neck region of the developing spermatid is identified as part of the original Golgi material of the cell. Not only was it traced from its origin, from the Golgi material which had moved away from the growing acrosome, but its appearance and staining reactions in Champy-Kull, Aoyama, and Kolatchev sections leave little doubt that it is composed of Golgi substance. The Golgi granule was identified in very late spermatids and in nearly ripe and ripe spermatozoa (fig. 59, Pl. 10).

Living spermatocytes and spermatids were examined in smear preparations mounted in a weak solution of neutral red, and unstained. While the idiosome complex was visible in a number of cells, the Golgi material was so faintly shown that it is unsafe to draw definite conclusions, regarding the form of the Golgi substance, beyond stating that appearances indicated that it is not in the form of a net work.

4. DISCUSSION.

The most complete account of the cytoplasmic inclusions during all stages of the spermatogenesis of a mammal appears to be that contained in the papers of Gatenby and Woodger (1921) and Gatenby and Wigoder (1929) for the guinea-pig. The history of the cytoplasmic inclusions of the male germ-cells of the mouse is in general similar to that of the guinea-pig. There are, however, certain differences.

In the guinea-pig, according to Gatenby and Woodger, the mitochondria are scattered through the cytoplasm during all stages of the spermatocytes. According to Duesberg (1910), however, they are at first situated in relation to the idiosome, and later form small groups around the nucleus. Finally, they become scattered through the cell. In the young primary spermatocytes of the mouse the mitochondria are situated in the vicinity of the nucleus, becoming dispersed prior to the prophase of the first division. During division the behaviour of the mitochondria is closely similar to that of the guinea-pig as described by Gatenby and Woodger. In the late spermatid of the guinea-pig the mitochondria do not form large clumps as in the mouse. The majority finally become arranged around the axial filament where they are figured by Gatenby and Woodger as large discrete granules/

granules. Those which remain in the residual cytoplasm run together and undergo degenerative changes. Thus, in both the mouse and the guinea-pig, the majority of the mitochondria take part in the formation of the middle-piece of the sperm, while a few are eliminated with the residual cytoplasm. According to Gatenby and Beams (1935) the mitochondria of the human spermatocyte tend to lie in the middle of the cell during division.

Sjövall (1906) describes and figures a structure which blackens with osmic acid and is situated in relation to the idiosome of the spermatogonia and spermatocytes of the mouse. He states that it is identical with the "Golgi-Kopsch network" of somatic cells. In the spermatid this structure leaves the nucleus and passes to the posterior part of the cell. Sjövall does not, however, give a detailed account of the behaviour of the Golgi material nor of the formation of the acrosome.

Duesberg (1908), in a paper on the spermateleosis of the rat, traces the origin of the acrosome. Clear areas make their appearance in the idiosome. Later, a granule arises in each, and these run together to form a single granule surrounded by a large vacuole-like structure. The subsequent stages in the history of the vacuole and granule appears to be similar to those of the mouse as described by the writer. Duesberg describes a deeply stained body which he calls the/

the "corps chromatoides". In the young spermatid it is situated in the neighbourhood of the idiosome. Later, it passes to the vicinity of the centrioles and renders their observations difficult. It is, at first, irregular in shape, but becomes spherical, fragments into two or three pieces which finally disappear in the cytoplasm after the elongation of the cell. Although Duesberg's figures are from Flemming preparations, and the "corps chromatoides" is not shown surrounding the idiosome of the young spermatid, there is little doubt that this structure is the Golgi material. He does not trace part of the material onto the middle-piece of the sperm.

Gatenby and Woodger believe that the Golgi material of the spermatocyte and of the spermatid is in the form of curved plates and rods surrounding the archoplasm. In silver preparations it appears as either a reticulum or as a homogeneous body. That the localised Golgi material is made up of discrete bodies was confirmed by Subba Rau and Brambell (1925) who carried out observations on the living spermatocytes and early spermatids of the guinea-pig. The present writer concludes that the Golgi material of the developing male germ-cells of the mouse is in the form of rods and granules. The Golgi elements are, however, small and closely packed together around the archoplasm making observation of their shape extremely difficult. In the Guinea-pig the Golgi bodies are fewer in number than/

than in the mouse, and Gatenby and Woodger were able to trace the proacrosomic granules from the spermatocyte until they ran together to form the proacrosome of the spermatid.

The number of mammals in which spermatogenesis has been investigated with methods for the demonstration of the Golgi material is small. The condition in the animals observed indicate that there is wide variation in the behaviour of the Golgi substance during the division of the spermatocytes. According to Gatenby and Beams (1935) the localised Golgi material of the human spermatocyte breaks up, during the prophase, into small granules. In the later stages of division the granules tend to lie near the spindle, but not necessarily near the asters. During the telophase they are situated close to the nucleus on the side away from the asters. In the young spermatid the Golgi granules are somewhat scattered to one side of the cell. Later, they come together to form a group situated close to the nucleus.

The location of the Golgi material during the stages of division of the spermatocytes of the mouse is in marked contrast to that of the rat as described by Ludford and Gatenby (1921) and to that of the guinea-pig, as described by Gatenby and Woodger. In the guinea-pig approximately half of the Golgi elements are grouped round each pole of the spindle. In the rat the Golgi bodies are scattered through the cell during the metaphase/

metaphase, and in the telophase form two groups situated towards opposite ends of the cell. The condition in the mouse more closely resembles that of the opossum as recorded by Duesberg (1920). In the opossum the Golgi material does not break up into granules until the anaphase when it forms one or more clumps situated between the chromosomes. The present writer observed, in silver preparations, a large number of spermatocytes undergoing prophase changes. The Golgi material was shown clearly as a clump of elements lying close to the nuclear membrane. Cells in the metaphase, anaphase and telophase were not numerous, but in some of those present the Golgi material was visible in the equatorial region. In no case were scattered Golgi elements observed near the spindle poles. It is concluded, therefore, that in the mouse the Golgi material remains in a single clump and is separated passively into two smaller groups with the division of the cell, or that, as in the opossum, it does not break up into granules until immediately before the division of the cell. Examination of a large number of young spermatids appears to add further support to the view that the Golgi material remains in a single clump. Cells were never seen in which the Golgi elements were coming together from the dispersed condition, as might be expected if the elements were distributed through the cytoplasm during division.

In/

In the mouse the acrosome is first visible after the Golgi material begins to move away from the nucleus: there is little doubt, therefore, that the acrosome is formed under the influence of the Golgi material. It is probable that it arises from proacrosomic granules which run together in a similar manner to those of the guinea-pig.

Gatenby and Woodger state that the proacrosome of the guinea-pig is surrounded by a vacuole and that it becomes divided into an inner and an outer zone. In the mouse the clear area which, at first, surrounds the developing acrosome corresponds with the vacuole of the guinea-pig. An inner and an outer zone were not identified. According to Gatenby and Beams (1935) the human acrosome is not divided into an inner and outer zone. The formation of the acrosome of the mouse is closely similar to that of the dog as described by Bell (1929), and also shows resemblance to that of the human spermatid. In the spermatid of the dog the Golgi material is in the form of granules which surround the idiosome. A large vacuole is formed between the idiosome and the nucleus. With the increase in size of the vacuole a pressure is exerted on the anterior pole of the nucleus, and a granule, which gives rise to the acrosome, is formed. The subsequent changes undergone by the vacuole and granule are closely similar to those of the corresponding structures of the mouse.

Gatenby/

Gatenby and Woodger state that, at the stage when the spermatid of the guinea-pig is elongating, a small part of the Golgi material and archoplasm become detached from the main mass and migrates to the protoplasmic bead of the middle-piece. The present findings on the origin of the Golgi material of the sperm confirm those of Gatenby and Woodger. Subba Rau and Brambell (1925) believe that the Golgi material of the middle-piece is, in some cases at least, budded off from the main mass at an earlier stage than that described by Gatenby and Woodger, and may become separated before the Golgi substance has moved away from the acrosome. During the present investigation it was found that the time at which the Golgi material of the middle-piece is budded off varies slightly. Budding was not observed while the Golgi substance was in contact with the acrosome. In most cases it occurs as the Golgi material migrates towards the posterior pole of the nucleus, but sometimes does not take place until it has reached the posterior part of the cell.

Subba Rau and Brambell observed Golgi material in living spermatocytes and spermatids of the mouse. According to their figures the Golgi elements are larger and fewer in number than those shown in the fixed material used during the present investigation. They do not describe the Golgi material of the late spermatid/

spermatid nor that of the sperm. The observations of the present writer on living material added little to the findings based on the examination of fixed and stained preparations.

Gatenby and Woodger figure the Golgi material of the sperm middle-piece of the guinea-pig as consisting of rod-shaped elements. Later, Gatenby and Wigoder (1929) give figures of ripe sperms in which the Golgi material appears to be in the form of a net-work. They do not, however, describe its structure. In the mouse the Golgi substance of the middle-piece originates as a small rounded body, which in some of the sections appeared to be in the form of a deeply impregnated cortex surrounding a small amount of archoplasm. After it has taken up its position in the middle-piece it is visible as a deeply impregnated granule. The present investigation shows that part of the original Golgi material of the spermatid is present in the middle-piece of the ripe sperm of the mouse. Golgi granules were noted by Bell in the middle-piece of the sperm of the dog. Gatenby and Woodger, and Gatenby and Wigoder have described Golgi material in the spermatozoa of the guinea-pig. Gatenby and Beams do not record the presence of Golgi material in the middle-piece of the human sperm. It is evident that Golgi material is present in the middle-piece of the large majority of those mammals examined with the aid/

aid of modern osmic and silver techniques.

Bell (1929) states that the Golgi granules of the spermatid of the dog become aggregated to form large spheres in the residual cytoplasm. He believes that the Golgi material is probably an unsaturated lipoid and that the larger spheres to which it gives rise are neutral fat. The present investigation shows clearly that the Golgi material in the residual cytoplasm of the mouse undergoes degenerative changes and is converted into fat. According to Gatenby and Beams the Golgi remnant of the human spermatid does not give rise to fat.

The post-nuclear granules and body described by Gatenby and Wigoder for the spermatid of the guinea-pig, or the post-nuclear cap recorded by Gatenby and Beams for the human sperm, were not observed during the spermatogenesis of the mouse.

5. SUMMARY.

1. Spherical mitochondria are present in the spermatogonia and spermatocytes. In the spermatogonia they are scattered through the cytoplasm, but are most numerous in the neighbourhood of the nucleus. In the resting primary spermatocytes they are, for the most part, in small clumps surrounding the nucleus. Prior to division they spread out through the cytoplasm, and remain scattered through the cell during the phases of division of the first and second spermatocytes.
2. The Golgi material of the spermatogonia is in the form of a flattened mass in close contact with one pole of the nucleus. In the spermatocytes it consists of individual elements surrounding the idiosome. During nuclear division it remains in the localised condition, in the equatorial region, and is, either divided into two smaller groups by the division of the cell, or is scattered through the cytoplasm immediately before cell division.
3. The mitochondria of the young spermatid are granular. Later, they increase in size, become clumped, and, finally, the majority surround the axial filament to form the mitochondrial sheath.

4. The Golgi material of the young spermatid is closely applied to the anterior pole of the nucleus. It moves away revealing a large vacuole which contains a deeply stained granule. The granule increases in size, at the expense of the vacuole, and gives rise to the acrosome.
 5. The Golgi material moves away from the nucleus. It buds off a small spherical piece of Golgi substance which migrates to the posterior pole of the nucleus and, later, is included in the middle-piece of the sperm.
 6. The Golgi remnant breaks up in the residual cytoplasm and is converted into fat.
-

DESCRIPTION OF PLATES 8, 9 and 10.Lettering.

Ac., acrosome; A.F., axial filament; Ar., archoplasm; c., centrioles; Ch., chromosomes; C.V. vacuole in residual cytoplasm; F., fat; G.B., Golgi bud; G.M., Golgi material; G.R., Golgi remnant; M., mitochondria; M.S., mitochondrial sheath; N., nucleus; S.H., sperm head; S.G.M., sperm Golgi material; V., vacuole surrounding developing acrosome.

PLATE 8.

Figs. 32-36 from Champy-Kull preparations. Figs. 37-41 from Kolatchev preparations. Fig. 42 from Aoyama preparations.

Fig. 32.- Resting primary spermatocyte.

Fig. 33.- First spermatocyte just before division.

Fig. 34.- First spermatocyte. Metaphase.

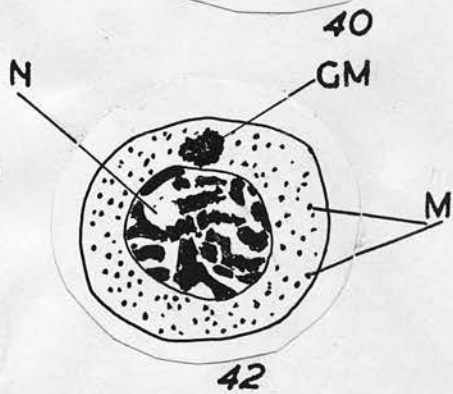
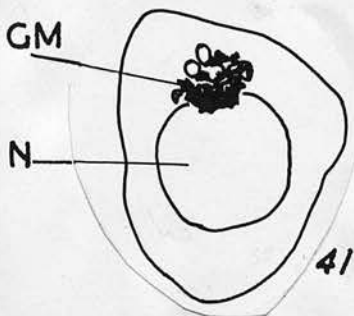
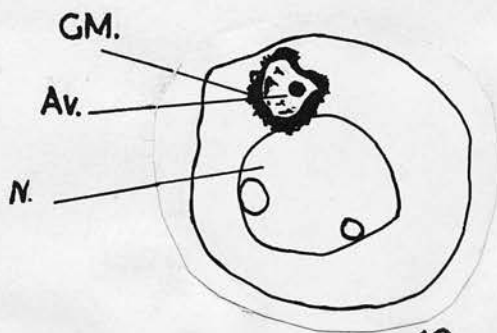
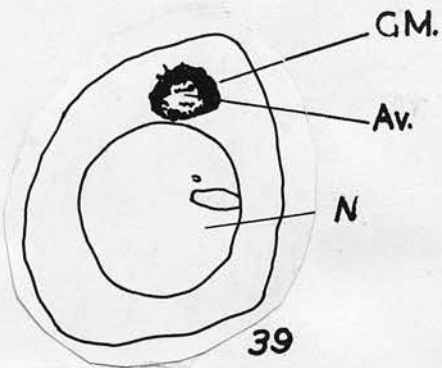
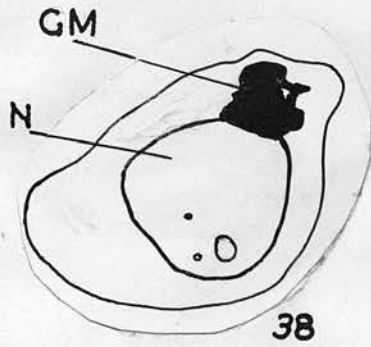
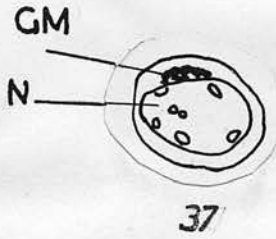
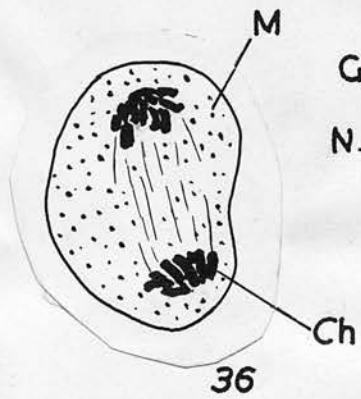
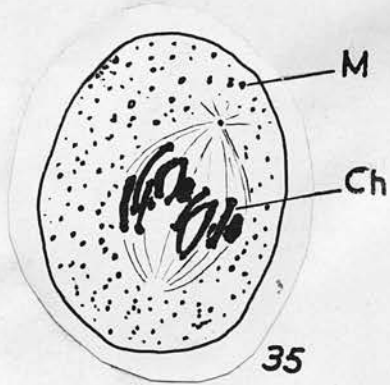
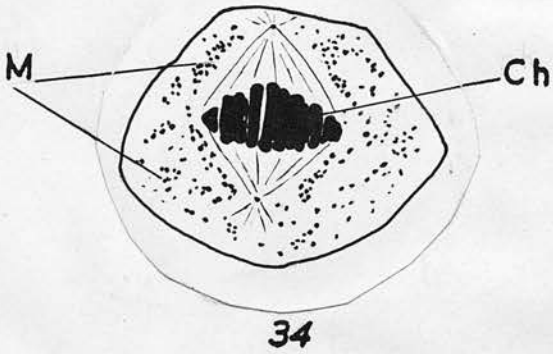
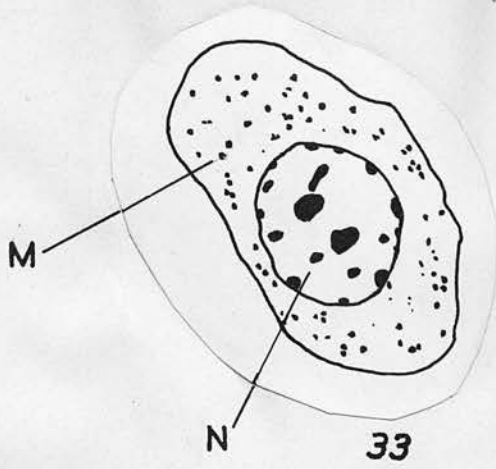
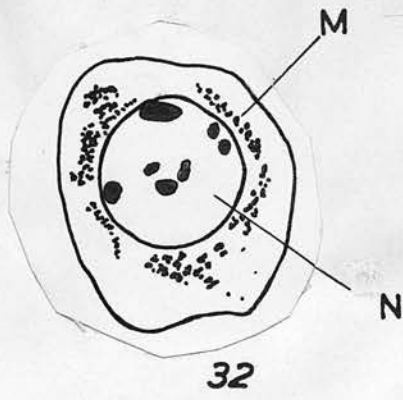
Fig. 35.- First spermatocyte. Early anaphase.

