Observations on the Origin of the Germ-cells of the Fowl (Gallus domesticus), studied by means of their Golgi bodies.

By

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With 10 Text-figures.

Introduction.

In 1908 Dantschakoff (**4**), in studying the development of the blood-cells in the Fowl, observed that certain large cells were budded off from the germ-wall endoderm in front of the primitive streak, to which she gave the name 'endodermal wandering cells'. Later, when the mesoderm penetrated this area and blood islands and vessels developed in it, she found that the endodermal wandering cells penetrated the walls of these vessels and were carried round in the circulation. At the stage of about twenty-one somites these cells disappeared from the blood-stream, and Dantschakoff concluded that they took no part in blood development. She described them as being larger than the blood-cells, with many yolk-spheres, and often showing pseudopodia.

In 1914 Swift (**7**) attempted to trace the primitive germ-cells of the Fowl back through earlier stages than those in which they were found in the genital ridge. He found that it was possible to find them in the splanchnic mesoderm of an embryo of about twenty somites. Others had found them in this region, but had been unable to trace them in stages prior to this. It occurred to Swift to seek them in the blood-stream, and he found that earlier than twenty somites large cells were

seen in the vessels which were very similar in all their features to the primitive germ-cells of the splanchnic mesoderm of slightly later stages. He concluded that these were the primitive germ-cells, and that they were identical with the endodermal wandering cells which Dantschakoff had shown to take no part in blood formation, and were originally derived from the germ-wall endoderm in a crescentic area in front of the primitive streak. Swift found that these cells, right up to the establishment of the genital ridge, were similar as regards size, nuclear characters, yolk-spheres, and mitochondria, except that as development progressed their yolk became less bulky.

Von Beremberg-Gossler (**1**) has described what he interprets as Golgi elements in the germ-cells of the 110-hour chick. They have the form of short rod-like bodies encircling the archoplasmic sphere in preparations fixed by Benda's method, and stained in Benda's alizarin-crystal violet. Von Beremberg-Gossler gives two figures of cells in the genital ridge of the 110-hour chick.

The observations recorded in the present paper were undertaken with a view to tracing these cells in preparations in which the Golgi elements had been rendered visible, in the hope that these structures would provide a means of distinguishing these cells from others in the embryo and perhaps of tracing them to the earliest stages.

The technique employed has been chiefly the reduced silver method of Cajal or Da Fano. I have also employed the osmic method of Kopsch, following a brief fixation in Mann's corrosive osmic. With the latter method I have, however, had very many preparations which failed to show any impregnation in the embryonic cells, and in the few which have apparently been successful the appearances seen are not consistent with those seen in silver preparations. Instances of this will be given below. Cajal (**3**) speaks of similar difficulties in connexion with the osmic method. He states: 'Del méthodo de Kopsch, muy constante, según proclaman Misch, Bergen, Holmgren, Sjowall, Weigl, &c., hemos hecho menos uso.

Cierto que con él consíguense tambien buenas coloraciones del órgano reticular, en donde destacan perfectamente los cordones; pero, en general, nos ha parecido que el ácido osmico da imágines demasido pálidas é incompletas.'

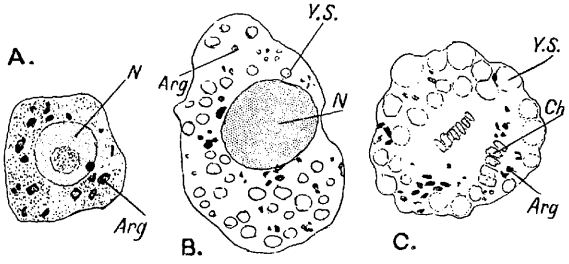
The appearance of the 'reticular organ' in the cells described by Dantschakoff and Swift in the various parts of the embryo of the Fowl will now be described, as it can be seen in my material, which consists of some forty chicks ranging from unincubated blastoderms to the day before hatching.

Primitive Streak Stages.

