The Cytoplasmic Inclusions of the Germ-Cells.[1] Part IX. On the Origin of the Golgi Apparatus on the Middle-piece of the Ripe Sperm of Cavia, and the Development of the Acrosome.

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With Plates 11 and 12 and 2 Text-figures.

Contents.

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1. Introduction.

It is well known from the work of Retzius that the middle-piece of the ripe spermatozoa of many mammals bears around itself a small clear bead of protoplasmic material which can be easily recognized in the fresh sperm.

In 1912 Weigl (**32**) published some comparative studies on the Golgi apparatus of the somatic- and germ-cells of different animals, in which he showed that the protoplasmic bead on the middle-piece of the spermatozoon of the guinea-pig contained structures possessing all the microchemical characteristics of true Golgi elements.

The work out of which the present paper arose was primarily undertaken with a view to discovering the mode of origin of these argentophile structures from the Golgi apparatus of the spermatid and spermatocyte.

The first part of this paper consists, therefore, of a description of our results in this field.

The study of the Golgi apparatus of the spermatocytes and spermatid naturally led, however, to the investigation of the relations of this structure to other cell constituents, especially to the acrosome.

The development of the acrosome in Cavia has been the object of repeated study by Niessing, Moore, Meves, and others, and quite recently by Papanicolaou and Stockard, but the exact relation of this body to the Golgi apparatus has not hitherto been described.

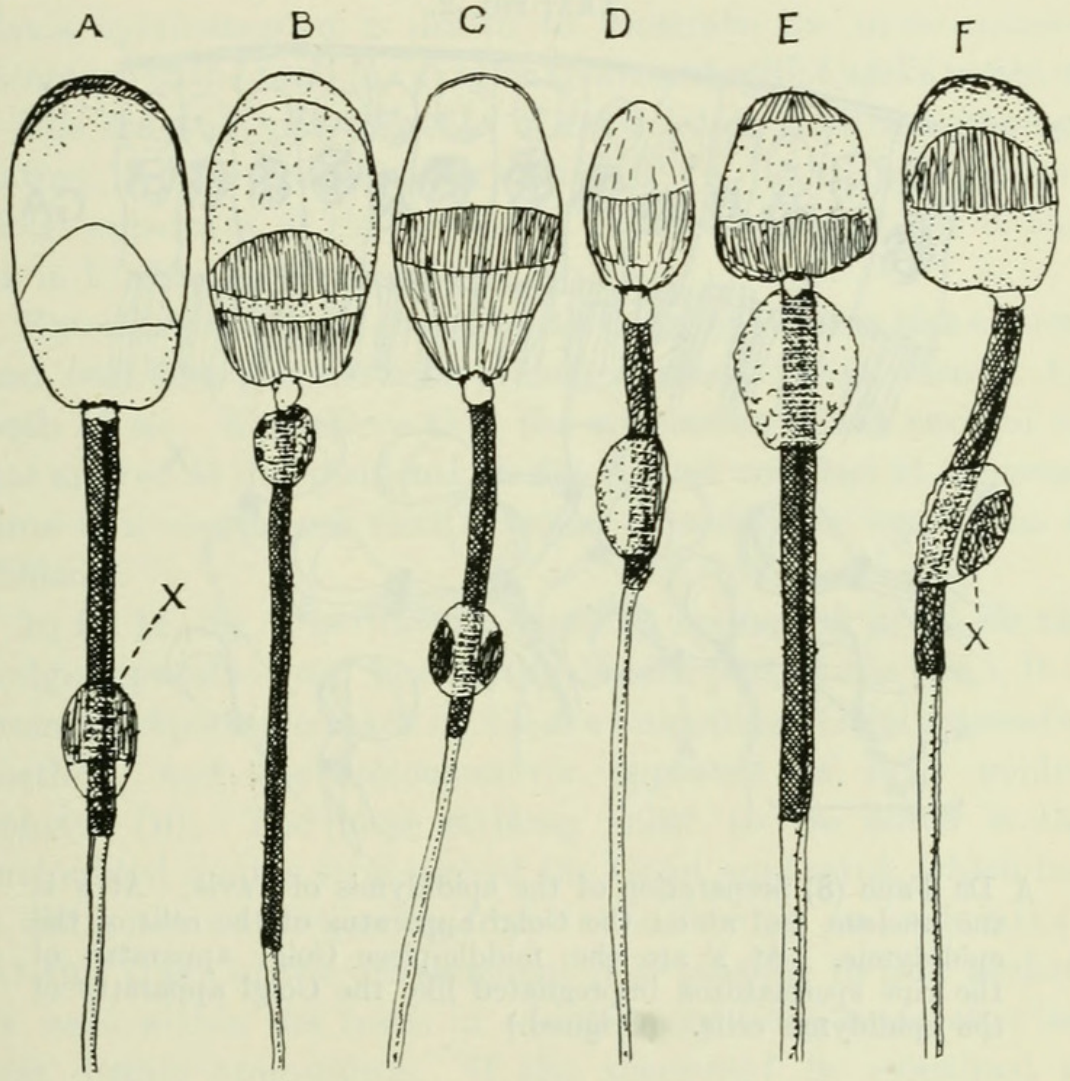
Our observations upon this point form the second part of the present paper, and we have also attempted to give a general account of the spermatogenesis of Cavia based upon the confirmed results of modern workers, together with certain suggestions for a revised and simplified English nomenclature of the subject.

2. Part I. The Development of the Definitive Middle-piece Golgi Apparatus.

Retzius, as is well known, has published a large number of drawings of various mammalian and other spermatozoa. If

we examine his figures (**29**), we find, as has already been mentioned, that Retzius has represented in many mammalian spermatozoa a small bead of protoplasm on some part of the middle-piece. In our Text-fig. 1 are reproduced six of this observer's figures, showing at x the bead of the middle-piece.

TEXT-FIG. 1.