Fig. 36.- First spermatocyte. Late anaphase.

Fig. 37.- Spermatogonium.

Figs. 38-41.- Spermatocytes to show Golgi material.

Fig. 42.- First spermatocyte. Early prophase.



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PLATE 9.

Figs. 43 - 45 from Aoyama preparations.

Figs. 46 - 53 from Champy-Kull preparations.

Figs. 43 and 44.- Spermatocytes. Metaphase.

Fig. 45.- Spermatocyte. Early telophase.

Fig. 46.- Young spermatid.

Figs. 47 and 48.- Young spermatids showing
developing acrosome.

Figs. 49 - 53.- Spermatids showing stages in
development of acrosome, and
history of the mitochondria
and Golgi material.

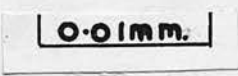
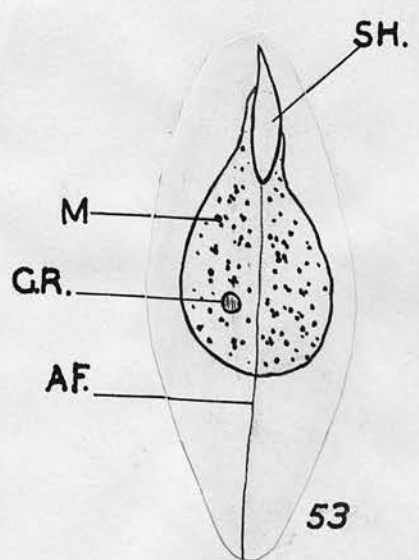
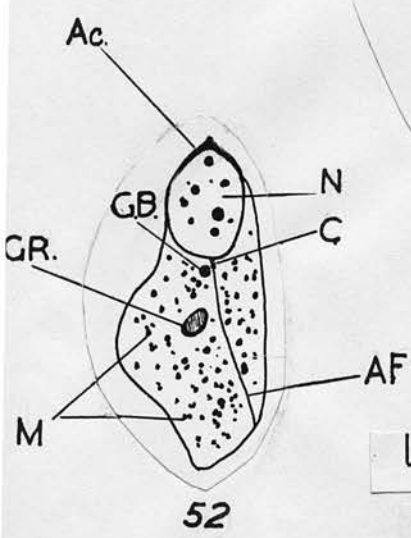
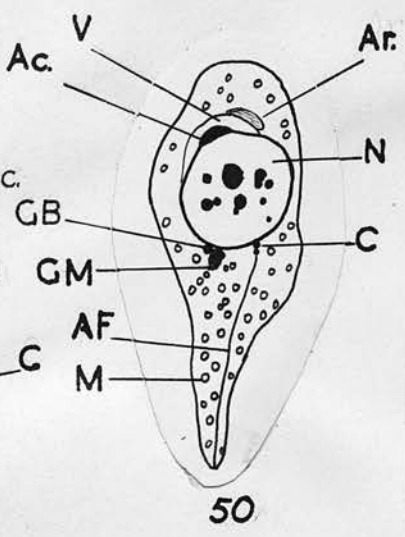
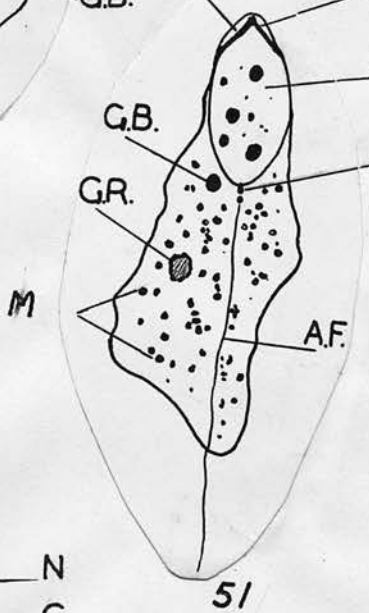
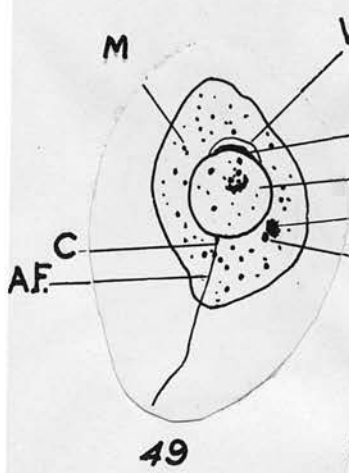
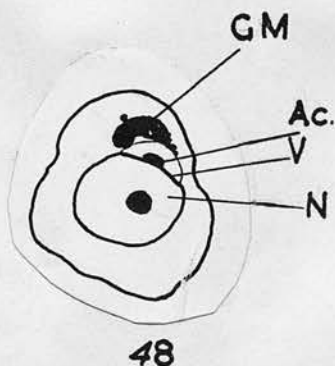
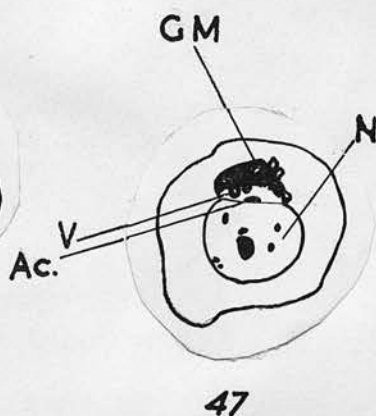
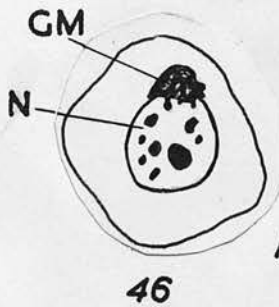
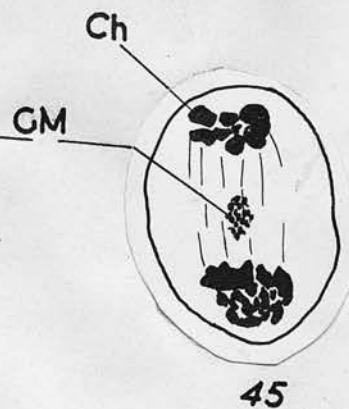
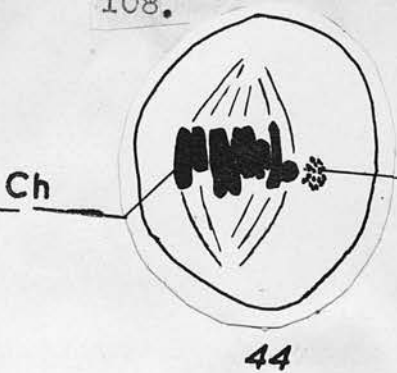


PLATE 10.

Figs.54-56 from Champy-Kull preparations.

Figs.57 and 59 from Kolatchev preparations.

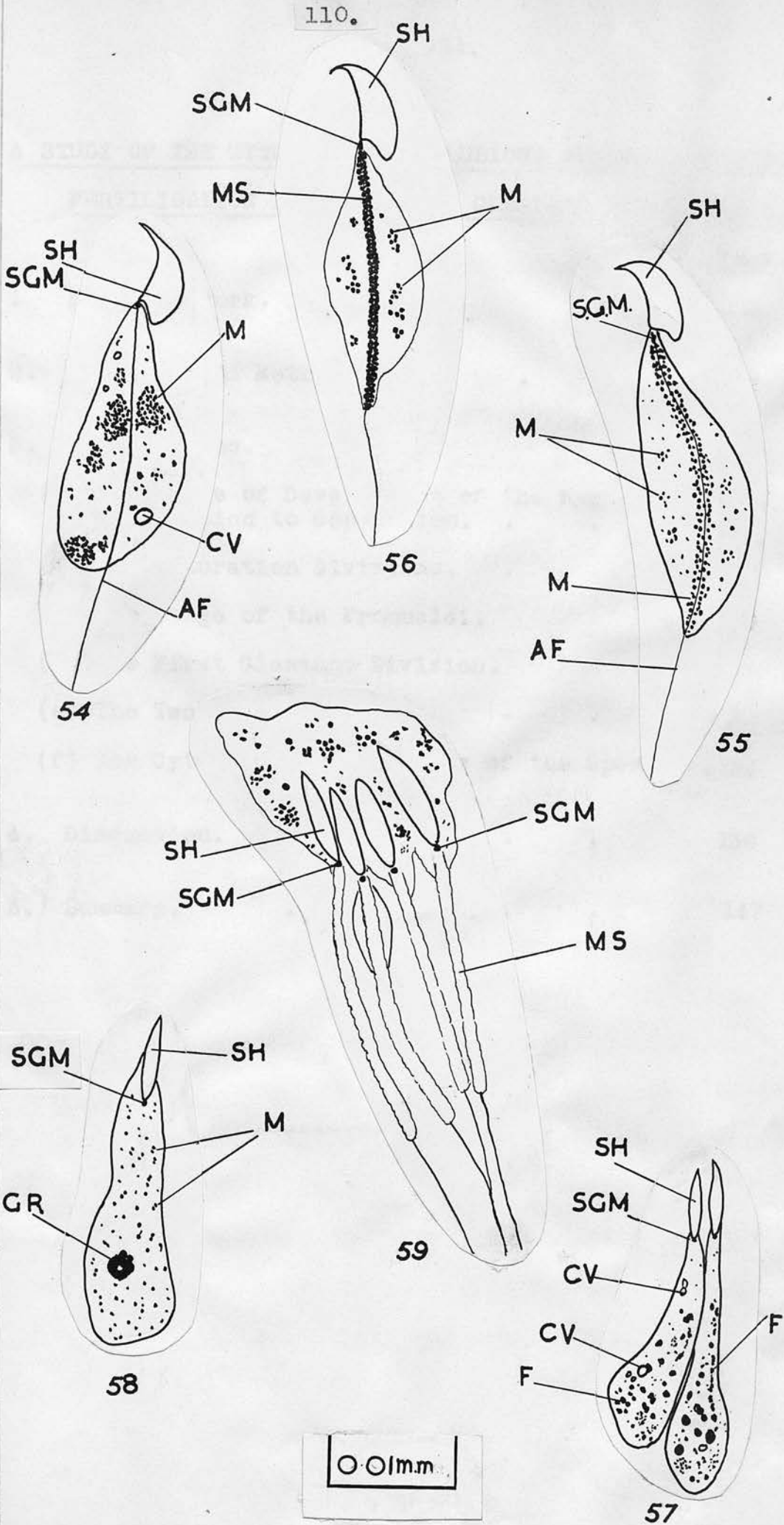
Fig. 58 from Aoyama preparation.

Figs.54-56.- Spermatids showing mitochondria and the Golgi material of the middle-piece.

Fig. 57.- Spermatids to show fat in residual cytoplasm.

Fig. 58.- Spermatid to show Golgi remnant.

Fig. 59.- Nearly ripe sperms to show Golgi material in the middle-piece.



A STUDY OF THE CYTOPLASMIC INCLUSIONS DURING MATURATION,
FERTILISATION AND THE FIRST CLEAVAGE DIVISION.

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During the stages of maturation and fertilisation, they were identified to cells of the two-cell-stage.

As the result of later investigations a detailed account is given, in this section of the Thesis, of the behaviour of the mitochondria during maturation, fertilisation, and the first cleavage division of the mouse-egg, and of the history of the cytoplasmic inclusions of the sperm immediately after the entry of the sperm into the ovum. The account is based

1. PREVIOUS WORK.

In the course of the earlier work on the oogenesis of the mouse preliminary investigations on the cytoplasmic bodies of the fertilised egg were carried out. According to these findings the Golgi elements are smaller and more numerous than are those of the late ovarian egg, while the mitochondria, like those of the ovarian oocyte, are granular and are collected into clumps distributed through the ooplasm; the clumps are, however, more numerous. Both the Golgi bodies and the mitochondria are slightly more numerous towards one pole of the egg. The yolk-globules are situated chiefly in one half of the cell, while in the two cell-stage they appear to be fairly evenly distributed between the two blastomeres. No attempt was then made to follow the history of the cytoplasmic inclusions and components during the stages of maturation and fertilisation, nor were they identified in cells of the two cell-stage.

As the result of later investigations a detailed account is given, in this section of the thesis, of the behaviour of the Golgi elements and mitochondria during maturation, fertilisation, and the first cleavage division of the mouse-egg, and of the history of the cytoplasmic inclusions of the sperm middle-piece after the entry of the sperm into the ovum. The account is based/

based on observations, on a large amount of material, carried out over a lengthy period.

The middle-piece of the sperm has previously been seen in the eggs of certain mammals, but little attempt appears to have been made to study in detail its cytoplasmic inclusions, except the centriole, nor to follow the subsequent history of these bodies in the ooplasm and in the cytoplasm of the early blastomeres.

Lams and Doorme (1908) describe the entry of the sperm during the second maturation division of the egg of the mouse. The whole tail is probably carried into the ovum, as a terminal filament, as well as the middle-piece was often observed. The tail remains attached to the head until the stage of the pronuclei. They state that the tail shows striations at all stages, and that these are well shown in osmic preparations. The middle-piece is, at first, figured as a homogeneous structure; later, it thickens and has a granular appearance. As the granules are well shown in osmic preparations there seems little doubt that, although not described as such, these granules are the mitochondria of the sperm. The subsequent history of the sperm-tail in the egg was not traced, nor was it seen in the cells of the two cell-stage. Lams and Doorme quote Gerlach (1906) as stating that the greater part of the tail of the sperm of the mouse is carried into the egg.

Rubaschkin (1905) describes a filament situated in the/

the vicinity of the sperm nucleus in the fertilised egg of the guinea-pig. As this filament was not seen in all of the ova examined, Rubaschkin concludes that, if this filament be the sperm-tail, it is not always carried into the egg.

Sobotta and Burckhard (1910) state that the whole tail does not always enter the rat egg, but the middle-piece only. They conclude that the middle-piece is resolved into mitochondria.

The tail of the sperm was observed by Lams and Doorme (1908) in the fertilised egg of the guinea-pig, and was later described in more detail by Lams (1913). Lams states that in some cases it remains in contact with the nuclear membrane throughout the growth of the male pronucleus, but that usually it becomes detached. As the pronucleus moves into the deeper cytoplasm the tail moves with it. The tail contains two filaments, and sometimes has a granular appearance in the region which corresponds to the position of the mitochondrial sheath. During the first cleavage division, it is situated in the cytoplasm close to the mitotic figure, and passes into one of the blastomeres of the two cell-stage. It was not observed in the cells of the four cell-stage.