This has been the most difficult stage to deal with from the point of view of the Golgi bodies. In ordinary stained preparations the large yolk-laden cells apparently budded off from the germ-wall in front of the primitive streak are easily seen, but I have experienced the greatest difficulty in obtaining satisfactory silver impregnations of cytoplasmic structures at this stage. The most I have been able to find has been an impregnation of coarse scattered granules of argentophile material in the ectoderm and germ-wall endoderm cells. These granules vary greatly in size but are for the most part coarse. The larger ones frequently show a clearer central region, but this may be merely an optical effect. One chick of less than twelve hours' incubation showed in its less yolk-laden ectoderm and endoderm cells bodies suggesting the 'banana-shaped' batonettes so often figured in the literature. A cell from this embryo is shown in Text-fig. 1, A. Whether these are the representatives of the Golgi bodies of the cells at this stage I do not feel able to say definitely at present. Cells from primitive streak stages with argentophile grains are shown in Text-fig. 1, B and C, and in Text-figs. 2 and 3. The large yolk-laden cells lying between germ-wall endoderm and the overlying ectoderm are packed with yolk-spheres, and resemble those of the germ-wall itself except that they do not contain yolk-spheres massed together in yolk-balls or clusters. While therefore I cannot make a positive statement about the Golgi bodies at this stage, I can say I have never seen them in the

concentrated condition, and I am strongly inclined to believe that they remain in a scattered condition in cells in which yolk-spheres are still present in great numbers. The earliest embryo in which I have found the ' reticular organ ' in a concentrated state is one in which the medullary folds have just met. In this the ectoderm and the mesoderm cells have the Golgi bodies in a typical condition. My hopes of finding a distinctive

TEXT-FIG. 1.[1]



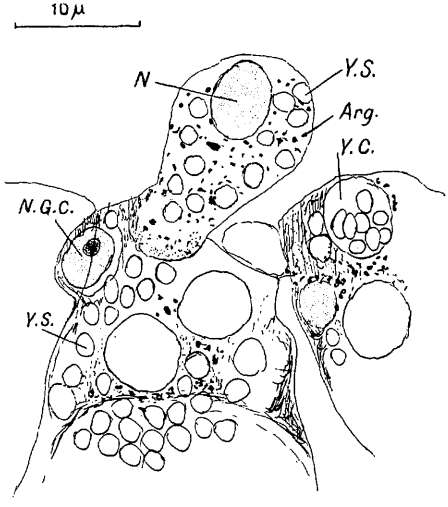
A, cell from the ectoderm of an embryo of under twelve hours' incubation. The argentophile material is arranged in a manner suggestive of ' nebenkern batonettes '. B, large cell lying between ectoderm and germ-wall endoderm of a chick in the primitive streak stage. The only argentophile material present is in the form of fine or coarse grains. C, similar cell in process of mitotic division. *Arg.*, argentophile granules; *N.*, nucleus; *Y.S.*, yolk-spheres; *Ch.*, chromosomes.

reticular organ in those cells of the germ-wall endoderm which are separated off into the space beneath the ectoderm have therefore not been realized. Nevertheless, I see no reason for doubting that Dantschakoff's original account of their origin is correct. A cell in process of separation from the germ-wall endoderm is shown in Text-fig. 2. I have, however, also seen

[1] All figures (except Text-fig. 6) were drawn with an Abbé drawing apparatus using Koristka 2 mm. oil immersion objective and Koristka No. 8C ocular, illuminated by Watson's holoscopic condenser, oiled to the slide. They are therefore all drawn to the same scale, which is indicated by the scale shown in Text-figs. 2, 4, and 9.

similar cells in the ectoderm, and in Text-fig. 3 is shown one apparently in process of making its way into the cavity beneath that layer. If the endoderm is formed at an earlier stage by delamination from the thick epithelium of the blastodisc stage,

TEXT-FIG. 2.