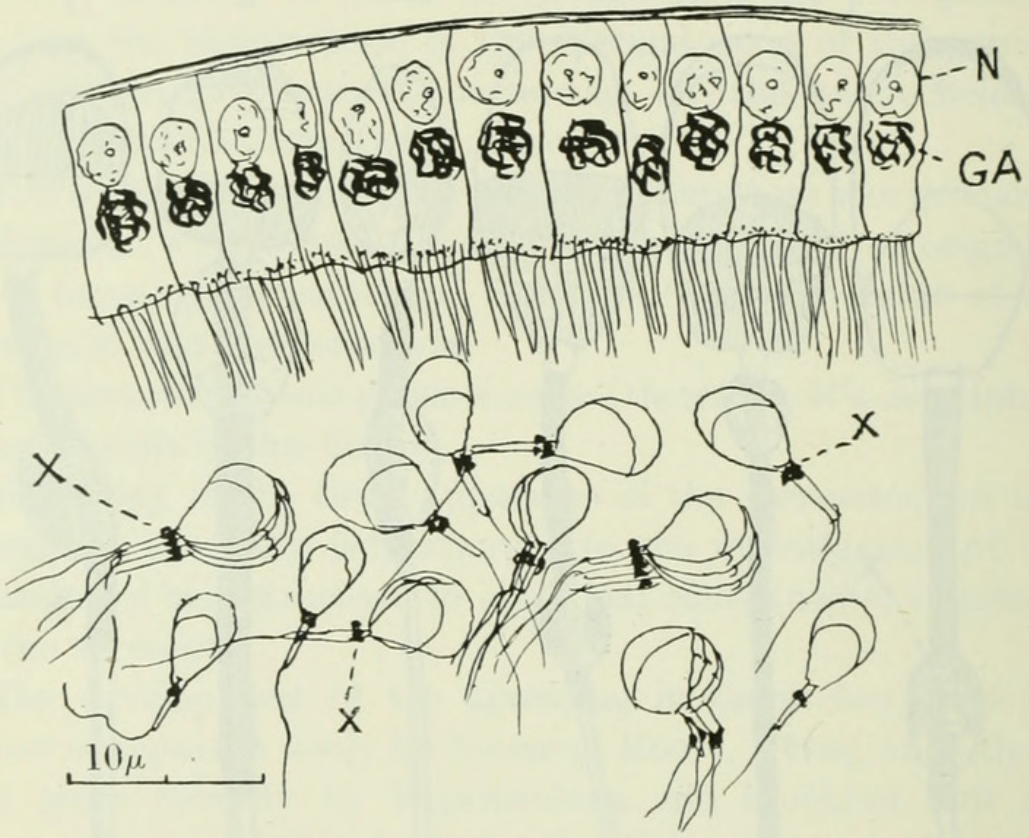


Ripe spermatozoa after Retzius (**29**). A = pig. B = sheep. C = rabbit. D = cat. E = lemur. F = hedgehog; showing at X the protoplasmic bead associated with the middle-piece.

In fig. 1, A is the spermatozoon of the pig; fig. B that of the sheep; fig. C, the rabbit; fig. D, the cat; fig. E, the lemur (Lemur catta); and fig. F, the hedgehog. A glance through the work of Retzius shows that this peculiar bead has been figured by him in several other mammals, namely: Sciurus

vulgaris, Cynomys, Myoxus glis, Cavia, Equus, Capra, Alces, Bos, Canis, and doubtfully in Dicotyles. In the spermatozoa of the following the bead does not appear in Retzius's figures: Homo, Didelphys, Talpa, Bradypus, Dipus, Hystrix, Lemmus, Mus, Myopotamus, Cervus, Rangifer, Globicephalus, Vulpes, Meles, Halichaerus, Hapale, and Innus. Some of

TEXT-FIG. 2.



A Da Fano (8) preparation of the epididymis of Cavia. At N is the nucleus, and at GA the Golgi apparatus of the cells of the epididymis. At X are the middle-piece Golgi apparatus of the ripe spermatozoa impregnated like the Golgi apparatus of the epididymis cells. (Original.)

these are, however, doubtful, and may possess the bead in a very reduced and atypical condition.

If now, as Weigl (32) has shown, the epididymis of Cavia be prepared by one of the Golgi apparatus techniques (Golgi, Cajal, or Da Fano), the protoplasmic beads of the free spermatozoa lying within the tubules are all found to contain a number of little rodlets or elongate platelets as shown in Text-fig. 2 at x. In this figure, drawn from a preparation by Da Fano's

cobalt-nitrate-silver method (**8**), the magnification is too low to show the minute structure of the bead ; at N is the nucleus of the cells of the epididymis wall ; and at GA the Golgi apparatus of these cells. In all preparations we possess, the Golgi apparatus of the epididymis wall and of the bead-contents of the middle-piece are the only objects which go black with the reduced silver. In Pl. 11, fig. 3, a nearly ripe Cavia spermatozoon is drawn to illustrate the more minute structure of the bead (GAX) after treatment with Cajal's method.

The question now arises: What relation does the impregnating middle-piece bead (GAX in Pl. 11, fig. 3) bear to the Golgi apparatus of the spermatid cell (GA in Pl. 11, fig. 3, and GE in Pl. 12, figs. 7, &c.) ?

Extensive trials were made with Golgi apparatus techniques, and our best preparations were examined independently by both of us. We believe that the conclusion which each of us has arrived at independently is the correct one, but at the same time it is recognized that to come to a definite conclusion is difficult.

In Pl. 11, fig. 2, is drawn a ripening spermatid in which the Golgi apparatus (GA) lies in the hinder part of the cell. It is from a preparation made by Cajal's unmodified Golgi apparatus method, and the mitochondria appeared as light golden spheres (M). The most striking point to be noted is the undoubted double structure of the Golgi apparatus, which has a distinct bead projecting from its surface on one side (GAX). At this stage in the development it is possible to find pockets of cells within the testis in which every Golgi apparatus has this double appearance. If the spermatid be examined at earlier stages such as in Pl. 11, fig. 13, the bead (GAX) can still be seen as a swelling on the surface of the Golgi apparatus.

With Cajal, Da Fano, or Kopsch methods, it is found that this outgrowing bead is not homogeneous—its centre being formed of a more lightly impregnating material, closely resembling archoplasm in its appearance. If, moreover, ripe spermatozoa are fixed in some such mixture as Flemming or Hermann, and stained in acid fuchsin, it will be noted that the

middle-piece bead stains like the archoplasm of the spermatid, i. e. a deep pink or reddish. We consider, therefore, that the outgrowing bead figured by us in Pl. 11, fig. 2, and Pl. 12, figs. 13 and 14, probably consists of detached portions of both archoplasm as well as Golgi apparatus elements.