Levi (1915) describes and figures the middle-piece of the sperm in a three cell-stage of Vespertilio murinus. He states that there are two lines of mitochondria in the/

the middle-piece and that these are smaller than are those of the egg.

Van der Stricht noted the presence of the sperm-tail in the eggs of mammals, and summarised his observations in one paper (1923). In the bat (Van der Stricht, 1902), the sperm enters the egg during the second maturation division. The tail becomes detached immediately, and during the first cleavage division is in contact with the centrosome at one pole of the spindle. The structure is not given in detail, but the tail is figured as possessing a granular middle-piece with a non-granular terminal part. The absence of the tail from some eggs is ascribed to faulty technique. The tail was seen in one cell of the two cell-stage. The sperm-tail has been identified in the fertilised egg of the rat (Van der Stricht, 1923); it was situated in the neighbourhood of the male pro-nucleus. No further observations appear to have been carried out on this animal.. A short part of the tail, attached to the sperm-head, was observed in the egg of the dog (Van der Stricht, 1908) during the second maturation division.

Kremer (1924) identified the sperm-tail in the fertilised egg of the rat, but did not describe its structure. He was unable to find the tail in the fertilised ovum of the mouse, and concluded that it disintegrates shortly after the entry of the sperm.

Nihoul/

Nihoul (1927), in a paper dealing with fertilisation in the rabbit, states that he is not convinced that the penetration of the sperm-tail is a constant feature.

Mainland (1930) identified the sperm-tail in a fertilised egg of the ferret; the structure was not described.

From the above account it will be seen that it has been recognised that part of the sperm-tail, seen in the fertilised ova of certain mammals, has a granular structure, and, in some cases, it has been stated that the granules are mitochondrial in origin. There does not appear, however, to be any detailed account of the history of the mitochondria of the sperm in the cytoplasm of the mammalian egg. The only reference to the Golgi elements of the vertebrate sperm, as seen in fertilised eggs, appears to be that of Nihoul (1927) in a paper on the egg of the rabbit. Nihoul considers that "la partie de l'élément mâle dans la constitution de l'appareil de Golgi de l'oeuf fécondé et des cellules embryonnaires ne saurait être que très petite".

If cytoplasmic inclusions are introduced into the egg by the sperm, then the study of the behaviour and fate of these bodies in the ooplasm is of great interest from the point of view of the contribution of the sperm to the cytoplasm of the embryo. The importance of such a study is clearly stated by Brambell (1930). He says - "virtually/

"virtually nothing is known of the fate in vertebrates of the sperm mitochondria and Golgi bodies contained in the middle-piece. Yet these various bodies constitute the cytoplasm received from the male parent and are equivalent, from the point of view of cytoplasmic inheritance, to the entire cytoplasm of the egg. It is highly desirable that these structures should be followed, by means of modern technique, through the stages of fertilisation and segmentation".

Considerably more detailed observations have been carried out on the behaviour of the mitochondria of the sperm in the eggs of invertebrates. Meves (1911) has shown that mitochondria are carried with the middle-piece into the egg of Ascaris. The mitochondria are, at first, larger than are those of the egg; they become distributed through the cytoplasm, and at the same time fragment into smaller bodies indistinguishable from those of the ovum. Meves, later, showed that in Filaria (Meves, 1916 a) mitochondria are introduced into the egg by the sperm, and their subsequent behaviour is closely similar to that of the sperm-mitochondria of Ascaris. In Phallusia (Meves, 1914 b) and in Mytilus (Meves, 1916 b) the fate of the sperm-mitochondria in the ooplasm could not be followed. In Echinus (Meves, 1914 a) the middle-piece becomes segregated into one of the first two blastomeres, and can be traced to the 32 cell-stage when it is situated in one of the blastomeres. In order to explain the /

the presence of the middle-piece in one blastomere of the 32 cell-stage, Meves suggests that the young animal is derived wholly, or mainly, from the cell containing the middle-piece, while the larval structures, which do not persist, are formed from the cells containing egg-mitochondria only.

Duesberg (1915) states that the sperm-mitochondria are carried into the egg of Ciona. Held (1917) has traced the mitochondria of the sperm of Ascaris (****) through the stages of fertilisation, and more recently, (1936) Collier_^ claims that sperm-mitochondria are liberated in the egg of Ascaris.

In the light of the clear demonstration of the presence of sperm-mitochondria in the eggs of certain invertebrates, and as there is no detailed study on the behaviour of the cytoplasmic inclusions of the sperm in the mammalian ovum, it is desirable that further observations be carried out on the fertilised egg of the mammal. Hence, the present work on the fertilised ovum of the mouse was undertaken.

Lams and Doorme (1908) gave a brief account of the mitochondria during fertilisation and the two cell-stage of the mouse; they did not describe the Golgi elements. Their observations, together with those of other authors, will be discussed later, in so far as they have a bearing on the present findings.

2. MATERIAL AND METHODS.

The material used in the present investigation consisted of fertilised eggs of the mouse fixed at various periods after pairing had taken place. The oviducts were dissected out and immediately placed in the fixing fluid. Sections were subsequently cut at 5μ and at 8μ in thickness.

In order to demonstrate the Golgi material oviducts were fixed according to the methods of Mann-Kopsch, Kolatchev, and of Aoyama. It was found that the material treated according to Aoyama's technique gave the best results: the sections were either mounted unstained, or were stained in Ehrlich's haematoxylin. The mitochondria were often well shown in the unstained preparations being a deep golden-brown in colour, while the Golgi elements were deeply impregnated. These sections were also favourable for the study of the middle-piece of the sperm during the early stages of fertilisation.

For the study of the mitochondria and of the yolk-globules material fixed in Flemming's fluid (without acetic) gave very good results. The sections were stained in Heidenhein's iron-haematoxylin. This material was also favourable for the study of the male and of the female pronucleus.

3. OBSERVATIONS.(a) THE STAGE OF DEVELOPMENT OF THE EGG IN RELATION
TO COPULATION.

Many of the animals used in the present investigation were examined for vaginal plugs twice a day; in these cases it was possible to obtain a rough estimate, only, of the time which had elapsed between pairing and fixation of the oviducts. Twenty-four animals were kept under observation, and an exact record kept of the time at which copulation had taken place. The oviducts of these animals were fixed at times varying from 5 to 43 hours later.

The oviducts of four individuals, fixed 5 - 6 hours after copulation, contained eggs undergoing the first maturation division. That this division may be completed earlier was shown by the presence of eggs, in phases of the second polar division, in oviducts fixed at 5 hours 10 minutes, and at 5 hours 30 minutes. The spindle of the second maturation division was also seen in a number of eggs fixed at times varying between 6 hours and 7 hours 15 minutes after pairing.

The present investigation shows that in the vast majority, if not in all cases, the sperm enters the ovum during the anaphase of the second maturation division, and that the sperm-tail is carried into the cytoplasm.

This/

This was very clearly shown in certain of the Aoyama preparations where part of the middle-piece was still outside the cytoplasm of the egg. As only one egg in the stage of the first polar division contained a sperm, it is evident that the entry of the sperm at this stage is very uncommon. It is unsafe to base a conclusion on one specimen only, and it may be that this egg was, in reality, undergoing the second division, the first polar body having been lost during the preparation of the material.

Eggs in the stage of the pronuclei were present in a number of oviducts placed in the fixing fluid at times varying from 6 hours to 9 hours 40 minutes after pairing. In one of these oviducts (about 6 hours) the male pronucleus was of approximately the same size, and closely similar in appearance to, the female pronucleus; in some of the eggs the middle-piece was still attached to the male pronucleus, while in others it was free. In all other preparations in which the male pronucleus had increased in size and assumed its final appearance the middle-piece had already become detached. The eggs in one oviduct (7 hours) contained a sperm-nucleus, which had increased but little in size, with a compact middle-piece in a stage prior to the spreading out of the mitochondria. The egg-nucleus had completed the two polar divisions. The ova in the other oviduct of the/

the same animal contained large male pronuclei detached from the middle-piece.

The first cleavage division was shown in eggs at 21 - 28 hours, and the two cell stage 24 - 43 hours after pairing. One oviduct, fixed at 21 hours, contained one egg in the prophase of the first cleavage division, while the remainder were still at the stage of the pronuclei. Another oviduct, fixed at 24 hours, showed eggs undergoing the first cleavage division and at the two-cell-stage. An examination of other material of which a less precise record was kept indicated that the four cell stage is reached in about 48 hours.

To summarize:- The first maturation division is completed within 6 hours after copulation. The second division takes place between 5 to about 7 hours. The stage of the pronuclei may be reached as early as 6 hours after pairing, but usually is not evident until slightly later; it persists until 20- 24 hours. The spindle of the first cleavage division is present in eggs fixed between 21 - 28 hours; the two cell stage is often reached in about 24 hours; the four cell stage in about 48 hours. Except in one case all the eggs within an oviduct were at the same stage.

lying in the peripheral cytoplasm (see page 121).

During the second maturation division the form and distribution of the Golgi elements and of the yolk-

globules is closely similar to that of the first division

(Fig. 7)

(b) THE MATURATION DIVISIONS.

In eggs in the anaphase or telophase of the first maturation division the yolk-globules are in the peripheral region, but are absent in the vicinity of the spindle; consequently the globules tend to be most numerous in the half of the egg opposite to that at which the polar division is taking place. The mitochondria are granular, and are collected into small clumps, while a few, chiefly towards the periphery of the cell, are scattered singly through the cytoplasm. The clumps are most numerous in the central region and in the neighbourhood of the spindle, and, in most cases, are particularly numerous in the equatorial region (fig. 60, Pl.11). Owing to their disposition relatively few mitochondria are eliminated with the first polar body; this was confirmed by an examination of polar bodies shown in preparations of later stages. The Golgi elements are in the form of granules and of small irregularly shaped bodies, and are more or less evenly distributed throughout the cytoplasm (fig. 70, Pl.12). In one egg, in an Aoyama preparation, which appeared to be in the telophase of the first polar division, the sperm-head and middle-piece is shown lying in the peripheral cytoplasm (see page 121).

During the second maturation division the form and distribution of the Golgi elements and of the yolk-globules is closely similar to that of the first division (fig./

(fig. 70, Pl.13). The arrangement of the mitochondria in relation to the spindle is, however, usually somewhat different. The spindle is always placed approximately parallel to the surface of the egg, and is nearly always situated at the extreme periphery; hence, owing to their scarcity in the peripheral region, mitochondria are not numerous at the poles or between the spindle and the surface of the cell. They are numerous at the other side of the spindle (fig. 62, Pl.11). In some few of the eggs examined the spindle of the second maturation division was situated in the deeper cytoplasm, and in these the arrangement of the mitochondria, in relation to the spindle, was closely similar to that of the first polar division. An examination of the second polar body showed that, like the first, it contained relatively few mitochondria. During maturation the mitochondria are slightly more numerous in the half of the egg at which the polar divisions are taking place (figs. 60 and 62, Pl.11).

In many of the eggs undergoing the second maturation division the sperm-head and middle-piece are shown lying in the peripheral cytoplasm of both Flemming and of Aoyama preparations. As the middle-piece, at this stage is deeply impregnated after treatment with silver nitrate it is particularly well shown in Aoyama material (figs. 66 and 68, Pl.13). The sperm-head is faintly stained and often difficult to identify (fig. 67, Pl.13).

The/

The stage of fertilisation at which the middle-piece becomes detached from the sperm-head appears to vary considerably, as in some eggs the male pronucleus is free of the middle-piece during the second maturation division while in others the two are still joined. In a few instances the middle-piece is lying close to, and apparently joined with, the male pronucleus after the completion of both polar divisions (fig. 63, Pl.12).

During the second maturation division the sperm-nucleus increases rapidly in size, and is usually differentiated into darkly and lightly stained regions. A faintly stained irregularly shaped mass of material can often be identified in the cytoplasm in the vicinity of the sperm nucleus: this is probably the remains of the acrosome.

The sperm nucleus contains, at first, a single large nucleolus, but with the rapid increase in the size of the nucleus several nucleoli make their appearance. The female pronucleus possesses several nucleoli, and in some cases as many as ten or eleven very small. By the time the two nuclei have approached the central region they both contain about the same number of nucleoli and are approximately equal in size. Hence, it is difficult, in the later phases, to distinguish with certainty between the male and the female pronucleus.

(c) THE STAGE OF THE PRONUCLEI.

In eggs at this stage the male and female pronuclei lie either far apart towards the periphery or close together in the central region. In the younger eggs the male pronucleus can be identified with ease, owing to its smaller size, and, in most preparations, to the presence of the sperm tail which may be still attached, or may lie in the cytoplasm in the neighbourhood of the nucleus.

In some preparations the middle-piece only is shown, while in other sections the whole tail appears to be present (fig. 61, Pl.11 and Fig. 64, Pl.12). It is likely, therefore, that the entire tail is carried into the egg, but that the flagellum is not always visible with the technique employed in the present investigation.

The sperm nucleus contains, at first, a single large nucleolus, but with the rapid increase in the size of the nucleus several nucleoli make their appearance. The female pronucleus possesses several nucleoli, and in some cases as many as ten or eleven were counted. By the time the two nuclei have approached the central region they both contain about the same number of nucleoli and are approximately equal in size. Hence, it is difficult, in the later phases, to distinguish with certainty between the male and the female pronucleus.

The yolk-globules, as in the preceding stages, are situated at the periphery, but are few or absent in the vicinity of the polar bodies. In the earlier phases the mitochondria are arranged in clumps which are fairly evenly distributed throughout the cytoplasm, except at the extreme periphery where they are few in number (fig. 61, Pl. 11; figs 63 and 64, Pl.12). In some few cases they are slightly more numerous in the half of the cell nearest to the polar bodies. These, in all probability, are eggs which have but recently completed the second maturation division, and consequently the mitochondria have not yet spread out from the position which they occupied during the polar divisions. Later, when the two pronuclei are lying close together in the central region of the cell, there is a concentration of mitochondria in the central region, particularly in the neighbourhood of the pronuclei. This concentration is, apparently, in preparation for the first cleavage division (fig. 69, Pl.13).

The Golgi elements are larger and much more numerous than during the maturation divisions. They are chiefly in the form of irregular and of rod-shaped bodies, while rings and granules are also present. Appearances indicate that the smaller elements, present during maturation, have increased greatly in size, and are now fragmenting to form smaller bodies which are becoming distributed throughout the egg (figs. 71 and

72, Pl. 14). The multiplication of the Golgi elements continues during all phases of this stage of fertilisation. The Golgi bodies are more or less evenly distributed through the cell, except in the peripheral region where they are less numerous than elsewhere.

The polar bodies contain granular mitochondria arranged in small clumps and scattered throughout the cell (fig. 63, Pl. 12). Each polar body contains small granular Golgi elements; these, however, increase slightly in size in the older polar bodies. In one of the Aoyama preparations the first polar body is undergoing division. The mitotic figure is in the early anaphase, and the Golgi bodies are scattered through the cytoplasm (fig. 74, Pl. 15).

(d) THE FIRST CLEAVAGE DIVISION.

As the pronuclei approach each other the concentration of the mitochondria in the central region of the egg becomes more marked, but the distribution of the Golgi elements does not appear to undergo any change (fig. 69, Pl.13). Although a large amount of material was examined ova showing the fusion of the pronuclei were not identified. One egg, in a Kolatchev preparation, was in the early prophase of the first cleavage division. The mitochondria are granular and are collected into clumps which are particularly numerous in the immediate vicinity of the nucleus. There is, therefore a further concentration, since the preceding stage, of the mitochondria in the neighbourhood of the nucleus. The Golgi elements, although slightly more numerous in the cytoplasm adjacent to the nucleus, appear to have undergone but little change in their distribution.

Several of the Flemming preparations contain cells in the metaphase and in the anaphase of the first cleavage division. The yolk-globules are, as in the preceding stages, distributed round the periphery of the cell. The mitochondrial clumps are now closely packed around the spindle, so that frequently the clumps have run together to form larger masses (fig. 73, Pl.14). In some of the anaphase stages examined, the clumps at the spindle poles are particularly large and dense.