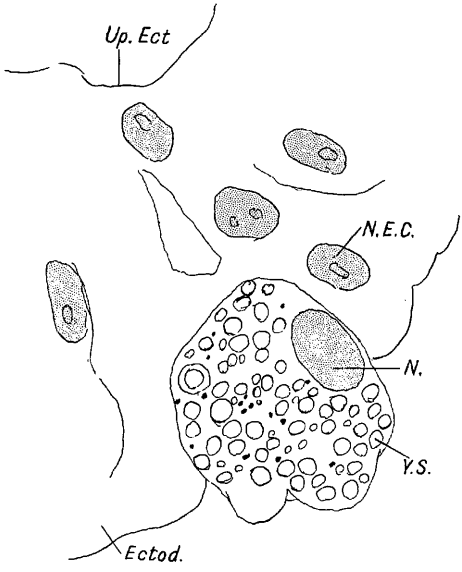
Cell similar to that in Text-fig. 1, B, apparently in process of being separated from the germ-wall endoderm. The yolk in the latter is in the form of large isolated spheres (*Y.S.*), or in spherical clusters of such spheres (*Y.C.*), held together by a membrane of cytoplasm. *N.G.C.*, nucleus of germ-wall cell. Other letters as in Text-fig. 1.

then it would not be remarkable to find some of the large yolk-laden cells lagging behind and delaying their delamination until the primitive streak stage.

In connexion with the difficulty of impregnating the Golgi bodies in early stages the words of Cajal (**3**) may be quoted

here: 'Durante la ontogenía el aparato de Golgi preséntase bien diferenciado desde las trienta y dos á trienta y cuatro horas de la incubación (embrión de pollo). La circunstancia

TEXT-FIG. 3.



Cell similar to that in Text-fig. 2, in process of separation from the ectoderm of the embryonal area. The same embryo showed such cells completely embedded in the ectoderm. *Ectod.*, lower surface of ectoderm; *N.E.C.*, nucleus of ectoderm cell; *Up.Ect.*, upper surface of ectoderm. Other letters as in Text-fig. 1.

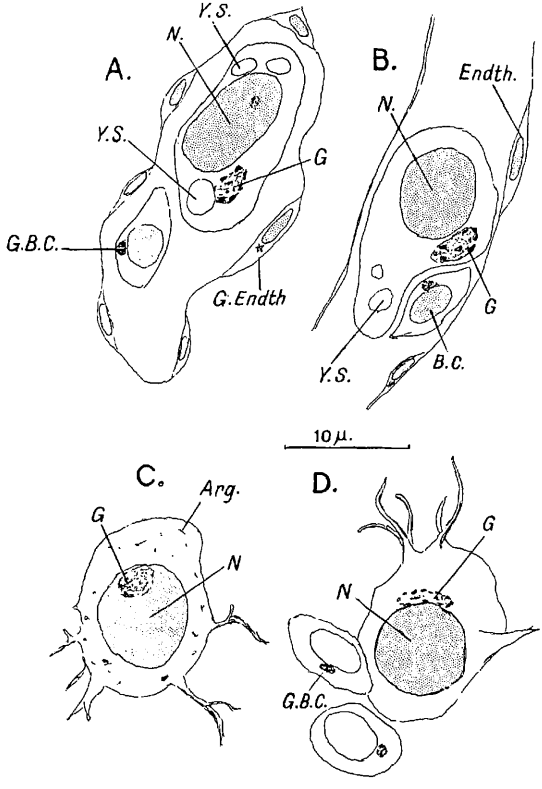
de contar ya con un aparato de esta clase las células germinales del canal primitivo, así como el óvulo y zoospermo, parece denotar que, de ser posible su temprana impregnación, cabría sorprenderlo hasta en las esferas de segmentación del óvulo.'

The Large Cells in the Blood-vessels.