Tracing now the history of the bead after the stage at which it still adheres to the main Golgi apparatus, we next find that it has become separated from the latter in the manner shown in Pl. 12, fig. 14. In a large number of cases the bead has been observed lying in a position intermediate between the main Golgi apparatus and the nucleus, that is, near the letter M in Pl. 11, fig. 2.

In the majority of cases the Golgi apparatus bead of the ripe sperm of Cavia lies in the position shown in Pl. 11, fig. 3, and less commonly in the position indicated in Pl. 12, fig. 16. Reference to Text-fig. 1 shows that the middle-piece beads in other animal sperms vary a good deal in position.

It seems probable that the small Gogli apparatus bead moves up from its position in Pl. 11, fig. 2, or Pl. 12, fig. 14, to its definitive position near the head centrosome-complex (Pl. 12, fig. 15), the bead becoming applied to the 'skeleton' of the middle-piece (MD in Pl. 12, fig. 14) at a time when the mitochondrial granules (M) are themselves becoming grouped around the skeleton.

3. Part II. Literature.

Meves (**20**), in his classical paper on the spermatogenesis, has given a detailed review of previous work on Cavia. To this the reader may be referred. More recently Papanicolaou and Stockard (**26**) have gone over the same ground, and also given a comprehensive review of the results of previous observers. The work of Papanicolaou and Stockard is chiefly concerned with the fate of the archoplasm (their 'idiosome') and its contents based on a study of material stained with a new methylene-blue-acid fuchsin combination after Zenker's fixation. The following is a brief résumé of their account, using their new and elaborate terminology.

(1) In the Primary Spermatocyte the idiosome is differentiated into an outer blue-staining ' idioectosome ' and an inner purple-staining ' idioendosome '. (2) During the preparation for the First Maturation Division the idioectosome disappears and, during the division, the substance of the idioendosome becomes scattered through the cytoplasm in the form of minute granules called ' idiogranulomes '. (3) In the Secondary Spermatocyte a new idioectosome is re-formed, containing the idiogranulomes. (4) During the Second Maturation Division the idiogranulomes are again scattered through the cytoplasm. (5) In the re-formed idioectosome of the spermatid each idiogranulome is seen to be surrounded by a clear vacuole—the ' idiogranulotheca '. (6) The idiogranulomes rapidly fuse to form a single large red-staining ' idiospherosome ' enclosed in a large vacuole, the ' idiosphaerotheca ' formed by the fusion of the idiogranulothecae. (7) The idioectosome now begins to move away to one side and is re-named the ' idiophthartosome '. Meanwhile the idiosphaerosome secretes a crescentic blue-staining ' idiocalyptosome ', and is itself known henceforth as the ' idiocryptosome '. (8) In the ripe spermatozoon the idiophthartosome disappears with the cytoplasm which is lost during metamorphosis. The idiocrypto- and idiocalypto-somes together form a double cap to the sperm-head called the ' spermiocalyptra ', and the idiosphaerotheca ' persists through all later stages and develops into a membranous cover for the cap and head of the sperm ', and is then known as the ' spermiocalyptrotheca '.

As we shall mention below, we have not been able to confirm the statement of these observers as to the scattering of the ' idiogranulomes ' during the maturation divisions, but we have adopted their account for several reasons.

We cannot, however, feel that Papanicolaou and Stockard have really improved the nomenclature of the subject by the introduction of these cumbersome new terms.

In the following table we have placed side by side the new terms of these authors and the corresponding synonyms used by previous workers. In the third column we have put forward

suggested English equivalents based upon those used by previous English authors, wherever these do not involve any ambiguity.

We object to the term ' idiosome ' because it has already been used by Whitman (**33**) to mean ' an ultimate hereditary unit '. The term ' archoplasm ' has been used by Moore (**22**), and we have adhered to it. We have avoided the ' archoplasmic vesicle ' of Moore because it has sometimes been applied to the whole of the archoplasm, but we have substituted ' archoplasmic vacuole ' instead. The only new term we have introduced is ' Proacrosomic granules ' for the minute granules (idiogranulomes) of Papanicolaou and Stockard, which ultimately fuse to form one large ' Proacrosome ', from which the acrosome is later differentiated. No one can object to this word for it is self-explanatory. It will be noted that we have explained all the complicated processes leading to the formation of the acrosome, without having recourse to the invention or adoption of a terminology of the type introduced by Papanicolaou and Stockard.

Papanicolaou and Stockard.	*Older Authors.*	*Suggested English Equivalent.*
Idiosome.	Idiozome (Meves). Sphäre (Niessing and Meves). Accessory corpuscle (Brown). Nebenkern (Hermann). Archiplasm (Benda). Archoplasm (Moore).	Archoplasm (AR).
Idioendosome.	Markschicht der Sphäre (Niessing).	Inner region of archoplasm.
Idioectosome.	Rinderschicht (Niessing).	Outer region of archoplasm.
Idiogranulomes.	Archosomes (Moore). Körnchen (Meves). Microsomenstrata (Niessing).	Proacrosomic granules (APG).
Idiogranulothecae.	Archoplasmic vesicles (Moore). Bläschen (Meves).	Archoplasmic vacuoles (VV).
Idiosphaerosome (becomes idiocryptosome).	The archosome (Moore). Das Korn (Meves), Die stark färbbaren Körper (Benda). Mitosom (Niessing).	Proacrosome (PRA).
Idiosphaerotheca.	Archoplasmic vesicle (Moore). Bläschen (Meves). Vacuole (Benda). Helle Membran (Niessing).	Archoplasmic vacuole (V)

Papanicolaou and Stockard.	*Older Authors.*	*Suggested English Equivalent.*
Idiocalyptosome.	Periphere Zone des Spitzenknopfs (Meves). Äusserer Teil des Mitosomes (Niessing).	Outer zone of acrosome (OZA).
Idiocryptosome.	Innenkorn (Meves). Dunkler Teil des Mitosomes (Niessing).	Inner zone of acrosome (IZA).
Spermiocalyptra.	Spitzenknopf or Spitzenkörper of German authors. Acrosom of v. Lenhossék.	Acrosome (A).
Spermiocalyptrotheca.	Kopfkappe of German authors.	Covering membrane of acrosome (CA).
Idiophthartosome (Idioectosome)	Archiplasmarest (Benda). Idiozomrest (Meves).	Golgi elements with archoplasmic remains (GA).