In/

In these eggs the mitochondria are less numerous in the remainder of the cytoplasm than in cells in which the concentration around the spindle is less intense (fig. 65, Pl.12).

It is evident, from the examination of the material described above, that the concentration of the mitochondria in the central region begins before the fusion of the pronuclei, and proceeds around the zygote nucleus during the prophase and the metaphase of the first division, and that in the anaphase the poles and sides of the spindle are surrounded by large dense masses of mitochondria. And, further, it is evident that this arrangement will result in the distribution of the mitochondria in approximately equal numbers between the two resulting cells.

The osmic and silver preparations did not show metaphase, or later stages of the zygote nucleus. An examination of the distribution of the Golgi elements in the prophase stage, described above, shows that although the Golgi bodies are slightly more numerous in the neighbourhood of the nucleus there is no marked change in their distribution correlated with the cleavage division. It is concluded, therefore, that, as the Golgi elements are more or less evenly distributed throughout the cytoplasm, the division of the cell results in their separation into two approximately equal groups.

(e) THE TWO CELL STAGE.

The nucleus of each blastomere contains large deeply stained nucleoli. The yolk-globules occur in the peripheral region (fig. 76, Pl.15).

The mitochondria, like those of the earlier stages, are granular and are arranged chiefly in clumps while a few are scattered singly through the cytoplasm. The mitochondrial clumps are most numerous in a fairly wide area surrounding the nucleus (fig. 76, Pl.15). In many cases this area extends to the region immediately adjacent to the other cell, while the distribution of the mitochondria in the second cell is closely similar to that of the first. In other cases the concentration of the mitochondria in the immediate neighbourhood of the nucleus is more marked. It is evident, therefore, that the first example represents an early two-cell stage which has but recently completed the first cleavage division, and that the mitochondria have not spread out to any great extent from the position which they occupied around the spindle. Later, the mitochondrial clumps become more closely concentrated in the vicinity of the nucleus, apparently in preparation for their arrangement around the spindle of the second cleavage division.

The Golgi elements are numerous and are present in approximately equal numbers in the two cells, thus further supporting the view that the first cleavage division/

division separates the Golgi bodies into two approximately equal groups. The Golgi elements are fairly evenly distributed throughout the cytoplasm, but are not numerous at the extreme periphery of the cell. Many of the Golgi bodies are very irregular in shape, and a few are much larger than are those present during the stage of the pronuclei (fig. 75, Pl.15). It would appear, therefore, that the Golgi elements rapidly increase in size and then fragment to form smaller bodies, so that there is a great increase in the amount of the Golgi material, presumably to keep pace with the rapid cell multiplication which will result from the cleavage divisions.

Cells in division were not shown in this material, and as the present investigation was undertaken primarily in order to study the cytoplasmic inclusions during the stages of maturation and fertilisation only, no attempt was made to obtain further preparations.

Golgi elements and mitochondria were identified in the polar bodies during the two-cell stage.

(f) THE CYTOPLASMIC INCLUSIONS OF THE SPERM.

The sperm-head with the middle-piece attached was identified in the peripheral region of a number of eggs undergoing the second maturation division. In most of the Flemming and Aoyama preparations the sheath of the axial filament is compact and deeply stained, and does not show any detail of structure except for a granular appearance and the appearance, on careful focussing, of a clear central line; this line marks the position of the axial filament. During the second maturation division the middle-piece is usually free of the sperm-head, but in some cases the two may still be joined. In some of the eggs the sheath is less compact and has a well marked granular structure (figs. 66 and 68, Pl. 13). The granules are identified as the mitochondria from which the sheath was originally formed, and which are now separating again into individual elements. The mitochondria of the sperm-tail are, at this stage, slightly larger and are more deeply stained than are those of the egg. This is well shown in Aoyama material, where the mitochondria of the sperm are deeply impregnated while those of the egg are golden-brown in colour.

Two of the eggs in an oviduct fixed in Flemming's fluid six hours after pairing had taken place show the male pronucleus situated in the vicinity of the female pronucleus, but with the middle-piece still attached. The mitochondria are well shown and are larger than those/

anterior end of the middle-piece. This structure is those of the egg (fig. 63, Pl.12). In all other material at the stage of the pronuclei the middle-piece is detached from the sperm-head, and may lie at the extreme periphery or, more often, extends into the deeper cytoplasm. The mitochondria have further separated out from their original position (fig. 61, Pl.11, fig. 64, Pl.12). Although a large number of Flemming preparations were examined further stages in the dispersal of the mitochondria were not identified. A few of the Aoyama preparations, however, gave a clear indication of their subsequent behaviour. As the mitochondria of the sperm spread out from their original position they become less deeply impregnated than formerly, but are still darker than those of the egg (fig. 71, Pl.14). Later, they form a loose broad band of a slightly deeper golden-brown colour than the other mitochondria in the cell (fig. 72, Pl.14).

The failure to trace the mitochondria of the sperm at a later phase of fertilisation than that described above is due to loss of intensity of staining, distribution of the granules through the cytoplasm, and also, in all probability, to a reduction in size, so that they become indistinguishable from the mitochondria present prior to the entrance of the sperm.

In eggs treated by the silver technique a small mass of deeply impregnated material is present at the anterior/

anterior end of the middle-piece. This structure is identified as the Golgi material of the sperm previously described in the account of spermateleosis. In a few of the preparations in which the middle-piece has become detached from the head of the sperm, a deeply impregnated mass of material was observed situated at the proximal end of the middle-piece. As this structure is darker than the mitochondria of the sperm, which are now a deep golden-brown colour, and as it is larger than the Golgi elements of the egg situated in the peripheral region, it is identified as the Golgi material of the sperm (fig. 71, Pl.14). No later stage in the history of the sperm Golgi substance was identified. It is highly probable, therefore, that, with the disaggregation of the mitochondria of the sheath, the Golgi material of the sperm is rapidly dispersed through the cytoplasm, where it increases in amount and fragments to form smaller bodies.

The present investigation shows clearly that the mitochondrial sheath of the middle-piece separates into granules which are, at first, slightly larger and stain more deeply than the mitochondria of the egg. The granules spread out and, during the stage of the pronuclei, form a broad band; at the same time they become less deeply stained in Flemming material and less deeply impregnated in silver preparations. Finally, these mitochondria, having become similar in staining properties/

properties and in size, at least, to those of the egg, become scattered through the cytoplasm. As no later stage than those described above was observed the final scattering of the granules must take place suddenly and with great rapidity, and at a sufficiently early phase to allow of their distribution throughout the cytoplasm before the first cleavage division. Such a scattering must result in the arrangement of the mitochondria derived from the sperm around the spindle along with those of the egg, and consequently to their distribution to the two resulting cells.

It should be pointed out that the appearance and staining properties of the Golgi elements and of the mitochondria of the sperm give no indication that they undergo degenerative changes in the ooplasm. They differ only in size from those of the egg, and in that the mitochondria are, at first, more deeply impregnated. If the cytoplasmic bodies carried into the egg by the sperm underwent degeneration it is evident that marked abnormalities in appearances and in staining properties, similar to those described for degenerating ovarian eggs, would be manifest. As no such abnormalities were observed it is clear that both the mitochondria and the Golgi elements of the sperm persist, become indistinguishable from those of the ovum, and are transmitted to the two cells formed as the result of the first cleavage division.

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In some of the Flemming preparations, two deeply stained granules were observed at the proximal end of the middle-piece of sperms which had recently entered the egg. These are, in all probability, the centrioles of the sperm. Centrioles derived from the sperm were not observed during the later phases. This is probably due to the large number of granular mitochondria, rendered visible by the methods of technique employed, making their identification uncertain.

The acrosome becomes detached from the sperm-nucleus very soon after the entry of the latter into the egg. As a faintly stained body was frequently observed in the cytoplasm close to the sperm nucleus, it is concluded that the acrosome undergoes rapid degeneration. That its degeneration is completed at an early stage of fertilisation is indicated by the absence of any structure which could be identified, during the later stages, as the remains of the acrosome.

According to the present findings, the acrosome enters the ovum during the second maturation division, usually when the egg-nucleus is in the metaphase. This agrees with the observations of Lillie and Searge (1908), who state that the sperm enters the ovum of the mouse during the second polar division, of Van der Stricht (1913) for the bat and for the cat, of Kramer (1924) for the mouse and rat, and of Nilsson (1927) for the rabbit. Van der Stricht (1913)/

4. DISCUSSION.

The earlier literature on ovulation in the mouse is conflicting and, as the present work is primarily concerned with maturation, fertilisation and the first cleavage division with special reference to the cytoplasmic inclusions, need not be dealt with here. It may be mentioned, however, that in one of the more recent papers on the mouse Long and Mark (1911) state that ovulation occasionally takes place at the stage of the first polar spindle, sometimes during the telophase, but usually not until the egg contains the second spindle. The presence of eggs undergoing both the first and the second maturation divisions in oviducts fixed five to six hours after pairing seems to support the findings of Long and Mark. It would appear, however, that ovulation during the first polar division is not uncommon.

According to the present findings the sperm enters the ovum during the second maturation division, usually when the egg-nucleus is in the anaphase. This agrees with the observations of Lams and Doorme (1908), who state that the sperm enters the ovum of the mouse during the second polar division, of Van der Stricht (1923) for the bat and for the cat, of Kremer (1924) for the mouse and rat, and of Nihoul (1927) for the rabbit. Van der Stricht (1923)/

(1923) states that the sperm enters the egg of the dog during either the first or the second polar division. In the course of the present investigation one egg, which was apparently undergoing the first maturation division, contained a sperm. While no reliable conclusion can be drawn from this single specimen, it should be borne in mind that, in the mouse, the sperm may occasionally penetrate the ovum before the first polar body has been formed. Long and Mark (1911), for the mouse, state that the sperms reach the ova in four to seven hours or more after copulation. According to the present observations the sperm enters the egg between about five hours and seven hours after pairing.

The stage of the pronuclei lasts from about six or seven hours up to about twenty to twenty-eight hours. The spindle of the first cleavage division was observed in eggs of twenty-one hours up to twenty-eight hours, but usually is present at about twenty-four hours after copulation. This agrees with Lewis and Wright (1935) who have carried out investigations on the fertilised egg of the mouse. They believe that "the stage of development of the egg in relation to copulation indicates that there is a definite though not exact relationship between the two events". They show that most of the eggs examined between one-quarter and twenty-four hours were in the one cell-stage, while most of those examined between twenty-four and forty-eight hours were/

were in the two to four cell stage.

During the two polar divisions the yolk-globules are absent in the neighbourhood of the spindle and are, consequently, more numerous in the half of the egg opposite to that at which the divisions are taking place. As yolk in the mouse ovum is scanty, there is no marked separation into deutoplasmic and non-deutoplasmic poles. The mitochondria are most numerous in the half of the egg at which the maturation divisions are taking place, and during the first division and the earlier phases of the second division the mitochondria are particularly numerous in the vicinity of the spindle. This partial segregation of the yolk and of the mitochondria towards opposite poles disagrees with the findings of Lams and Doorme (1908), who claim that most of the mitochondria and the yolk-globules are situated at the opposite side of the egg to the polar spindle. Lams and Doorme do not describe the arrangement of the mitochondria in relation to the spindle, but state that few mitochondria are eliminated in the first polar body.

During the maturation divisions the mitochondria of the guinea-pig egg are scattered through the cytoplasm (Lams, 1913). In the bat (Levi, 1915) they are arranged in a peripheral zone. According to Van der Stricht (1923) they are arranged in a cortical layer in the egg of the bat, cat and dog. In the bat the cortical/

cortical layer is thicker around the first polar spindle, and in the dog it is sometimes thicker around the spindle and around the sperm-nucleus. Nihoul (1927) states that the mitochondria are at first slightly more numerous at the periphery of the rabbit-egg, but when the two pronuclei approach each other the mitochondria are arranged radially around the area containing the pronuclei and are, consequently, less numerous at the periphery of the cell. Thus it appears that an arrangement of the mitochondria similar to that of the egg of the mouse during the maturation divisions, as described in the present paper, has not been recorded previously for the eggs of mammals.

Van der Stricht (1923) states that during fertilisation the egg of the bat and of the guinea-pig undergo a change of polarity, brought about by a movement of the yolk to the former animal pole, and that eggs of the dog, cat and mouse preserve their primitive polarity. According to the present findings there is no marked polarity in the egg of the mouse. That the globules appear to be fairly evenly distributed between the first two blastomeres would indicate that they spread out slightly from their original position before the first cleavage division.

Lams and Doorme (1908) describe the globules present in the ooplasm of the mouse, as fat. These globules have been shown conclusively, by the present writer, to/

to be non-fatty in nature (see p. 22).

During the first cleavage division of the egg of the mouse there is a marked concentration of the mitochondria around the spindle. The first cleavage division of the egg of the mouse was not described by Lams and Doorme, and a marked concentration of the mitochondria in relation to the spindle does not appear to have been previously recorded for the mammalian ovum. The nearest approach seems to be in the egg of the rabbit where, according to Nihoul (1927), the two pronuclei when close together are surrounded by a clear area. The mitochondria have a radial disposition around this zone, but during division they appear to be numerous in the neighbourhood of the spindle.

Such an arrangement of the mitochondria in relation to the spindle, as that described for the mouse egg, would lead to their segregation in approximately equal numbers between the first two blastomeres. In the maturation divisions, however, owing to the position of the spindle and to the small size of the polar bodies, a few mitochondria only are eliminated from the egg. That the mitochondria are segregated into two groups of approximately equal numbers by the first cleavage division was confirmed by observations on the two cell stage. As previously pointed out they are sometimes most numerous in the part of the blastomeres adjacent to each other. It is of interest that Lams and Doorme have/

have described a two cell stage in which the mitochondria are most numerous in the adjacent parts of the cell.

Nihoul (1927) gives an account of the Golgi material of the oocyte and of the fertilised egg of the rabbit. During the stage of the pronuclei the majority of the Golgi elements are, at first, concentrated around the male pronucleus; later, when the female pronucleus approaches the male, the Golgi elements tend to surround it also. The area occupied by the Golgi bodies is shown as a clear perinuclear zone in other preparations. The first cleavage division was not observed.

During fertilisation and the first cleavage division of the mouse-egg there is no marked concentration of the Golgi elements in the vicinity of the pronuclei or of the spindle. They remain fairly evenly distributed throughout the cytoplasm, and with the division of the cell are divided into two approximately equal groups. The fragmentation of the Golgi elements during the stages of fertilisation is of interest, and is, apparently, in preparation for the rapid cell multiplication which takes place during cleavage. Nihoul does not describe fragmentation of the Golgi bodies in the fertilised egg of the rabbit, but states that the size of the "Golgi apparatus" becomes reduced as the cleavage divisions proceed, and that the material, which is localised in the blastomeres, must fragment during/

during mitosis.

The rabbit and the mouse are, so far as the writer is aware, the only mammals in which the mitochondria and Golgi elements have been studied in detail during fertilisation and the first cleavage division. There are marked differences between the present account and that of the rabbit egg, which would indicate that there is considerable variation in the behaviour of the cytoplasmic inclusions during the stages of fertilisation in the ova of different mammals. The papers of Lams (1913), of Levi (1915), and of Van der Stricht (1923) add evidence in support of this view.

The present investigation shows clearly that the sperm middle-piece, and in some cases at least the entire sperm-tail, is carried into the egg. The work of Lams (1913), Levi (1915), and of Van der Stricht (1923), showing that the sperm-tail is present in one of the early blastomeres of certain mammals, does not support the present conclusion that the mitochondria of the sperm become scattered through the cytoplasm prior to the first cleavage division. Lams pointed out that the cytoplasm of the blastomere containing the sperm-tail ought to be considered as male and female in origin, while the cytoplasm of the other blastomere is exclusively female. Van der Stricht suggests that the embryo is formed from the cell containing the sperm middle-piece while the other blastomere gives rise to the trophoblast. During/

During the present investigations a number of cleavage and two cell-stages were examined, but in no case was the middle-piece identified. It appears, therefore, that the mitochondria of the sperm are scattered through the ooplasm during the stage of the pronuclei. Further, as the male mitochondria show marked activity after their introduction into the ooplasm, and as there is no evidence of their undergoing degenerative changes, it is reasonable to conclude that they become distributed throughout the cytoplasm, undergo fragmentation, and later are arranged around the spindle together with those of the egg, and are consequently shared out in approximately equal numbers between the first two blastomeres.