Soon after the blood-vessels are established large amoeboid cells appear in them having the characters described by Swift. They are larger both as regards their cytoplasm and nucleus than the blood-cells, and they are rich in yolk. The appearance of these cells in preparations in which the Golgi bodies are impregnated is shown in Text-fig. 4. It will be seen that the apparatus consists of granules or short rods arranged round the archoplasmic sphere. The latter has already been figured by Swift (7), and shown to contain two centrosomes by von Beremberg-Gossler (1). It will also be noticed that the Golgi bodies are in a much less condensed state than in the other cells of the embryo, exemplified here by the blood and endothelium cells. These large cells, which are undoubtedly the 'endodermal wandering cells' of Dantschakoff, are evidently amoeboid. When in the larger blood-spaces, e.g. the heart and vitelline veins, they show numerous slender pseudopodia, much as an amoeba does when suspended freely in water and not in contact with a solid object (Text-fig. 4, C and D). In the smaller vessels, in which the large cells cannot escape contact with the wall, such fine pseudopodia are not seen, but the cells have a smooth contour, with sometimes a short blunt pseudopodium. The appearance of a pseudopodium seen in Text-fig. 4, B, may simply be due to compression.

These cells present much the same appearance at all stages in which they are visible in the blood-vessels. At the stage of about forty-four hours, from which Text-fig. 4 was taken, they are rarely seen in the peripheral vessels, but begin to concentrate in the vessels in or near the body of the embryo—in the large vitelline veins behind the heart, in the heart itself, in the ventral and dorsal aortae, and in the small vessels of the head. They show a marked tendency to occur in small groups of two or three.

TEXT-FIG. 4.

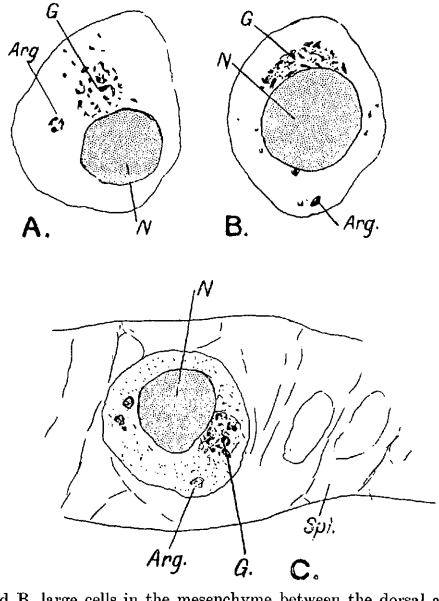


A and B, from a preparation made by Cajal's method, toned and counter-stained in safranin, showing large cells in small blood-vessels of the head of a forty-four-hour chick. The large yolk-spheres are less numerous than in the cells of previous stages. C and D, cells from same embryo (untoned) lying in a large vitelline vein near the heart, and showing numerous slender pseudopodia. In C the Golgi bodies are superimposed upon the nucleus; a few argentophile granules are seen in the cytoplasm. *B.C.*, blood corpuscle; *Endth.*, endothelial cell; *G.*, Golgi bodies of the large cells; *G.B.C.*, Golgi bodies of blood corpuscles; *G.Endth.*, Golgi bodies of endothelial cells. Other letters as before.

The Large Cells in the Embryonic Tissues.

After the stage of about twenty-one somites the large cells above described are no longer seen in the vessels. In the

Text-fig. 5.



A and B, large cells in the mesenchyme between the dorsal aorta and the splanchnic mesoderm of a sixty-seven-hour chick prepared by Cajal's method, untoned. In A the Golgi elements are arranged in a sphere; in B this is more spread out over the nucleus. C, large cell in the splanchnic mesoderm from the same embryo. *Spl.*, splanchnic mesoderm. Other letters as before.

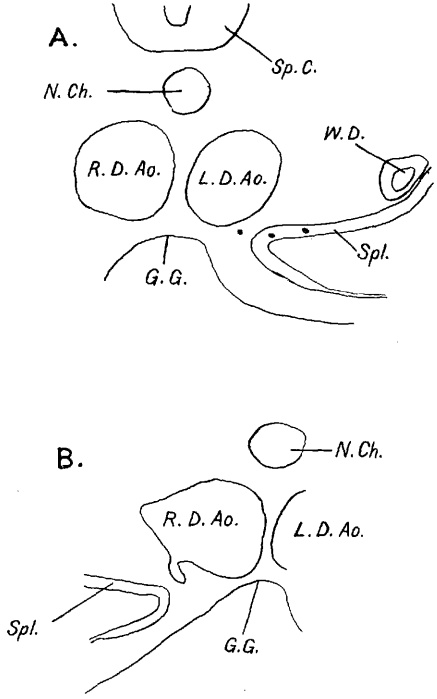
mesenchyme between the dorsal aorta and the splanchnic mesoderm, however, and in the splanchnic mesoderm itself, are seen large cells precisely similar in every way to those