4. Technique.

The guinea-pigs used for this work were nearly all supplied to us by Mr. H. M. Carleton and Mr. J. S. Haldane of New College, Oxford, to whom our thanks are given.

We used especially the Golgi apparatus techniques of Cajal and Mann-Kopsch, as well as many other methods. One of us (J. H. W.) carried out a large number of tests with the Cajal method in order to ascertain the best time to leave the testes in the formalin fixative. It was found that twenty-four hours in the fixative and twenty-four hours in the silver bath gave the best results, though it was always very difficult to get really satisfactory preparations with any of the formalin-silver nitrate methods.

We used the methods of Stockard and Papanicolaou with fairly satisfactory results, but never got preparations quite so clear as drawn in their figures. At a later stage in this work we tried Da Fano's new cobalt formalin method, which gave useful results. We also made some excellent Mann-Kopsch preparations (three hours Mann's fluid, two weeks 2 per cent. OsO_4), but Flemming without acetic acid and Champy gave poor results.

5. General Description of the Behaviour of the Inclusions of the Cytoplasm in Cavia Spermatogenesis.

We have compiled the following descriptions, and also Pl. 12, after a personal study of many preparations of guinea-pig testes, and also after a careful examination of the literature of the subject. The works of Niessing (**24**), Meves (**20**), Brown (**2**), Benda (**1**), v. Lenhossék (**18**), Moore (**22**), and Stockard and Papanicolaou (**26**), have been considered especially with reference to the formation of the acrosome. Regaud (**27**) and Duesberg (**6**) have also been consulted and their various statements examined. A good many of our results are quite new, especially with reference to the Golgi apparatus.

6. Period I. Growing Spermatocyte.

The mitochondria and Golgi apparatus are to be found in the so-called germinal epithelial cells ; during the growth of the spermatogonium, the mitochondria, which hitherto tended to surround the region of the archoplasm, become spread throughout the cytoplasm, while the Golgi apparatus and archoplasm increase in size. Some time before the spermatocyte has become full-grown the archoplasm becomes distinguishable into two regions—an outer clearer part, and an inner chromophile part formed by the proacrosomic material.

In Pl. 12, fig. 5, is drawn the spermatocyte just about to begin the first maturation division. The chromosomes are appearing within the nucleus and are connected to one another here and there by chromatic or linin filaments. Throughout the cytoplasm the mitochondria (m) are scattered haphazardly. At chb is the enigmatic chromatoid body, which later may be found in each spermatid, and which apparently therefore may divide during cell-division. The Golgi apparatus and the archoplasm are at ge. By this stage the inner region of the archoplasm containing the proacrosomic material has resolved itself into a large number of discrete granules which have been figured by Moore, Meves, Niessing, and Stockard and Papanicolaou, and which we propose to call the proacrosomic granules

(APG), as it is they which ultimately form the acrosome, or head-cap of the sperm.

In Pl. 12, fig. 5, the Golgi apparatus is seen to consist of a large number of semilunar platelets, rodlets, or dictyosomes (GE), which lie upon the outer surface of the archoplasm. By Mann-Kopsch technique the Golgi apparatus is not a reticulum, but is as drawn in Pl. 11, fig. 1 (AR), and Pl. 12, fig. 5. Examined after Cajal's method, or by Da Fano's modification of Cajal's formalin-silver nitrate method, the Golgi apparatus is seen to be in the form of a reticulum, or of flat plates joined here and there, as shown in figs. 2 and 3 of Pl. 11.

7. Period II. Maturation Divisions.

The periods of division of the spermatocyte are difficult properly to study. In very little of our material were mitoses to be found, and this part of our work is the section about which we feel the most diffident to write. Meves, Niessing, and Moore all failed to follow the proacrosomic granules through the phases of the maturation divisions, and we have been unable to establish Papanicolaou and Stockard's claim that these granules retain their individuality and become sorted out to the daughter cells during cell-division. Meves, Niessing, and Moore all agree that the proacrosomic granules soon become visible after the archoplasm is re-formed subsequent to division—that is in the late telophase. We have adopted Papanicolaou and Stockard's description for two reasons: firstly, it is extremely unlikely that the proacrosomic granules would gradually accumulate and grow, especially before the first maturation division—only to become disintegrated at the mitotic prophase; and secondly, we are aware that the Golgi elements or dictyosomes hitherto had not been followed through division, but we now know that in mammals as well as invertebrates the Golgi elements may become sorted out during division and do not lose their individuality.

In Pl. 12, fig. 6, we give a diagram illustrating the interpretations we at present consider to be the most likely to be correct: the mitochondria are spread haphazardly throughout

the cytoplasm, and they offer no remarkable behaviour for study. Around each mitotic aster are grouped approximately one-half of the Golgi elements or dictyosomes; for confirmation of this phenomenon in cells other than those of the guinea-pig testis see Deinecka (**3**), Golgi (**14**), Murray (**23**), Perroncito (**25**), Fauré-Fremiet (**9**), and Gatenby (**11, 13**). This behaviour of the Golgi element or dictyosome does not entail any sort of division of the element itself, but only a haphazard, though subequal, sorting out of the whole elements between the daughter cells.[1]

At APG in fig. 6 of Pl. 12 are the proacrosomic granules, which become scattered in the cytoplasm during division. As with the Golgi elements, the individual granules in the spermatocyte archoplasm are not themselves divided, but sorted out whole between the daughter cells.

At CHB is the chromatoid body whose fate in the maturation divisions has not been followed out; one fact, however, may be mentioned, it is that by far the majority of spermatids contain a chromatoid body (Pl. 12, fig. 7, CHB). In many animals the spermatocyte and spermatid contain a chromatoid body of some kind, and in the case of Smerinthus strong evidence has been accumulated which indicates that this body has the power of binary fission (**10**).