The fate of the Golgi material of the mouse-sperm is more difficult to follow than that of the mitochondria. As the entire middle-piece is introduced into the egg, and as, in silver preparations, a small deeply impregnated mass was observed at the proximal end of the middle-piece, it is evident that the sperm Golgi material enters the ooplasm. The presence, at a later stage, of a larger mass of Golgi material at the proximal end of the middle-piece suggests that the Golgi substance increases in amount. Later, in all probability, it undergoes fragmentation and further increase in amount, at the same time becoming scattered through the ooplasm, and is, therefore, transmitted, together with that/

that of the egg, to the first two blastomeres.

If the present findings be correct, then it is evident that the sperm contributes directly to the cytoplasmic inclusions of the zygote, and consequently to those of the resulting embryo. It must be remembered, however, that the present conclusions are based on the study of one species only. As Meves (1911, 1914 a, 1914 b, 1916 a. 1916 b), Duesberg (1915), Held (1917), and Collier (1936) have shown clearly that the sperm-mitochondria are carried into the eggs of certain invertebrates, and as Lams (1913), Levi (1915), and Van der Stricht (1923) claim that the middle-piece is segregated into one of the blastomeres of certain mammals it is desirable that further investigations be undertaken on the behaviour of the cytoplasmic inclusions of the sperm in the fertilised egg of other animals.

4. During the maturation divisions the yolk granules are most numerous in the half of the egg opposite to that at which the divisions are taking place. The mitochondria are granular and are collected into small clumps; a few, chiefly towards the periphery are scattered singly through the cytoplasm. They are most numerous in the central region. During the first division, and in the early stages of the second division, they are numerous around the spindles. Few mitochondria are eliminated in the polar bodies. The Golgi elements are in the form

V. SUMMARY.

1. Ova of the mouse were fixed at times varying from 5 - 43 hours after copulation.
2. Ovulation may take place at either the stage of the first or the second maturation division.
3. The sperm enters the egg during the anaphase of the second maturation division. As indicated by one egg the sperm may occasionally enter during the first division. The second maturation division takes place between 5 to about 7 hours after pairing. The stage of the pronuclei may be reached at about 6 hours after pairing, but usually is not reached until later. The first cleavage division takes place between 21 - 28 hours.
4. During the maturation divisions the yolk-globules are most numerous in the half of the egg opposite to that at which the divisions are taking place. The mitochondria are granular and are collected into small clumps; a few, chiefly towards the periphery, are scattered singly through the cytoplasm. They are most numerous in the central region. During the first division, and in the early stages of the second division, they are numerous around the spindle. Few mitochondria are eliminated in the polar bodies. The Golgi elements are in the form of/

of granules and of small irregularly shaped bodies; they are fairly evenly distributed through the cytoplasm.

5. During the stage of the pronuclei the distribution of the yolk-globules is similar to that during the polar divisions. In the later phases there is a concentration of the mitochondria in the neighbourhood of the pronuclei. The Golgi elements are scattered through the cytoplasm; they increase in size and undergo fragmentation.
6. The mitochondria become arranged around the spindle of the first cleavage division and are transmitted with approximate equality between the first two blastomeres. The Golgi elements remain scattered through the cytoplasm but are slightly more numerous in the neighbourhood of the zygote nucleus. The Golgi elements and the yolk-globules are fairly evenly distributed between the first two blastomeres.
7. The middle-piece of the sperm is carried into the egg. The mitochondria of the sheath are, at first, larger than those of the egg; they spread out through the cytoplasm and undergo fragmentation. It is concluded that the mitochondria of the sperm are transmitted to the first two blastomeres. There is/

is evidence that the Golgi material of the middle-piece fragment and that the individual elements become scattered through the ooplasm before the first cleavage division.

8. Granular mitochondria are present in the blastomeres of the two cell-stage; they are collected into small clumps and are scattered singly through the cytoplasm. The Golgi elements undergo fragmentation; they are fairly evenly distributed throughout the cytoplasm.

DESCRIPTION OF PLATES 11 - 15.Lettering.

F.Pr., female pronucleus; G.E., Golgi elements; M., mitochondria; M.Pr., male pronucleus; N., nucleus; P.B., polar body; S.G.E., Golgi elements of sperm; S.H., sperm-head; S.M.P., middle-piece of sperm; Y., yolk-globules.

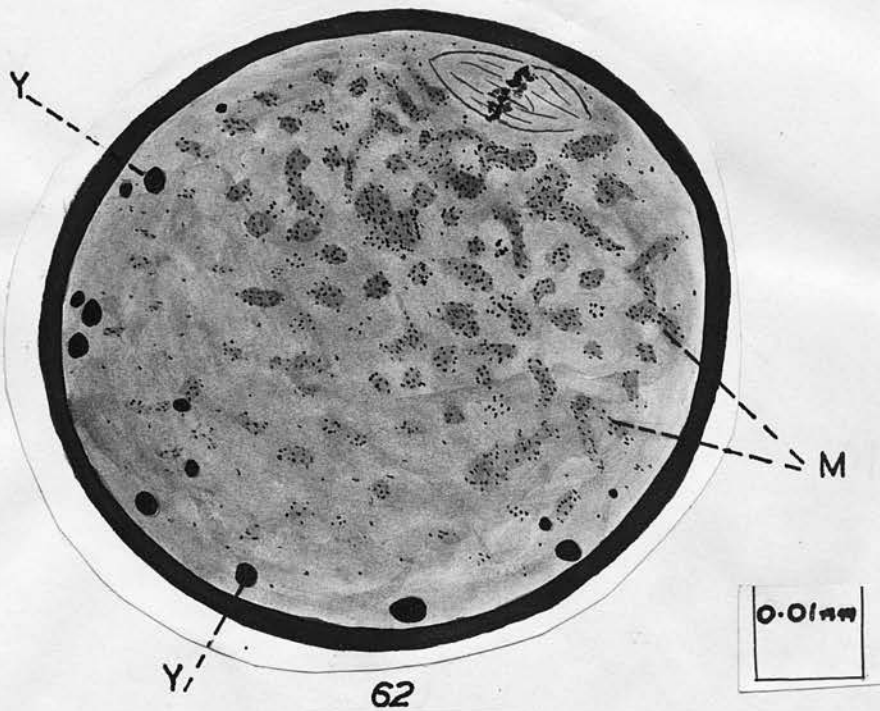
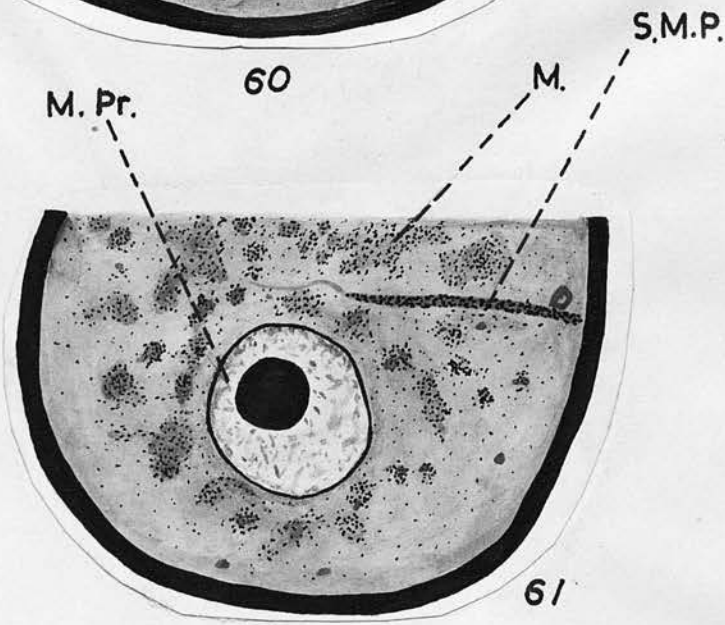
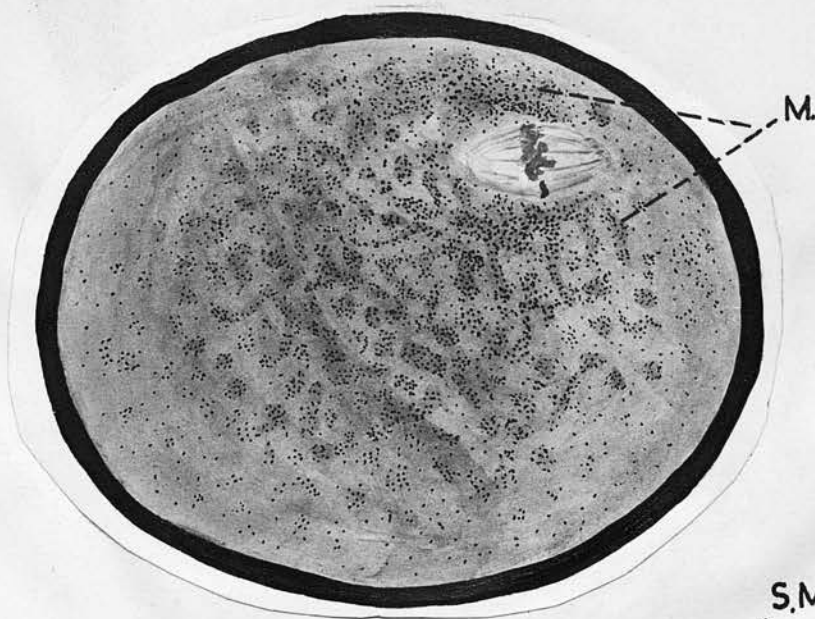
PLATE 11.

All figures from material fixed in Flemming's fluid.

Fig.60.- First maturation division; early anaphase.

Fig.61., Stage of the pronuclei. Mitochondria, male pronucleus, and sperm-tail shown.

Fig.62.- Second maturation division.



0.01mm

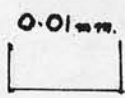
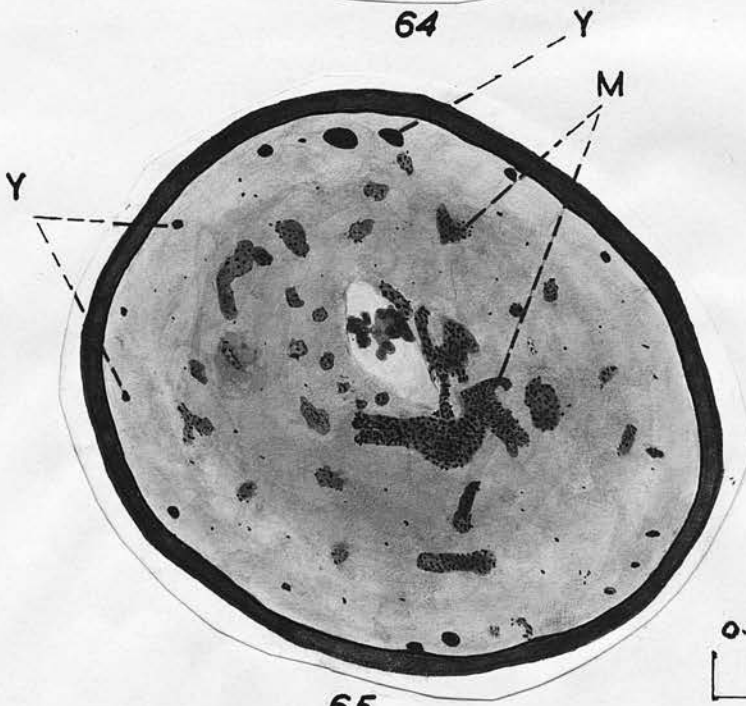
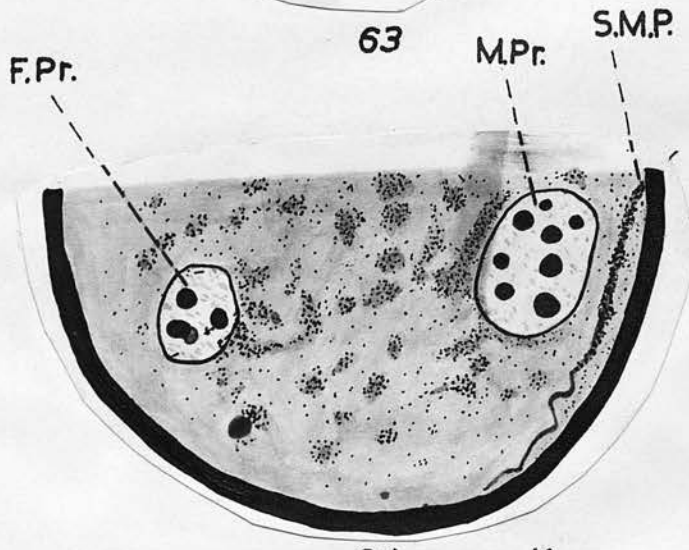
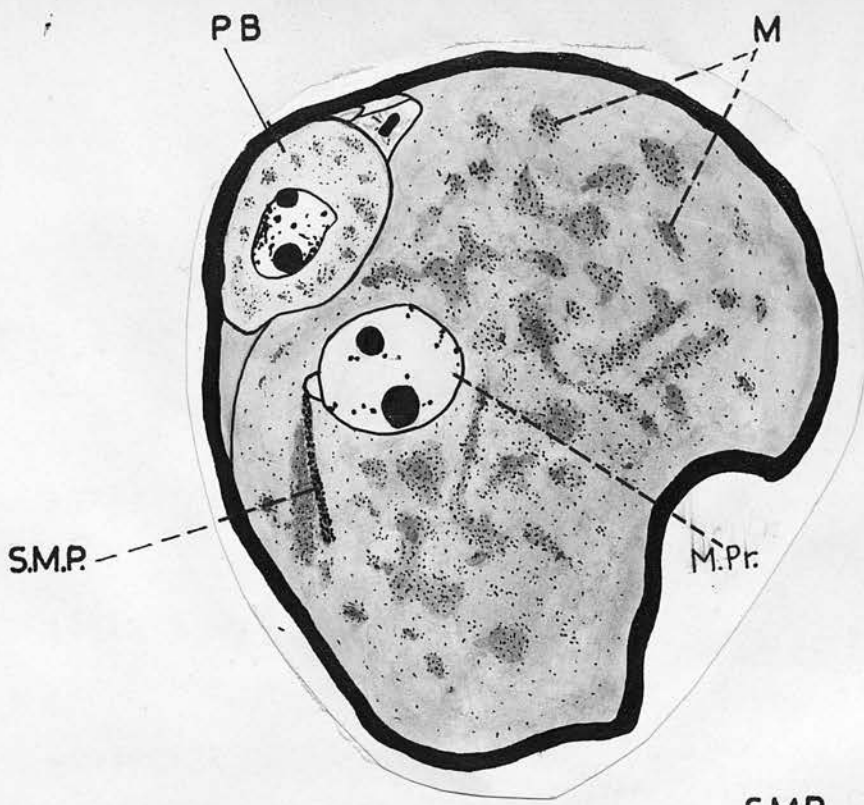
PLATE 12.

All figures from material fixed in Flemming's fluid.

Fig.63.- Male pronucleus with middle-piece still attached.

Fig.64.- Male and female pronucleus, mitochondria, and sperm-tail shown.

Fig.65.- Early anaphase of first cleavage division showing concentration of mitochondria around the spindle.



65

PLATE 13.

Fig. 69 from Kolatchev preparation. All other figures from Aoyama preparations.