previously met with in the vessels, except that they never exhibit slender pseudopodia, but they still frequently show short blunt processes perhaps indicating amoeboid potentialities. There is no change in the Golgi bodies, as will be seen in Text-fig. 5. Large yolk-spherules are still present (not shown in Text-fig. 5 from Cajal preparations). It should be emphasized that there is not the slightest difficulty in recognizing these cells and in distinguishing them from other cells in the embryo, either in this stage or in the preceding one, especially in silver preparations in which the Golgi bodies are impregnated.

The situation of these cells in a transverse section through the trunk region of a sixty-seven-hour chick is shown in the low-power sketch in Text-fig. 6. The appearances seen in my preparations strongly suggest that the cells leave the dorsal aortae and migrate in the direction of the splanchnic mesoderm. I have not witnessed their actual passage from the aorta, but I have seen them tightly wedged in the small paired branches of that vessel. The position of one such branch is shown in Text-fig. 6, B.

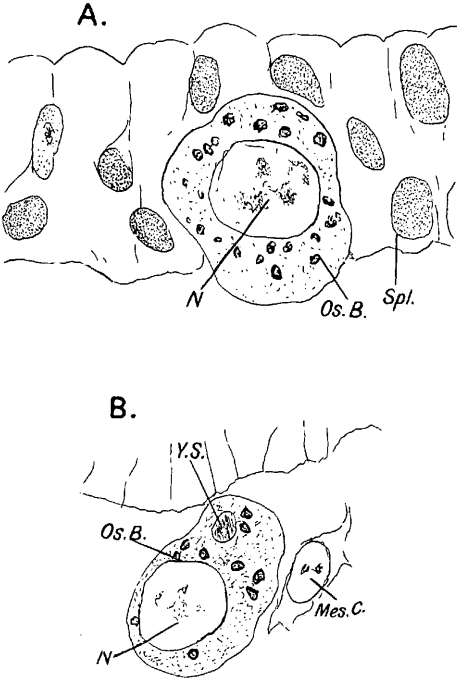
I have now to record some curious appearances in a chick of this age (sixty-seven hours) prepared by the Mann-Kopsch-Altmann method. Some of the large cells of this embryo are shown in Text-fig. 7. In addition to yolk-spherules the cells show a number of curved rods blackened by osmic acid and looking remarkably similar to the ' banana-shaped ' batonettes so commonly figured in the cells of invertebrates, e.g. in the neurones of Helix described by Brambell and Gatenby (**2**). This is the most fully impregnated specimen I have obtained of this stage by the osmic method, most of the others showing little or no impregnation. In the embryo figured in Text-fig. 7 these large cells in or near the splanchnic mesoderm are the only cells in the embryo which show such blackened curved rods. If these represent the Golgi bodies in a scattered condition this chick is unique in my collection, for in none of those prepared by the silver method does the reticular organ present such an appearance in this or any other stage, with the excep-

TEXT-FIG. 6.



A, low-power drawing of a section of the sixty-seven-hour chick from which Text-fig. 5 was drawn, showing the positions of three germ-cells indicated by large dots. B, low-power drawing of the same chick, showing small branch from dorsal aorta. *G.G.*, gut groove; *L.D.Ao.* and *R.D.Ao.*, left and right dorsal aortae; *N.Ch.*, notochord; *Sp.C.*, spinal cord; *Spl.*, splanchnic mesoderm; *W.D.*, Wolffian duct.