8. Period III. The Newly-formed Spermatid.

In Pl. 12, fig. 7, is a drawing of the newly-formed spermatid; it contains the same categories of cytoplasmic elements as the spermatocyte, only they are approximately one-quarter in amount. With reference to the fact that the spermatid cell is generally much more than one-quarter the size of the spermatocyte, it may be pointed out that between the stages drawn in Pl. 12, figs. 5 and 7, there must be a period during which the cells are rapidly growing. While it is certain that the spermatid Golgi apparatus and archoplasm is usually

[1] Dictyokinesis in the maturation of the germ-cells of Mus, Cavia, Stenobothrus, Limnaea and Helix is the subject of a forthcoming paper by Ludford and Gatenby. The process is even more haphazard than depicted in fig. 6.

more than one-half the size of the same structures in the spermatocyte, it is difficult to obtain satisfactory evidence of any increase in size of the individual mitochondria.

With reference to the sorting out of the Golgi elements or dictyosomes during the maturation divisions, attention is drawn to recent work on Limax agrestis, where it has been demonstrated that the number of dictyosomes in the spermatocyte is eight, and in the spermatid two (**13**). In all probability, though no count is possible in Cavia, the number of platelets or dictyosomes in the spermatid is approximately one-quarter the number in the spermatocyte.

Within the archoplasm of the spermatid the proacrosomic granules have collected (or according to Meves, Niessing, or Moore, now become visible again) (Pl. 12, fig. 7, APG); but very soon around each proacrosomic granule a clear ring appears, so that the granule reposes in a vacuole—the archoplasmic vacuole: the proacrosomic granules together with their vacuoles in which they lie, now tend to run together, so that one obtains the appearance of a number of granules, some larger than others (Pl. 12, fig. 7, APG).

At this stage the centrosome is dividing in the cytoplasm, near, but outside, the archoplasm (Pl. 12, fig. 7, c).

In the next stage the proacrosomic granules have run together so as to form two or three large grains, each surrounded by the clear vacuolar ring—the archoplasmic vacuole (Pl. 12, fig. 8, APG). The whole Golgi apparatus and archoplasm gradually passes to the anterior pole of the cell, i. e. that part of the cell which gives rise to the head end of the sperm, and which most commonly is directed towards the germinal epithelium. In Pl. 12, fig. 8, the Golgi apparatus and archoplasm are shifting in an upward direction (according to the way this cell has been drawn on the Plate). From the posterior end of the cell, the axial filament grows out from the centrosomes (c^1 and c^2).

The next stage in the formation of the acrosome is depicted in Pl. 12, fig. 9. A part of the nucleus is shown at N, and the Golgi apparatus plus the archoplasm lie nearly in front but to one side of the nucleus. The whole apparatus lies in contact

with the nucleus at one spot. A change has come over the proacrosomic structures: these have finally fused to form a single large bead, the proacrosome, within its vacuole (v), and around the entire periphery of the inner granule an outer rind has been secreted (iza). These two regions are known as the outer and inner region of the proacrosome (hitherto proacrosomic granules). The proacrosomic apparatus moves through the archoplasm and finally becomes stuck upon the surface of the nuclear membrane, towards the front end of the nucleus, and hereafter may be called the acrosome (Pl. 12, fig. 10). On the side of the acrosome which touches the nuclear membrane the outer region of the acrosome is completely pushed away, so that the inner region of the acrosome alone touches the nuclear membrane in the mid-region of the acrosome; at the edges, however, as shown in Pl. 12, fig. 11, the outer region of the acrosome lies in contact with the nuclear membrane.

The Golgi apparatus (i.e. all the dictyosomes), and the archoplasm upon which it lies, keeps its position, partly embracing both the acrosome and one side of the nucleus (as shown in Pl. 12, figs. 10 and 11) some considerable time, during which the two parts of the acrosome grow rapidly. Eventually, however, the apparatus and the archoplasm break away as shown in Pl. 12, fig. 12, and begin to drift back towards the tail end of the spermatid (Pl. 12, fig. 13).

The inner region of the acrosome gradually becomes flattened out on the front of the spermatid nucleus, and the whole structure undergoes the changes shown in Pl. 12, figs. 12–15.

9. On the Subsequent Behaviour of the Golgi Apparatus and Archoplasm.

By the stage drawn in Pl. 12, fig. 12, the Golgi elements and archoplasm have begun to drift down the elongating sperm cell, and in Pl. 12, fig. 13, this apparatus has completely flowed away from the nucleus. Between the stages depicted in Pl. 12, figs. 13 and 14, the definitive middle-piece Golgi apparatus appears as described by us on p. 269.

Between the stages in Pl. 12, figs. 15 and 16, the apparatus

and the archoplasm flow into the bead, which sloughs off, and take no part in the subsequent development of the spermatozoon. In Pl. 12, fig. 16, the apparatus and archoplasm have undergone degeneratory changes.

10. The Case of the Rat Spermatozoon.

Retzius (**29**), as we have mentioned above, does not figure a protoplasmic (Golgi) bead on the ripe spermatozoon of the rat or mouse, and apparently it would have seemed to be one of the exceptions to the rule that the ripe mammalian sperm carries a Golgi apparatus. Our friend Dr. Da Fano of King's College, London, who has made preparations of the rat testis by his new cobalt methods, examined at our request his preparations of rat epididymis, with the result that he found that each ripe sperm does carry a small bead which impregnates with silver nitrate. Retzius, therefore, overlooked this bead in the rat sperm, and may have done likewise in the other forms in which he does not draw the characteristic bead.

11. Discussion.

(*a*) On the Origin of the Acrosome in Animal Spermatogenesis.

The evidence that the Golgi apparatus is in some way intimately associated with the formation of the acrosome or perforatorium has accumulated considerably within the last few years.

In Paludina (**12**) and in Columbella (**30**), two molluscs, it has been shown that the Golgi apparatus adheres to the head end of the nucleus of the spermatid, and before breaking away deposits or secretes a small granule from which the acrosome finally develops. In *Smerinthus populi*, a moth (**10**), it has been shown that the acrosome is developed by changes which take place in crescentic 'acroblasts', which we now know as the dictyosomes or individual units of the Golgi apparatus. In the testis of *Stenobothrus viridulus* we have endeavoured to follow out the formation of the acrosome: in this cricket it seems likely that the Golgi apparatus

is intimately associated with the formation of the acrosome, but the form chosen did not provide the very clear evidence wanted. In the spermatogenesis of the louse, Doncaster and Cannon (**5**) observed that the acrosome was formed from a body which they took to represent the Golgi apparatus.