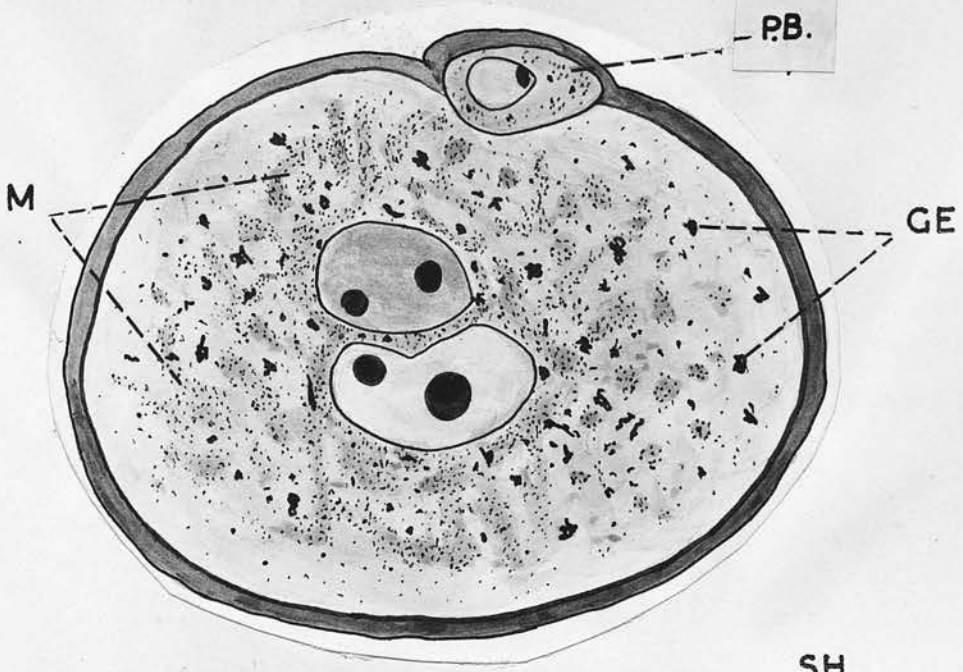
Fig.66.- Anaphase of second maturation division.
To show middle-piece.

Fig.67.- Section through the same egg as fig.66;
showing head of sperm.

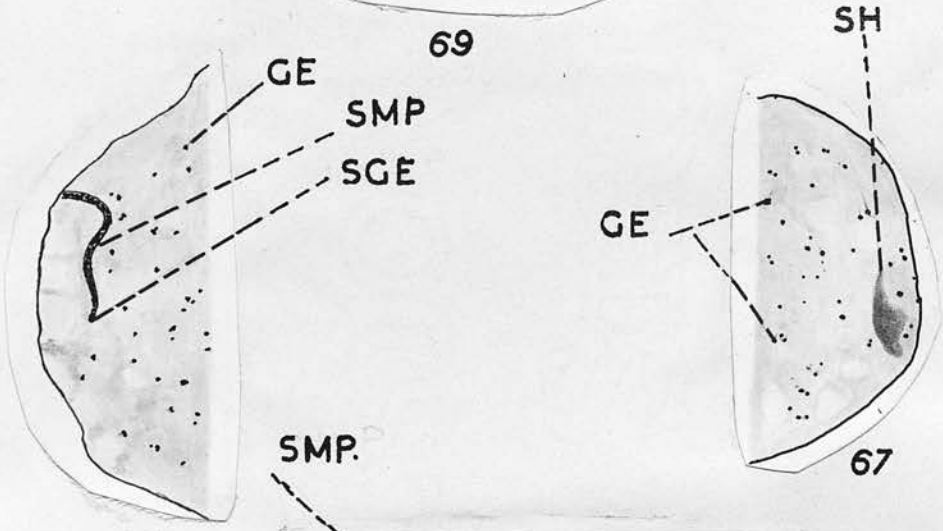
Fig.68.- Anaphase of second maturation division.
To show middle-piece.

Fig.69.- Male and female pronucleus in central
region of egg prior to fusion.

Fig.70.- Anaphase of second maturation division.
Middle-piece shown entering the egg, some
of it is still outside the cytoplasm.

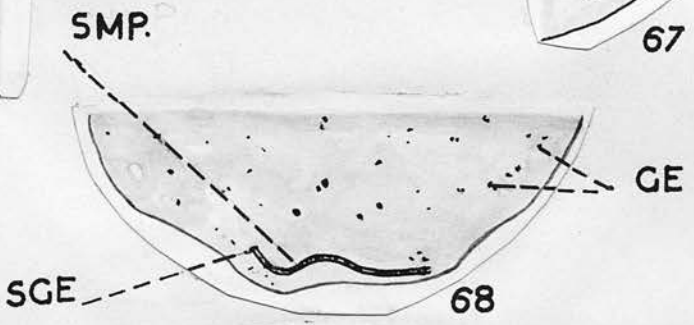


69

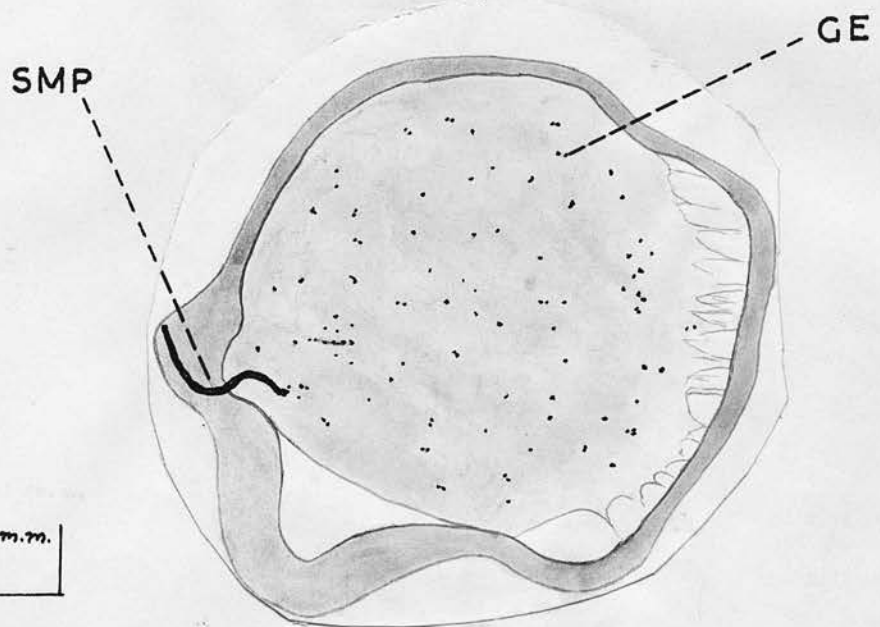


66

67



68



70

0.01m.m.

PLATE 14.

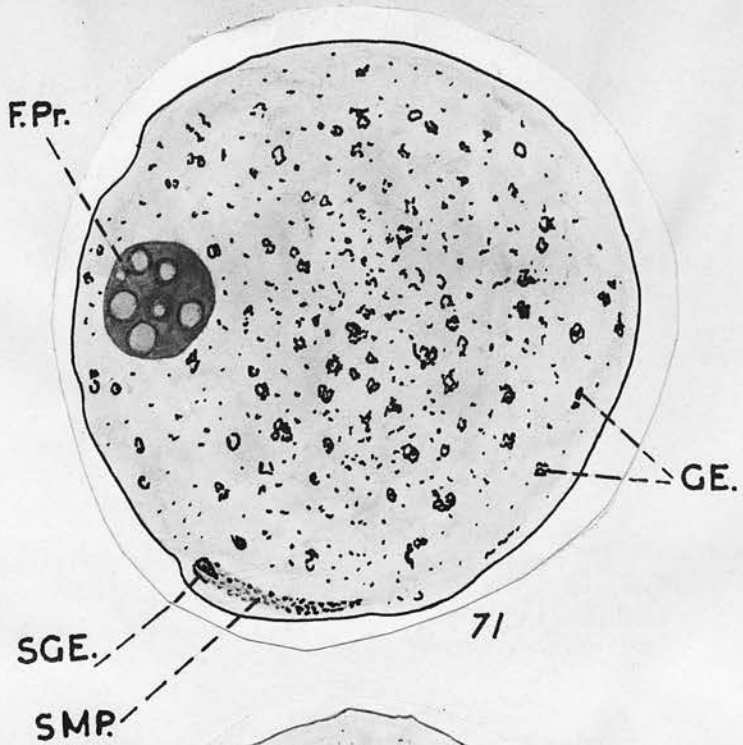
Figs. 71 and 72 from Aoyama preparations.

Fig. 73 from Flemming preparation.

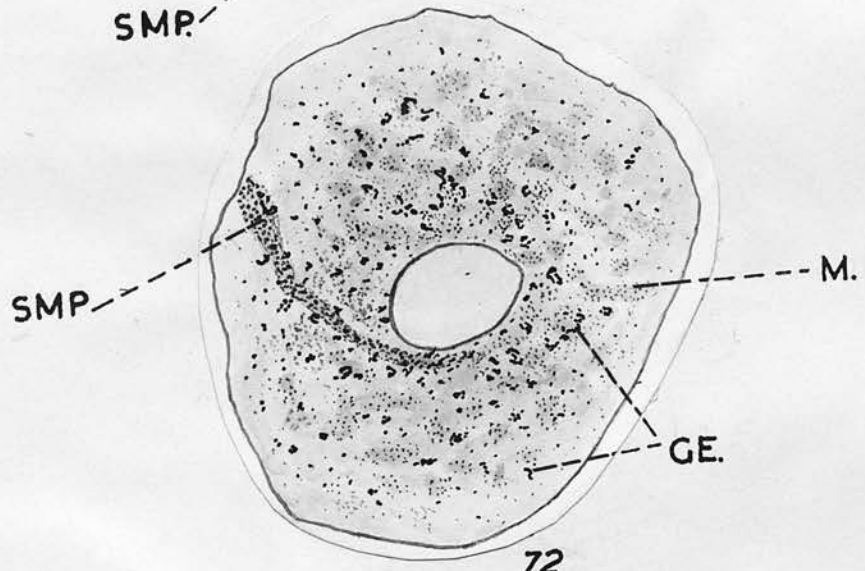
Fig.71.- Mitochondria of sperm middle-piece.
The deeply impregnated material is
the sperm Golgi material.

Fig.72.- Sperm-mitochondria spreading out
through the cytoplasm.

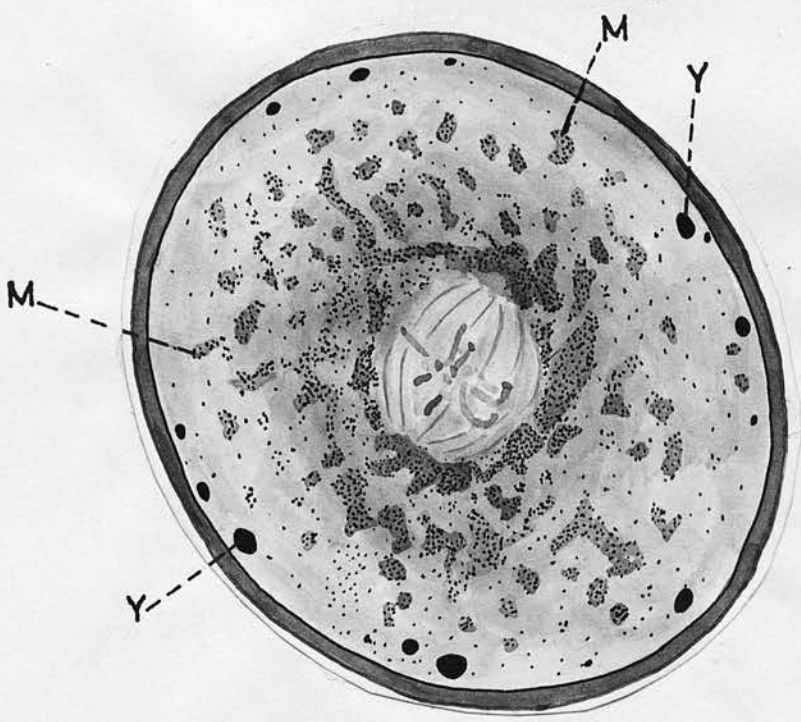
Fig.73.- Metaphase of first cleavage division.



71



72



73

0.01 μm.

PLATE 15.

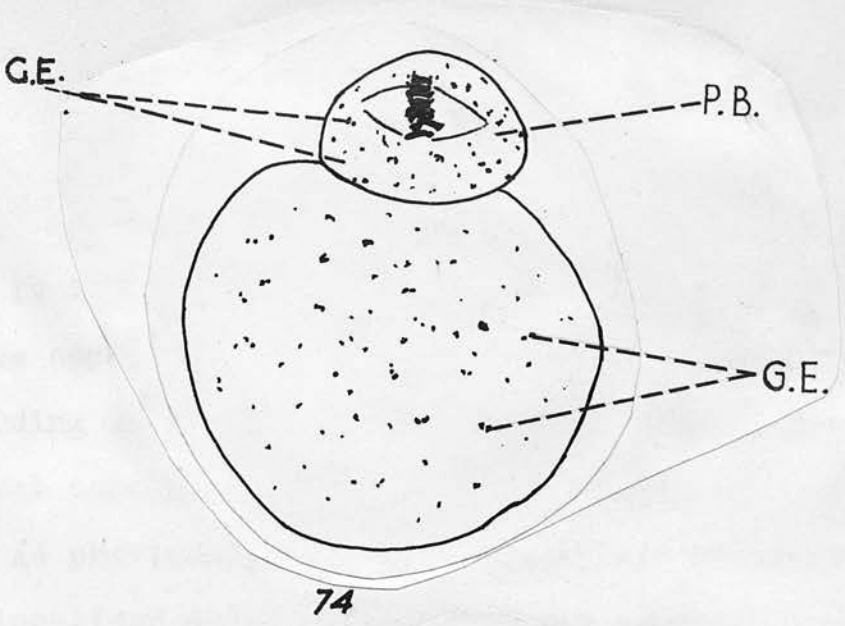
Figs. 74 and 75 from Aoyama preparations.

Fig. 76 from Flemming preparation.

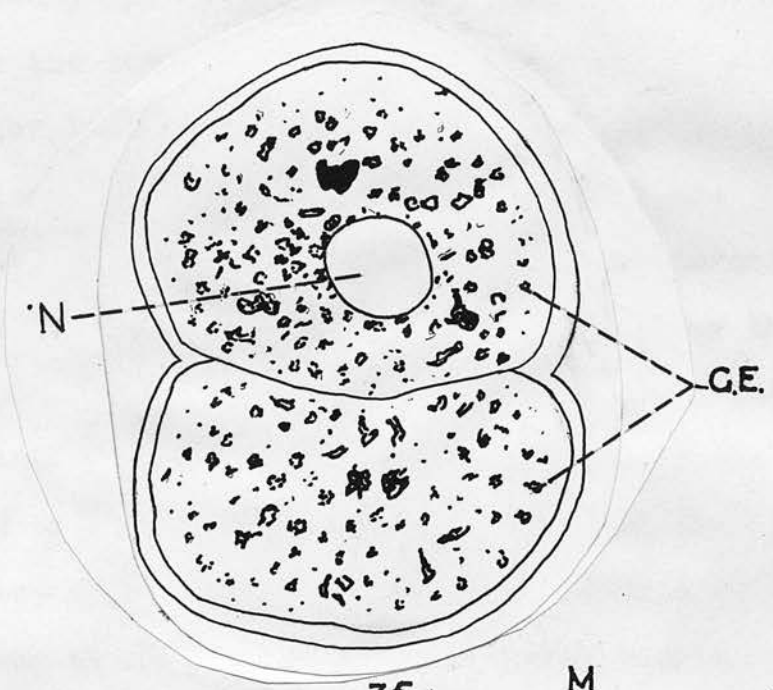
Fig.74.- Stage of the pronuclei. First polar body dividing. Golgi elements scattered through the cytoplasm of the polar body and through the egg.

Fig.75.- Two cell-stage. Showing Golgi elements.