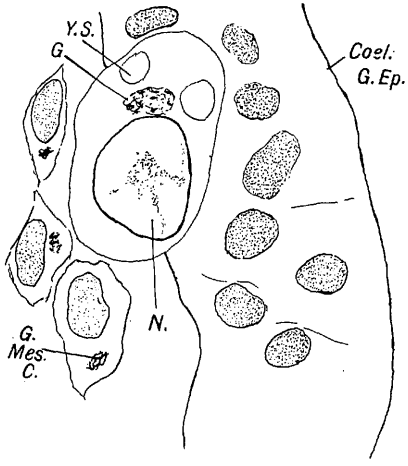
TEXT-FIG. 7.



Large cells from a sixty-seven-hour chick prepared by the Mann-Kopsch-Altmann method. In A the cell is nearly included in the splanchnic mesoderm; that in B is still outside in the mesenchyme. *Mes.C.*, mesenchyme cell; *Os.B.*, bodies blackened by osmic acid (there were no such bodies in the mesoderm and mesenchyme cells of the same embryo); *Y.S.*, yolk-sphere stained red. Other letters as before.

tion of the very early stage illustrated in Text-fig. 1, A. I therefore feel that the greatest caution should be exercised in the interpretation of such bodies in Kopsch preparations, and do not feel prepared in the present instance to regard them as genuine Golgi elements until I find them in the same condition in silver preparations of this stage.

TEXT-FIG. 8.



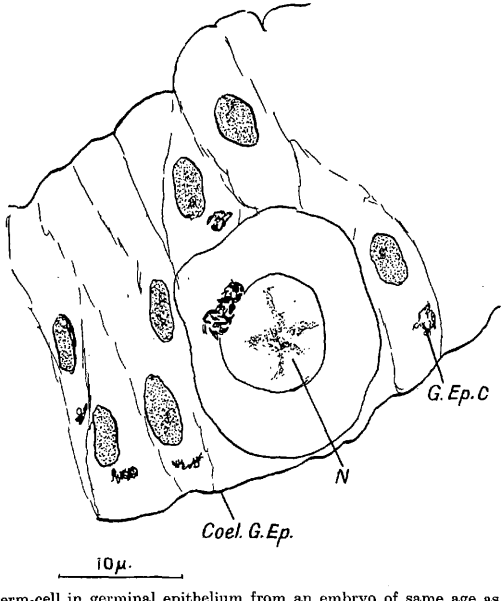
Germ-cell in the germinal epithelium of a chick embryo of 103 hours incubation, prepared by the method of Cajal, toned and counter-stained in haematoxylin. *Coel.G.Ep.*, coelomic surface of germinal epithelium ; *G.Mes.C.*, Golgi bodies of mesenchyme cell. Other letters as before.

THE LARGE CELLS OF THE GENITAL RIDGE.

These cells, the acknowledged primitive germ-cells, are depicted in Text-figs. 8 and 9. They are somewhat larger and have a reticular organ in which the elements are rather more condensed together than in preceding stages. A few

yolk-spherules are usually still visible. The cells lie either in the germinal epithelium or just beneath it. Text-figs. 8 and 9 were drawn from chicks of 103 hours of incubation.

TEXT-FIG. 9.



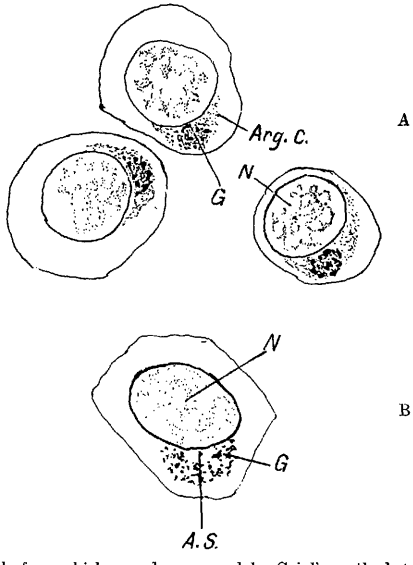
Germ-cell in germinal epithelium from an embryo of same age as that in Text-fig. 7, prepared in the same way. *G.Ep.C.*, Golgi elements of a germinal epithelium cell. Other letters as in Text-fig. 7.