According to the account given for Smerinthus (**10**) by Gatenby, and for Pediculus by Doncaster and Cannon, all the Golgi apparatus is taken up in the formation of the acrosome. Our recent observations on Stenobothrus, and on several other moths (e.g. Biston), have shown that in these insects much of the apparatus finally passes as isolated crescents, spheres, or dictyosomes into the elongating tails of the spermatozoa : this matter is far from being cleared up, but of one thing we may feel certain—that the Golgi apparatus of insects is related to the formation of the acrosome.

Turning now to our observations on the acrosome of the cavy, we note that the account we give agrees in general with that previously described for Paludina (**12**). In both animals we find a Golgi apparatus (plus archoplasm) which moves up to the front end of the nucleus of the spermatid, deposits a granule there, remains for a time, and finally passes away from the head end of the sperm into the lengthening tail.

Papanicolaou and Stockard describe the proacrosomic material as appearing inside the archoplasm as a differentiated area of the latter, which stains specifically in acid fuchsin. Here we have the crux of the whole matter : is the proacrosomic material, which later forms the acrosome, to be regarded as a product of the archoplasm, or of the dictyosomes or Golgi elements ? We believe that this matter may be settled after the events in the formation of the acrosome of insect spermatids have been more fully examined : this remark refers especially to the Smerinthidae.

Another point to which we would like to draw attention is the fact that in the guinea-pig the Golgi apparatus (the ' Nebenkern ' of some older authors) embraces the forming acrosome from the stage when the proacrosomic granule first touches the nuclear membrane, up to the stage when the

acrosome has reached almost its greatest size ; the natural inference being that the Golgi apparatus and not the nucleus is concerned with the growth and perfection of the rudimentary acrosome. In this connexion it will be remembered that one of us has shown in Smerinthus (**10**) that the acrosome may form completely, while the nucleus lags behind in development, as occurs in degenerating spermatids.

We conclude at present that the animal acrosome is formed directly in association with the Golgi apparatus, and that the nucleus has little if any influence in the process.

(*b*) The Middle-piece of the Spermatozoon after Entry into the Egg.

That the middle-piece of the mammalian spermatozoon is carried into the egg is well known, and it is now established by the work of van der Stricht (**31**), Lams (**16**), and Levi (**19**), that excepting the centrosome the entire middle-piece of Vespertilio and Cavia, after having become carried bodily into the egg, remains inert and complete, and is passively borne into one or other of the two blastomeres (or one of three in Levi's case), and is ultimately lost sight of, probably degenerating at a later stage in the cleavage of the egg.

Lams' (**16**) work is particularly worthy of mention. Alone, and also in conjunction with Doorme, he showed that in the white mouse and the cavy the middle-piece (excepting the centrosome) remains unchanged after entry into the ovum. Many of the figures of Lams show the mitochondria lying upon the middle-piece, but in no case did he find any activation of these bodies. In both the cavy and the rat we are aware that the middle-piece bears a Golgi bead, but since Lams used no methods for the Golgi apparatus, it is hardly justifiable to use his work as evidence with regard to the behaviour of the Golgi bead after introduction into the ovum.

Henneguy, at the discussion following Lams' communication to the Brussels congress of 1910, suggested that the blastomere

containing the tail of the sperm became transformed into the embryonic part of the germ, the other blastomere into the trophoblast.

Meves (**21**) likewise suggests something similar for the case of Echinus. That part of the pluteus containing the sperm middle-piece is supposed to bud off the Echinus rudiment, a very unlikely suggestion indeed.

Levi (**19**) in remarking on these facts and suggestions says :

'Le ipotesi di Henneguy e di Meves non furono finora suffragate da alcun fatto, ed il solo argomento nuovo che io adduco, la possibilità della persistenza del pezzo intermedio dello spermatozoo in uno dei blastomeri provenienti dalla 2ª segmentazione, non contribuisce ancora ad illustrare il significato del condrioma maschile nello sviluppo ulteriore.'

As one of us pointed out before, the explanation of Henneguy for the case of the mammal does not accord with the generally accepted interpretation as to the origin of identical twins, for if the presence of a middle-piece was a factor of any sort of differentiation, the two separating blastomeres would not produce the identical twins.

We see no reason to suppose that the middle-piece Golgi apparatus is stripped off the sperm and left outside ; there seems every justification for the supposition that the apparatus is carried into the egg with the mitochondria. What fate lies in store for this middle-piece Golgi apparatus is unknown to us, nor do the works of van der Stricht, Lams, or Levi bring forward any sort of evidence with regard to this point.

In all probability, the apparatus, like the mitochondria, first remains complete and inert and ultimately degenerates, after having fulfilled its function, whatever that may be.

The meaning of the stages in spermatogenesis during which most of the mitochondria and part of the Golgi apparatus become applied to the middle-piece of the spermatozoon, is difficult to understand. If the mitochondria and Golgi apparatus of the spermatozoon remain inert, unlike those of Ascaris which persist in the egg and live (**15**), we are forced to conclude that the function fulfilled by these bodies is carried

out between the time the sperm leaves the spermatic tubule, and enters the egg.

Two suggestions are obvious and may be set forth : (*a*) Both mitochondria and Golgi apparatus are concerned with the production of the energy used up by the movements of the sperm tail. (*b*) Either the mitochondria or the Golgi apparatus (or both) carry some active substance which is set free just as the sperm enters the egg, or after it has penetrated the egg, and whose function is related in some obscure way to the phenomenon of heredity.

It seems to be established that every mammalian sperm is partly formed of mitochondria, and we may find that every such sperm has a Golgi apparatus. The experimental evidence which is necessary for the elucidation of the function of these two categories of cell inclusions within the structure of the spermatozoon would be very difficult to procure, and it appears to be very doubtful whether mere observation of the behaviour of these inclusions during fertilization will provide any conclusive facts.