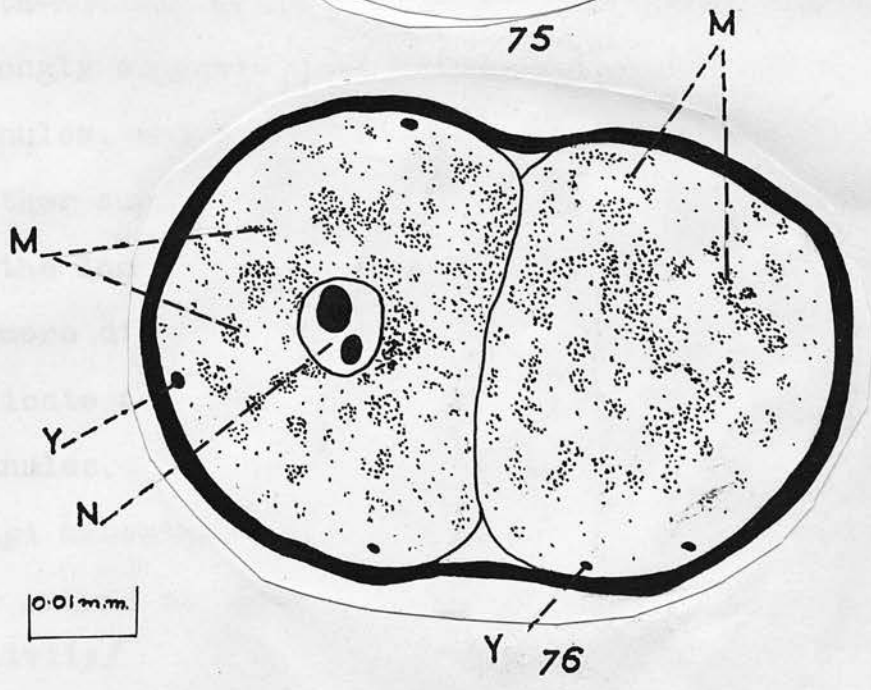
Fig.76.- Two cell-stage. Showing mitochondria and yolk-globules.



74



75



76

0.01mm.

GENERAL DISCUSSION.

It is desirable to discuss in greater detail, than in the separate sections of this work, certain findings regarding the cytoplasmic inclusions, and to draw some general conclusions from these findings.

As previously mentioned the writer believes that the localised Golgi material of the germ-cells of the mouse is in the form of individual elements which have come together to form a juxta-nuclear body. Although there is disagreement as to the structure of the localised Golgi material of germ-cells, considerable evidence has been produced in favour of the view that it is made up of filaments, rods or granules. The writer's conclusions are based on the study of the centrifuged and uncentrifuged oocyte, and on the spermatocytes and spermatids. The appearance of the juxta-nuclear Golgi material of the young oocyte strongly suggests that it is made up of rods and granules, and the study of centrifuged ovaries adds further support in favour of this view. The structure of the localised Golgi material of the male germ-cells is more difficult to determine, but appearances indicate that it is composed of filaments, rods and granules. The writer believes, therefore, that the Golgi material is to be regarded as a substance which may assume various forms at different phases of the activity/

activity of the cell, but which in the germ-cells of the mouse, at least, is never in the form of a network. It is not denied that the Golgi substance may, in certain cells, assume the form of a network, but it is stressed that the Golgi material is a substance which may take various forms in different types of cell, and may also undergo changes of form correlated with the phases of activity of the cell. It is of interest to note in this connection that Bowen (1926) stated that the "Golgi apparatus" is a substance which may be distributed through the cytoplasm as discrete bodies or may be concentrated in the form of a network; the exact form in which it is present in the cell is of secondary importance. Subramaniam and Gopala Aiyar (1937) review the work of certain authors on the structure of the Golgi material, particularly in the eggs of invertebrates. They believe that the Golgi bodies of the eggs of invertebrates undergo changes of shape during oogenesis, and suggest probable modes of origin of the Golgi network from discrete elements.

The term Golgi apparatus was originally used to designate the network of Golgi substance described for somatic cells. Consequently, the writer believes that the term Golgi apparatus should be reserved for such a structure, and that when the localised Golgi material is made up of the individual bodies, which have/

have not run together to form a network, it should be referred to as the Golgi material or Golgi substance. As is usual for the dispersed condition, the individual elements making up the localised mass may be referred to as the Golgi elements or Golgi bodies.

It is now well known that the Golgi material has been shown to play a part in the elaboration of granules of secretion, and that it is believed to take part in the formation of the acrosome, and of the yolk in the egg cells of many animals. The literature dealing with the relationship of the Golgi substance to secretion and to yolk-formation is well known and need not be cited here.

It is evident that the Golgi material performs various functions in different types of cells. This is well shown in the present investigation on the gametogenesis of the mouse. In the follicle-cells of the ovary the Golgi material undoubtedly plays an important part in the elaboration of secretory material which is passed into the cavity of the follicle. It is probable that it is also concerned with the formation of some nutritive substance passed from the follicle-cells to the young oocyte. There is no conclusive evidence that the Golgi elements take a direct part in the elaboration of the yolk of the mouse egg. It is probable, however, that, owing to their activity and migration/

migration through the ooplasm of the young oocyte, they play some part in the formation of the nutritive material of the egg. Further evidence of the many activities of the Golgi substance is shown by its participation in the formation of the acrosome, and of the ripe sperm. It would appear, therefore, that the function of the Golgi material in the germ-cells and follicle-cells of the mouse, is to play a part in the separation of substances from the general cytoplasm and the elaboration of these substances into formed components of the cell. Further, the end products of the activity of the Golgi material varies in the different types of cells, and, as is evident in the follicle-cells, in different phases of development of the same cell.

The above conclusions regarding the functions of the Golgi material were elaborated independently by the writer as the result of his work on the germ-cells and follicle-cells of the mouse, and were primarily based on the earlier part of the investigation on the ovary. Since these conclusions were first formulated a series of papers by Subramaniam have been published. In these contributions Subramaniam reviews the work of certain authors on the activity of the Golgi material of gland-cells and of germ-cells, and makes some interesting suggestions regarding the function of the Golgi/

Golgi substance. In a paper on the oogenesis of Salmacis bicolor (1934) he concludes that the function of the Golgi bodies is the secretion of various intracellular enzymes and that secretory products, such as fat, yolk, the acrosome, mucous etc., are secondary products resulting from the action of these enzymes. In a later paper Subramaniam (1935) points out that although considerable work has been carried out on the form and function of the Golgi material "no serious attempt has been made to formulate any general theory regarding its function, irrespective of the nature of the cell in which it is found". Further, he states that - "It is also rather surprising that an inclusion which has been shown to be universal in occurrence should be attributed special functions in certain cell groups, forgetting that it may have a general function in all cells". In the eggs of Acentrogobius neilli the Golgi bodies enlarge forming a rim around droplets of fatty yolk which is secreted in their interior. Some of the Golgi bodies remain unmodified, then migrate to the periphery where they form a concentration below the zona radiata. On fertilisation the inner part of the zona is transformed into mucilaginous matter. At the same time the Golgi bodies below the zona enlarge, and the Golgi rim of the fatty yolk droplet becomes beaded. The beads separate and divide giving rise to minute irregular Golgi bodies, the/

the majority of which, pass to the periphery. Secretion of mucous then appears to be more rapid. Subramaniam states that the behaviour of the Golgi elements strongly suggests that they are concerned with the conversion of the zona radiata into a mucilagenous envelope. During the oogenesis of Acentrogobius the Golgi bodies initially secrete fatty yolk and are later concerned with the secretion of mucous. This "warrants the suggestion that the function of the Golgi apparatus in oogenesis is only secretory, the nature of the secretion being incidental to the needs of the hour". These conclusions add support to the view that the Golgi material may take part in the elaboration of various substances at different phases of the development of the cell.

In a paper on the oogenesis of Meretrix casta Subramaniam (1937) states that the neutral red vacuoles of the egg represent enzymes, and suggests that there is a continuous secretion of enzymes by the Golgi material. He believes that the main function of Golgi ^{in eggs} bodies, in which there are no deutoplasmic inclusions appears to be the control of metabolism. "In specialised cells in addition the enzymes are engaged in the production of various substances such as yolk, fat, lipoid, mucous, serous and other droplets". He also suggests that the acrosomic vesicle of spermatids contains/

contains enzymes secreted by the Golgi material.

The present writer believes that the function of the Golgi material is to aid in the separation and elaboration of secretory and other products, rather than to participate in the formation of these materials by the direct transformation of its own substance. He considers, therefore, Subramaniam's suggestion regarding the secretion of various enzymes by the Golgi material to be a useful one. If it be true that the Golgi material is concerned with the elaboration of various substances in different cells and also in the same cell at different phases of activity, then it is probable that these substances are formed by the action of enzymes which are themselves produced under the influence of the Golgi material. The evidence for identifying the neutral red vacuoles of the egg of Meretrix with intra-cellular enzymes appears to be insufficient. Even if, as suggested by several workers cited by Subramaniam (1937), neutral red indicates the presence of enzymes, further microchemical tests must be carried out before the neutral red vacuoles can be definitely identified with intra-cellular enzymes. Such a study is, however, beyond the scope of the present work.

That the Golgi material is an important living cytoplasmic/

cytoplasmic inclusion is shown by its growth during the development of the egg of the mouse, during spermatogenesis, and in the fertilised egg prior to the first cleavage division. Further evidence of its importance as a cytoplasmic inclusion is demonstrated by its segregation, in approximately equal amounts to the resulting cells, during the division of the spermatocytes and of the fertilised egg.

The present investigations on the cytoplasmic inclusions during fertilisation show conclusively that the sperm middle-piece, and probably the whole tail, is carried into the egg of the mouse. The structure of the middle-piece of the mammalian sperm, as seen in the ooplasm of eggs treated by modern methods for the demonstration of the Golgi material, is described for the first time. The writer's findings that the mitochondria and Golgi material of the sperm become distributed through the cytoplasm of the egg does not agree with the observations of earlier workers on the history of the sperm middle-piece in the fertilised eggs of mammals. More detailed studies, have, however, been carried out on the history of the sperm mitochondria in the ooplasm of invertebrates, and the conclusions regarding their distribution to the first two blastomeres of certain animals is confirmed by the present work. If the conclusions of the writer be correct, /

correct, then it is evident that the sperm middle-piece plays an important part in fertilisation, and that the sperm, as well as the egg, contributes to the cytoplasm of the embryo. That the sperm middle-piece does in reality contain Golgi material is confirmed by the study of spermateleosis.

The migration of mitochondria, as well as some of the Golgi material, into the region of the developing middle-piece, primarily ensures that they are included in the ripe sperm and subsequently are carried into the egg. It is probable, however, that, while situated in the middle-piece, both mitochondria and Golgi material are important structural and functional parts of the sperm. That the mitochondria of the sheath differ in staining properties, as well as in size, from those of the spermatid and of the egg, suggests that they undergo a chemical change correlated with functional activity. After their liberation into the cytoplasm of the fertilised egg the mitochondria of the sperm become smaller and regain their original staining properties, so that they become indistinguishable from those of the egg. Their reduction in size is brought about by multiplication, so that at the time of the first cleavage division the mitochondria which have origin from the male are very numerous, if not equal in number to those of the egg. The Golgi material of the/

the sperm increases in amount and fragments into smaller bodies prior to the first cleavage division. It is probable, therefore, that numerous Golgi elements originate from the Golgi substance of the sperm and are distributed between the first two blastomeres. Owing to their distribution throughout the ooplasm, prior to the first cleavage division, the sperm mitochondria and Golgi material are mixed with those of the egg and are consequently transmitted to the zygote. The importance of the contribution of the sperm to the cytoplasm of the embryo is clearly recognised by Brambell (1930) when he states that - "It is unfortunate that little is known of the function or ultimate fate of the cytoplasmic constituents of the sperm, other than the proximal centrosome, which are introduced into the cytoplasm of the ovum on fertilisation". Further, he states that - "these various bodies constitute the cytoplasm received from the male parent and are equivalent, from the point of view of cytoplasmic inheritance, to the entire cytoplasm of the egg".

In view of the work on the sperm mitochondria in the eggs of certain invertebrates, and the present findings on the Golgi material and mitochondria of the mouse sperm, it is improbable that the sperm tail of the other mammals which have been investigated, is segregated into one of the blastomeres of the two cell stage./

stage. It is desirable, therefore, that other animals be investigated regarding the part played by the cytoplasmic inclusions of the sperm during fertilisation.

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PAPER SUBMITTED AS ADDITIONAL EVIDENCE OF RESEARCH.

"The Effect of Increased Daily Illumination and of Reversed Day and Night on the Oestrus Cycle of the Mouse (*Mus musculus*)",

'Proc. Roy. Soc. Edin.', vol. 60, 1940.

REPRINT FROM THE
PROCEEDINGS
OF THE
ROYAL SOCIETY OF EDINBURGH
SESSION 1939-1940

VOL. LX—PART III—(No. 25)

The Effect of Increased Daily Illumination and of
Reversed Day and Night on the Œstrus Cycle of
the Mouse (*Mus musculus*)

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EDINBURGH

* PUBLISHED BY ROBERT GRANT & SON, LTD., 126 PRINCES STREET, AND
WILLIAMS & NORGATE, LTD., 36 GREAT RUSSELL STREET, LONDON, W.C. 1

MCMXL

Price One Shilling

XXV.—The Effect of Increased Daily Illumination and of Reversed Day and Night on the Œstrus Cycle of the Mouse (*Mus musculus*). By R. A. R. Gresson, Ph.D., Department of Zoology, University of Edinburgh. *Communicated by* Professor J. RITCHIE, M.A., D.Sc.

(MS. received June 19, 1940. Read July 1, 1940.)

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I. INTRODUCTION.

IN the course of a recent investigation on the fertilised egg of the mouse (Gresson, 1940 *a*, *b*) it was necessary to keep a record of the time at which pairing took place. This revealed that copulation, especially in the winter, was very infrequent during the hours of daylight, and usually took place at about 12 midnight or between midnight and early morning. Similar observations were made by Lewis and Wright (1935), who found that in the autumn and winter mice seldom copulated during the day, and that while the majority of pairings took place between 10 p.m. and 1 a.m., several of their mice paired between 1 a.m. and 7 a.m., whereas in the spring pairing occurred more readily during all hours of the day.

In an attempt to obtain pairings during the day the writer decided to keep some female mice in total darkness during the day and under bright illumination during the night. It is well known that extra illumination accelerates the onset of sexual activity of certain mammals. In the ferret œstrus has been induced during the winter months by exposure to extra evening illumination (Bissonnette, 1932; Bissonnette and Bailey, 1936; Allanson, Rowlands and Parkes, 1934). Marshall and Bowden (1936) and Marshall (1940) found that ferrets treated with ultra-violet light usually remain on heat for a longer period than those subjected to light irradiation. Acceleration of the onset of sexual activity was induced in male and female raccoons by the application of increased daily periods of illumination (Bissonnette and Csech, 1937).

The shortening of the daily period of light from 15 to 9 hours almost prevents reproduction of the field mouse, *Microtus agrestis* (Baker and Ranson, 1932). Whitaker (1936) accelerated sexual activity in the white-footed mouse, *Peromyscus*, and later (1940), by varying the periods of artificial illumination, found that the highest percentage of white-footed mice in breeding condition occurred during the "long day" periods.

Although a considerable amount of work has been carried out on the effect of light on reproduction, there appears to be only one record of an attempt to induce copulation during the day in an animal which normally pairs at night. Hemmingsen and Krarup (1937) kept 16 female rats in complete darkness during the day and under illumination during the night. The animals normally pair at about 12 midnight. An examination of the experimental animals showed that the spontaneous muscular activity, mating instincts, and cyclic vaginal changes were shifted 12 hours—that is, a complete reversal of œstrus was produced, the new œstrus rhythm being established about 10 days after the reversal of the lighting conditions. The authors conclude "that the direct action of darkness does not immediately and directly produce the manifestations of œstrus. But once a certain rhythm of darkness and light has become established, the œstrus phenomena centre about the middle of the dark periods." Hemmingsen and Krarup found that alternation of 8 hours dark and 8 hours light does not abolish the 24-hour activity rhythm, and that constant light stimulated œstrus in 4 females in which a previous reversal of sexual activity had been obtained.

The experiments described in the present paper were undertaken in order to obtain cytological preparations of the stages of fertilisation. They show that a "long day" period and reversed lighting affect the sexual activity of the mouse.

II. METHODS.

The experiments described in the present paper were carried out between November 1937 and July 1938. The stock was a mixed one of white and coloured mice.

Female mice were housed in a constant-temperature room maintained at 74° F. and illuminated by electric light for 16 to 17 hours a day commencing at about 6 p.m. The light was turned off in the morning so that the animals were kept in total darkness during 7 to 8 hours of the solar day. The conditions approximated to those of summer in which day and night were reversed. The mice were provided with a small quantity of fine wood shavings for bedding placed in one corner of the

cage, and the cages were placed in such a position that all parts were brightly illuminated when the light was turned on.