It has been said that the animal spermatozoon is merely a much modified cell, and it has been shown in this paper that the remark is true to the smallest detail, for a sperm such as that of Cavia is a complete cell with nucleus, mitochondria, Golgi apparatus, and centrosome. In one fact, however, the two gametes differ widely : While the nuclear matter (chromosomes) of both gametes is similar in quantity, the mitochondria and Golgi apparatus of the spermatozoon are infinitely less in quantity than those of the ripe ovum. Are we to look upon the presence of the mitochondria and Golgi apparatus in the animal spermatozoon as being merely of phylogenetic importance, and indicative of a period when the two gametes were equal in size and metabolic potentialities, or should we entertain the view that the mitochondria and Golgi apparatus are specially concerned with a ' cytoplasmic heredity ', as apposed to a ' nuclear ' one ?

It has never been shown satisfactorily that either the mitochondria or the Golgi apparatus can originate from the nucleus,

though some indications of this have been noted (see **11**, p. 581), and until such is established we are not justified in dismissing the hypothesis of a special ' cytoplasmic heredity '.

More than this we cannot at present write ; the very function of the mitochondria and the Golgi apparatus is not understood, and those paths which will lead to this understanding are only now being entered.

University College, London,
April 12, 1920.

12. Summary.

(*a*) The Middle-piece Golgi Apparatus.

1. The middle-piece of the mammalian spermatozoon is formed from part of the mitochondria of the spermatid which become grouped around a central rod or skeleton. Not all the mitochondria of the spermatid pass into the middle-piece, a certain proportion always sloughs off.

2. On the middle-piece of many mammalian spermatozoa there is a protoplasmic bead which can be seen in the fresh, and which, on fixation, stains in plasma dyes.

3. With formalin and silver nitrate techniques the protoplasmic bead is found to contain a number of argentophil platelets or rods, which impregnate exactly like the Golgi apparatus of younger sperm cells.

4. The spermatid of Cavia contains a Golgi apparatus consisting of an inner core of archoplasm, and a cortical region formed of curved plates and rods—the dictyosomes. With formalin-silver nitrate techniques, the Golgi apparatus either appears as a reticulum, or the whole cortex of the apparatus reduces the silver, and then appears homogeneous : with Mann-Kopsch techniques the individual dictyosomes are often very clearly marked.

5. At a stage when the spermatid is elongating the Golgi apparatus buds off a small part of itself. This part becomes

separated from the main Golgi apparatus, and ultimately comes to lie in the middle-piece bead referred to in paragraph 2.

6. The rest of the Golgi apparatus of the ripening spermatozoon sloughs off.

7. While all the chromatinic substance of the young spermatid eventually goes to form the nucleus of the spermatozoon, only the majority of the spermatid mitochondria, and a very small part of the spermatid Golgi apparatus, form the representatives of these cell organs in the ripe spermatozoon.

8. Attention is drawn to the works of Lams and Doorme, van der Stricht, and Levi, where it has been shown that the whole middle-piece of the mammalian sperm (Cavia or Vespertilio) enters the egg at fertilization, but, so far as these authors could observe, thereafter remains inert, and is carried whole and haphazardly into one of the blastomeres.

(*b*) The Formation of the Acrosome.

9. The acrosome of the spermatozoon of Cavia is formed from the proacrosomic granules which are differentiated within the archoplasm during the later growth stages of the spermatocyte.

10. The archoplasm in the spermatocyte of Cavia is covered by the Golgi elements or dictyosomes, which in all probability are associated with the differentiation within the archoplasm of the proacrosomic granules.

11. Each of the spermatids derived from the spermatocyte contain an equal share of Golgi elements, archoplasm, and proacrosomic granules. According to Papanicolaou and Stockard the latter granules do not disintegrate during mitosis, but, keeping their individuality, become scattered in the cytoplasm, are subequally sorted out among the daughter cells, and eventually come to lie within the re-formed spermatid archoplasm.

12. Each proacrosomic granule has a liquid-filled space formed around it, so that it comes to lie in an archoplasmic vacuole.

13. The several proacrosomic granules within their archo-

plasmic vacuoles approach and fuse into fewer larger granules, which eventually all come together to form a single large granule lying in a single archoplasmic vacuole. This structure is known as the proacrosome.

14. The Golgi apparatus complex now consists of numbers of dictyosomes lying on the surface of the archoplasm : the latter contains near its centre the proacrosome. The latter soon becomes distinguishable into an inner darkly-staining bead surrounded by a paler cortical zone, the whole lying in the archoplasmic vacuole.

15. The Golgi apparatus complex has moved up towards the anterior end of the spermatid nucleus, and it now becomes applied to the nuclear membrane. Where the complex touches the membrane the Golgi elements or dictyosomes are pushed aside, so that the archoplasm comes into direct contact with the spermatid nuclear membrane.

16. From its more or less central position the proacrosome passes through the archoplasm and becomes applied to the nuclear membrane, upon which it becomes flattened so as to form a hemisphere. The proacrosome is now spoken of as the acrosome : it has an inner zone, an outer zone, and it is still covered on its outer side by the archoplasmic vacuole. Where the latter comes into contact with the archoplasm there is differentiated the covering membrane of the acrosome, which is rarely very clear.

17. The acrosome grows rapidly, and at a stage when it has differentiated to form a conspicuous cap at the anterior end of the spermatid nucleus, the Golgi elements with archoplasmic remains, which hitherto covered and embraced the developing acrosome, gradually drift away and pass towards the posterior end of the spermatid.

18. The acrosome now develops by itself. The lower part of the archoplasmic vacuole spreads down past the equator of the spermatid nucleus, and the lower edges of the outer zone of the acrosome cover the equatorial region of the nucleus. The archoplasmic vacuole becomes less evident.

19. The outer zone of the acrosome grows very rapidly,

becomes cone-shaped, and later flattened and crescentic in shape when the broad side of the sperm is examined. In the fully formed acrosome the outer zone of the acrosome is much greater in extent than the inner zone of the acrosome.

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14. DESCRIPTION OF PLATES 11 AND 12.

Illustrating Dr. J. Brontë Gatenby and Mr. J. H. Woodger's paper on the 'Cavy Sperm'.

Explanation of Lettering.