The controls were kept with the general stock, in an inside animal room, and were housed and fed in exactly the same manner as the experimental animals. The room was heated and the temperature maintained as close as possible to that of the constant-temperature room; it was impossible, however, to avoid slight fluctuations, but these were so slight as to be unimportant. The room was illuminated by daylight and was kept in darkness during the night.

As male mice will pair at any time of the day throughout the year, the males were kept with the general stock.

The males were introduced into the experimental cages, containing females on œstrus, at intervals during the day beginning at about 11 a.m., and were finally removed at about 5 p.m. The females were examined every half-hour for vaginal plugs, and the time of copulation determined to the nearest half-hour. In some cases the exact time of pairing was recorded.

Males were introduced into the control cages at intervals between about 11 a.m. and 5 p.m. When pairings took place the time was recorded.

My thanks are due to Professor James Ritchie for placing the facilities necessary for the carrying out of the experiments at my disposal, and for reading the manuscript.

III. RESULTS OF EXPERIMENTS.

In the majority of the experiments a careful control was kept, consisting of litter mates or, where this was impossible, of mice of the same age. In the first experiment, however, no special controls were kept, the general stock, consisting of about 12 mice, being used as control.

An examination of Table I shows that 7 mice, 4 weeks old, were subjected to reversed lighting on November 19. One of these paired after 17 days at 5.45 p.m., and in 4 of the others a complete reversal of the time of pairing was established in between 27 and 68 days. The remaining 2 failed to pair and were removed from the experimental cage slightly later. Three mice were added on January 17; these showed a reversal of œstrus in 4 to 47 days. In this experiment 6 mice or, if the individual which paired at 5.45 p.m. be excluded, 5 out of 10 mice underwent a reversal of œstrus in December and January, a time of year when no pairings were recorded, during the day or night, in the general stock.

Table II represents a second series of experiments set up in March

and April: the general stock was used as control. Nine of the ten mice paired during the day in 9 to 38 days after the beginning of the experiment. Amongst the stock animals pairings were more frequent during the daytime than in the preceding 2 months.

Tables III, A and B, represent the first experiment of which a careful control, consisting of litter mates, was kept. Three of the five experimental animals paired within 32 days, at times varying from 11 a.m. to 4.15 p.m., and two controls paired at 12 noon in 24 and 34 days.

In the experiment dealt with in Tables IV, A and B, all the experimental mice paired during the daytime, while only 2 of the 5 controls did so although the experiment was continued until after May 18, and thus extended into a period in which copulation during the day is more frequent than during the winter months.

In an experiment commenced in March (Table V, A) all the mice paired during March, April, and May, while all the controls paired in April, May, and June (Table V, B). Only one of the experimental animals paired in an appreciably shorter time than the controls; it must be remembered, however, that these pairings took place during the spring and early summer.

An experiment was started on April 25 (Tables VI, A, B). All the experimental animals paired within 38 days. Two of the controls paired in 44 and 49 days; the remaining 2 did not pair although the control was continued until the middle of June.

Seven of the nine mice dealt with in Table VII, A, paired within 25 days, while none of the 7 controls did so although kept until the end of June.

Table VIII deals with 8 mice which had been kept as controls for various periods, and were then subjected to reversed light and dark periods. None of these animals had paired while in the control groups. The 3 mice transferred on March 3 paired within 35 days; 2 of the five transferred on June 27 paired in 9 days' time. The experiment was discontinued about the middle of July.

In Tables I-VII, A, each entry represents one mouse.

TABLE I.

1.	2.	3.	4.	5.
Age.	Beginning of Experiment.	Date of Pairing.	Days Elapsed between 2 and 3.	Time of Day.
4 weeks	Nov. 19	Dec. 6	17	5.45 p.m.
"	" 19	" 16	27	12.0 noon
"	" 19	" 23	34	11.0 a.m.
"	" 19	" 31	42	11.15 "
"	" 19	Jan. 25	68	11.0 "
"	" 19	Did not pair
"	" 19	Did not pair
"	Jan. 17	Feb. 10	25	11.40 a.m.
Adult	" 17	Jan. 21	4	11.0 "
"	" 17	Mar. 4	47	1.30 "
Total No. of mice 10.			Paired 8.	

TABLE II.

1.	2.	3.	4.	5.
Age.	Beginning of Experiment.	Date of Pairing.	Days Elapsed between 2 and 3.	Time of Day.
4 weeks	Mar. 4	April 4	31	11.0 a.m.
"	" 4	" 5	32	3.45 p.m.
"	" 4	Did not pair
3 weeks	April 4	May 12	38	1.0 p.m.
"	" 4	" 12	38	11.30 "
"	" 11	" 3	22	12.0 noon
"	" 11	" 7	26	12.30 p.m.
"	" 11	" 14	31	12.0 noon
Adult	" 4	April 13	9	1.40 p.m.
"	" 4	May 2	28	12.30 "
Total No. of mice 10.			Paired 9.	

TABLE III.

A.				
1.	2.	3.	4.	5.
Age.	Beginning of Experiment.	Date of Pairing.	Days Elapsed between 2 and 3.	Time of Day.
4 weeks	Dec. 17	Dec. 31	14	11.0 a.m.
"	" 17	Jan. 13	27	4.15 p.m.
"	" 17	" 18	32	12.0 noon
"	" 17	Did not pair
"	" 17	Did not pair
Total No. of mice 5.			Paired 3.	

B.

Control.

4 weeks	Dec. 17	Jan. 10	24	12.0 noon
"	" 17	" 20	34	12.0 "
"	" 17	Did not pair

Total No. of mice 3. Paired 2.

TABLE IV.

A.

1. Age.	2. Beginning of Experiment.	3. Date of Pairing.	4. Days Elapsed between 2 and 3.	5. Time of Day.
4 weeks	Feb. 3	Mar. 10	35	12.10 p.m.
"	" 3	" 17	42	3.0 "
"	" 7	" 15	37	2.15 "
3 weeks	" 7	April 8	58	2.0 "
"	" 7	May 18	98	4.30 "

Total No. of mice 5. Paired 5.

B.

Control.

4 weeks	Feb. 3	Did not pair
"	" 3	Did not pair
"	" 7	May 2	82	1.0 p.m.
3 weeks	" 7	Mar. 28	27	12-1.0 "
"	" 7	Did not pair

Total No. of mice 5. Paired 2.

TABLE V.

A.

1. Age.	2. Beginning of Experiment.	3. Date of Pairing.	4. Days Elapsed between 2 and 3.	5. Time of Day.
3 weeks, 3 days	Mar. 4	April 13	40	1.40 p.m.
3 weeks, 3 days	" 4	May 4	61	11.45 "
8 weeks	" 7	Mar. 17	10	2.45 "
"	" 7	May 18	72	10.45 a.m.
"	" 7	" 27	81	12.0 noon

Total No. of mice 5. Paired 5.

B.
Control.

3 weeks, 3 days	Mar. 4	April 11	38	12.0 noon
3 weeks, 3 days	" 4	May 27	84	12-1.0 p.m.
8 weeks	" 7	April 11	35	11.30 a.m.
"	" 7	May 12	66	1.0 p.m.
"	" 7	June 2	87	12.0 noon
Total No. of mice 5. Paired 5.				

TABLE VI.

A.

1. Age.	2. Beginning of Experiment.	3. Date of Pairing.	4. Days Elapsed between 2 and 3.	5. Time of Day.
4 weeks	April 25	May 20	25	12.0 noon
"	" 25	" 28	33	1.0 p.m.
"	" 25	" 30	35	12.35 "
"	" 25	June 2	38	12.0 noon
Total No. of mice 4. Paired 4.				

B.

Control.

3 weeks	April 25	June 6	44	12.0 noon
"	" 25	" 13	49	1.0 p.m.
"	" 25	Did not pair
"	" 25	Did not pair
Total No. of mice 4. Paired 2.				

TABLE VII.

A.

1. Age.	2. Beginning of Experiment.	3. Date of Pairing.	4. Days Elapsed between 2 and 3.	5. Time of Day.
3 weeks	May 2	May 20	18	12.0 noon
"	" 2	" 20	18	11.0 a.m.
"	" 2	" 23	21	10.15 "
4 weeks (approx.)	" 26	June 6	11	4.30 p.m.
4 weeks (approx.)	" 26	" 9	14	12.0 noon
4 weeks (approx.)	" 26	" 9	14	1.0 p.m.
4 weeks (approx.)	" 26	" 20	25	12.0 noon
4 weeks (approx.)	" 26	Did not pair
4 weeks (approx.)	" 26	Did not pair
Total No. of mice 9. Paired 7.				

The present findings on the effect of the "long day" period of artificial light on the reproduction of the mouse agrees with the observations of Bossette (1932) and others referred to in the introduction to this paper. Increased daily lighting periods appear to have no effect on the reproduction of some mammals. A summary of the work on mammals will be found in Whitaker's paper (1940).

That reversed day and night brings about a reversal of the time of mating is evident from an examination of Table I. Seven of the 8 mice which copulated did so between 11 A.M. and 3:30 P.M. It is possible that the animal which paired at 2:45 P.M. may have also undergone reversal, as mice are known to copulate in the early hours of the morning. In the experiment set up on December 27 (Tables III, A, B, C) 25 of the controls paired in January. As the number of animals used in the first group was small, and in view of the fact that no daylight pairings were made at this time of the year among the 10 animals of the general stock, it is unsafe to draw any definite conclusions from this experiment, primarily that these animals pair more readily in the night than others. The experiments begun in the spring may be considered together (Tables IV, A, B, VI, A, B) in view of the mice referred to in Table III already during the hours of daylight with 38 days. Tables V and VI give more precise information, as careful controls of these experiments were kept. The experimental animals and of the controls paired during the daytime. This confirms the findings of Lewis and Wright (1935) that in the spring mice pair more readily during the day. Reversed lighting, however, appears to have accelerated daytime pairing, as the majority of the experimental animals copulated at an earlier date than corresponding controls.

Tables VII, A, B, indicate that during late May and June pairing does not usually occur during the day, but that this may be induced by subjecting the animals to reversed day and night.

Table VIII is of interest in that it shows that daytime pairing can be induced in animals which have previously spent a considerable time as controls. Unfortunately, this experiment had to be discontinued at an earlier date than was intended.

As the proportion of daytime pairings amongst the experimental mice is higher, at all times of the year, than amongst the corresponding controls, it is concluded that reversed day and night, and not the "long day" conditions, is the determining factor in increasing the proportion of daytime pairings. It is concluded that (1) mice can be brought into estrus in mid-winter when subjected to "long day" conditions; reversed lighting during the winter induces daytime pairing; (2) in

B.

Control.

3 weeks (2 mice)	May 2	Did not pair
4 weeks (5 mice)	„ 26	Did not pair
Total No. of mice 7. Paired 0.				

TABLE VIII.

1. Age.	2. Time in Control.	3. Transferred on.	4. Date of Pairing.	5. Days Elapsed between 3 and 4.	6. Time of Day.
4 months (approx.)	3 months	Mar. 3	Mar. 24	6	12.20 p.m.
10 weeks	6 weeks	„ 3	April 8	21	12.0 noon
„	„	„ 3	„ 22	35	12.20 p.m.
2 months (2 mice)	1 month	June 27	July 5	9	11.0 a.m. and 12.30 p.m.
2 months (3 mice)	„	„ 27	Did not pair
Total No. of mice 8. Paired 5.					

IV. DISCUSSION.

In considering the effect of reversed light conditions on the sexual activity of the mouse, the results of the present work, as indicated by the tables, are somewhat unconvincing if taken by themselves; but if considered in relation to the time of year certain important effects are evident.

The majority of animals represented in Table I were first subjected to reversed day and night conditions on November 19. The conditions as regards length of "day" and temperature approximated to those of summer. When it is remembered that under ordinary conditions pairing is infrequent during mid-winter, it is seen that the "long day" accelerated sexual activity, bringing 4 of the 7 mice into œstrus during December and 1 in January. The oviducts of these mice were subsequently sectioned, and the ova were found to have been fertilised. That the acceleration was not due to temperature, but to the long light period, was indicated by the absence of pairing during December and January amongst the animals in the general stock. When mice are illuminated during the short winter day by natural light only, œstrus seldom occurs. Such pairing as normally takes place during mid-winter would appear to be due to the extensive use of electric light during the whole or part of the working day.

The present findings on the effect of the "long day" period of artificial light on the reproduction of the mouse agrees with the observations of Bissonnette (1932) and others referred to in the introduction to this paper. Yet increased daily lighting periods appear to have no effect on the reproduction of some mammals. A summary of the work on mammals will be found in Whitaker's paper (1940).

That reversed day and night brings about a reversal of the time of pairing is evident from an examination of Table I. Seven of the 8 mice which copulated did so between 11 a.m. and 1.30 p.m. It is possible that the animal which paired at 5.45 p.m. may have also undergone reversal, as mice are known to copulate in the early hours of the morning.

In the experiment set up on December 17 (Tables III, A, B) 2 of the 3 controls paired in January. As the number of mice used in the control group was small, and in view of the fact that no daylight pairings occurred at this time of the year amongst the 12 animals of the general stock, it is unsafe to draw any definite conclusions from this experiment, but it may be that some strains pair more readily in the daytime than others.

The experiments begun in the spring may be considered together (Tables II; V, A, B; VI, A, B). Nine of the mice referred to in Table II paired during the hours of daylight within 38 days. Tables V and VI give more precise information, as careful controls of these experiments were kept. The 9 experimental animals and 7 of the 9 controls paired during the daytime. This confirms the findings of Lewis and Wright (1935) that in the spring mice pair more readily during the day. Reversed lighting, however, appears to have accelerated daytime pairing, as the majority of the experimental animals copulated at an earlier date than the corresponding controls.

Tables VII, A, B, indicate that during late May and June pairing does not usually occur during the day, but that this may be induced by subjecting the animals to reversed day and night.

Table VIII is of interest in that it shows that daytime pairing can be induced in animals which have previously spent a considerable time as controls. Unfortunately this experiment had to be discontinued at an earlier date than was intended.

As the proportion of daytime pairings amongst the experimental animals is higher, at all times of the year, than amongst the corresponding controls it is concluded that reversed day and night, and not the "long day" conditions, is the determining factor in increasing the proportion of daytime pairings. It is concluded that (1) mice can be brought into œstrus in mid-winter when subjected to "long day" conditions; (2) reversed lighting during the winter induces daytime pairing; (3) in

the spring pairing takes place more frequently during the hours of daylight than at other times of the year; under reversed lighting conditions the proportion of pairings during the daytime is slightly higher than under natural conditions; (4) daytime pairings are not common during late May and June, but may be induced by reversed lighting.

Hemmingsen and Krarup (1937) found that in the rat *œstrus* is reversed in about 10 days from the beginning of the experiment. The time taken to bring about reversal in the mouse varies considerably. In some cases reversal is established in less than 10 days, while some mice did not pair until more than 60 days had elapsed. It should be noted that only 2 of the controls paired in less than 30 days from the beginning of the experiment. In considering the number of days required to bring about reversal it should be remembered that the majority of the mice were 3 to 4 weeks old when first subjected to reversed lighting, and that the mice used in these experiments usually came on the first *œstrus* when about 5 weeks old. An examination of the tables shows that few of the young mice underwent a reversal at the first *œstrus*. Six of the 12 adult mice used in the course of the present investigation paired within 10 days of the beginning of the experiment.

No record was made of the spontaneous activity of the experimental mice, but observations indicated that, under reversed day and night, a reversal of activity took place.

It is possible that some strains of mice may pair more readily during the day than others. Further experiments, using a large number of pedigree mice, would have to be carried out in order to obtain information on this point, but this was not possible during the present investigation.

V. SUMMARY.

1. Female mice were kept in darkness for 7 to 8 hours during the day, and were kept under bright illumination (electric light) for 16 to 17 hours beginning at about 6 p.m. The general stock was used as control for some of the experiments, while for others, controls consisting of mice of the same age were used.

2. The "long day" conditions accelerated *œstrus* and induced pairing in mid-winter. Reversed day and night conditions induces daytime pairing. In the spring the mice in the controls paired more readily during the daytime than at other times of the year, but the proportion of daytime pairings among the experimental animals was somewhat higher than among the control mice.

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