A =acrosome. APG =proacrosomic granules. AR =archoplasm (centrosphere). C, C^1, C^2 =centrosome (first and second). CA =covering membrane of acrosome. CH =chromosome. CHB =chromatoid body. GA =Golgi apparatus plus archoplasm. GAX =middle-piece Golgi apparatus elements. GAXC =cytoplasmic bead containing the Golgi elements of the middle-piece. GE =Golgi element or dictyosome. IZA =inner region of acrosome. LPV =lower part of archoplasmic vacuole embracing nucleus. M =mitochondrium. MC =manchette. MD =middle-piece. MX =degenerate mitochondria coalescing to form von Ebner's granules. N =nucleus. OZA = outer zone of acrosome. T =tail of sperm. V =archoplasmic vacuole. V.V. =archoplasmic vacuoles.

In each plate the scale is on the left-hand side.

PLATE 11.

Fig. 1.—Spermatid of Cavia, at the time when the Golgi apparatus (GE and AR) is still in contact with the forming acrosome: the latter is distinguishable into two regions, an inner zone applied to the nucleus (IZA) and an outer zone (OZA). The mitochondria are scattered throughout the ground cytoplasm. This cell is drawn from a Mann-Kopsch preparation; note the discrete dictosomes, GE.

Fig. 2.—Later spermatid, showing the double appearance of the Golgi apparatus (GA). At GAX is the bead which later becomes attached to the middle-piece, as in fig. 3 at GAX. Preparation by Cajal formalin-silver nitrate method.

Fig. 3.—Ripe sperm just before the residue cytoplasmic bead strips off. The middle-piece Golgi apparatus bead is at GAX, the definitive Golgi apparatus, which is cast off, at GA. Preparation by Cajal's method.

Fig. 4.—Sperm at same stage showing mitochondria heavily impregnated by reduced OsO_4. Preparation by Mann-Kopsch method. The Golgi apparatus did not impregnate in the region of the testis from which this cell was drawn. The bead protoplasm is seen at GAXC.

PLATE 12.

This plate is drawn from three separate sets of preparations by (*a*) Mann-Kopsch, (*b*) Cajal's Golgi apparatus technique, (*c*) a mitochondrial method.

So far as possible we have utilized the work of previous observers. All the figures are drawn to scale excepting fig. 16, in which the tail of the sperm is much shorter than it should be.

Fig. 5.—Ripe spermatocyte, I, in the prophases of the first maturation division, chromosomes appearing in the nucleus. The Golgi complex contains in the middle the divided centrosome (C). Around the latter are the proacrosomic granules (APG) which constitute the inner zone of the archoplasm. Between the Golgi elements or dictyosomes (GE) which lie on the surface of the archoplasm, and the inner region of the archoplasm, is a space free of proacrosomic granules. The space constitutes the outer zone of the archoplasm. The whole Golgi complex is drawn in optical section. In the ground cytoplasm lie the mitochondria (M) and the chromatoid body (CHB).

Fig. 6.—Second spermatocyte division metaphase viewed from side. The mitochondria lie haphazardly around the spindle. Following Papanicolaou and Stockard we have drawn the proacrosomic granules (their idiogranulomes) as preserving their individuality and becoming distributed here and there in the cytoplasm around the spindle (APG).

Fig. 7.—Newly-formed spermatid showing the same elements as the spermatocyte in fig. 5, only the proacrosomic granules are now surrounded by the archoplasmic vacuoles (V.V.). The centrosome is dividing. The mitochondria tend to pass to the periphery of the cell.

Fig. 8.—Later stage: the Golgi complex begins to move towards the anterior pole of the cell. The proacrosomic granules have fused one with another till only three are left, the large main one in the middle (APG) being surrounded by its archoplasmic vacuole (V.V.). The mitochondria tend to lie on the periphery of the cell. The centrosome has divided into two, and from one part the flagellum is growing out.

Fig. 9.—Golgi complex and part of nucleus of later spermatid. The proacrosomic granules have all run together to form the proacrosome (PRA), lying in the archoplasmic vacuole (V); the proacrosome is differentiated into an outer (OZA) and an inner zone (IZA). The proacrosome has left its position in the middle of the archoplasm and has approached the nuclear membrane (N).

Fig. 10.—Later spermatid, after the proacrosome has become partly flattened against the nuclear membrane. The outer and especially the inner zones (OZA, IZA) of the acrosome have become much larger. The Golgi apparatus and archoplasm surround the entire acrosome. The archoplasmic vacuole has begun to grow down on each side of the nucleus (LPV). The Golgi complex is placed to one side of the nucleus, but in later stages the acrosome comes to lie at the head end of the nucleus, possibly by a partial rotation of the latter.

Fig. 11.—Spermatid at a later stage just before the Golgi apparatus flows away from the acrosome. The front and back parts of the nucleus

show thickenings. The mitochondria have left the periphery and are collecting towards the middle of the posterior end of the cell.

Fig. 12.—The Golgi apparatus has left the head end of the cell, and is beginning to regain its spherical shape. The centrosome apparatus and flagellum have moved up towards the posterior end of the nucleus. From this region of the latter manchette fibres (MC) begin to grow back. The acrosome become plastered over the entire front of the nucleus. Nearly all the mitochondria have left the anterior pole of the cell.

Fig. 13.—Later stage showing great development of the outer zone of the acrosome (OZA). The manchette has become tubular (MC). From the spermatid Golgi apparatus (GA) has begun to grow out a small bead (GAX) which later forms the middle-piece Golgi apparatus. The mitochondria are collecting in the region of the manchette. The centrosome ring is beginning to pass from the posterior part of the nucleus.

Fig. 14.—The acrosome become more oval in contour. The centrosome ring is passing near the Golgi apparatus (c^2). From the latter the middle-piece Golgi apparatus bead is just separating (GAX). The manchette is less evident, and around the axial filament or flagellum a distinct thickening is visible. It was not settled whether the parts in figs. 13 and 14, MC and MD, were inter-related.

Fig. 15.—The acrosome is now fully formed. The nucleus has gained its characteristic shape. The middle-piece Golgi bead (GAX) has become fixed to the middle-piece (MD) just behind the nucleus. The mitochondria begin to become attached to the middle-piece skeleton (MD) from before backwards. The Golgi apparatus is drifting down and undergoes staining changes.

Fig. 16.—Spermatozoon viewed edgewise, just before skinning off of residue bead. The middle-piece bead is at GAX, but not all the mitochondria (M) have become applied to the middle-piece skeleton ; in the residue protoplasm many of the mitochondria run together and undergo changes, forming von Ebner's granules (MX). The Golgi apparatus is degenerating.