THE GERM-CELLS IN YOUNG GONAD STAGES.

Preparations of these stages were obtained by dissecting out the kidneys and attached gonads from chicks of both sexes of 12, 14, 15, 16, 17, 18, 19, 20, and 21 days of incubation.

The small mass of tissue thus obtained was fixed and passed through the silvering process without further dissection. The germ-cells of a chick of fifteen and of seventeen days of

TEXT-FIG. 10.



Germ-cells from chick gonads, prepared by Cajal's method, toned and counterstained in safranin. A, group of three cells in a cortical cord of an embryo of fifteen days' incubation. In addition to the Golgi elements there is an argentophile cloud spreading round part of the nucleus. B, germ-cells from a cortical cord of the ovary of a chick embryo of seventeen days' incubation. No argentophile cloud is seen in these cells, but the Golgi elements are more voluminous. The archoplasmic sphere is hemispherical (*A.S.*) and applied by its flat side to the nucleus.

incubation are shown in Text-fig. 10, A and B respectively. The cells are now smaller than the primitive germ-cells of the preceding stage, but possess large nuclei and are much

more numerous. Yolk is no longer seen. The Golgi elements in the cells of the embryo from which Text-fig. 10, A, was drawn were surrounded by a cloud of argentophile material. In some specimens this is not present; Text-fig. 10, B, for example, shows a voluminous reticular organ but no cloud. The archoplasmic sphere in this specimen has a hemispherical shape with its flat side in contact with the nucleus.

It will be noticed that there is considerable variation in the condition of the Golgi bodies in the large cells described in the above pages, even in cells from the same embryo, mounted on the same slide. Compare, for example, Text-fig. 4, C and D, and Text-fig. 5, A and B; also Text-fig. 8 with Text-fig. 9. In some cases the Golgi elements tend to be arranged in a spherical form; in others they are more spread over the nucleus.

The later history of the Golgi bodies in the young oocytes of pullets is described by Rogers Brambell (**2** *a*).

Conclusion.

I feel no doubt about the continuity of the primitive germ-cells of the genital ridge with those of the splanchnic mesoderm of earlier stages, and with the large cells of the blood-stream in still earlier ones. They are easily distinguished by their size, pseudopodia, yolk-content, and Golgi bodies, both from the blood-cells and, when lying in the mesenchyme, from other cells which surround them. About their continuity with the cells separated from the germ-wall endoderm in front of the primitive streak I feel less able to speak. At this stage the Golgi bodies have not given the help I hoped they would in the identification and separation of these cells. The conspicuous cells of the blood-stream must, however, have an origin independent of the blood islands, and we have the observation of Dantschakoff, working without any preconceived notion about their possible connexion with the germ-cells, that they are derived from the germ-wall endoderm and take no part in blood-formation; to this is added the corroboration of Swift. I would then invite comparison between

the cell drawn in Text-fig. 1, B, and that in Text-fig. 4, A. It is my impression that the latter condition is derived from the former by the assimilation of the yolk, accompanied by the assumption by the Golgi bodies of a concentrated condition.

As my observations have not been directed to this problem I am unable to say anything about the ultimate fate of the primitive germ-cells. It may be mentioned, however, that while Swift (**8**) believes that they give rise to the definitive germ-cells, Firket (**5**), who has given much attention to this subject, is of opinion that they are all destined to degenerate, and that the definitive germ-cells arise from the germinal epithelium. A study of the Golgi bodies of these stages might assist in settling this point.

It should also be added that Reagan (**6**) claims to have supplied an experimental proof of Swift's conclusions regarding the origin of the primitive germ-cells by extirpating the germ-wall endoderm in front of the primitive streak in chicks. He claims that the genital ridge which later develops in such experimental eggs shows no primitive germ-cells. He gives very little information about the technical procedure followed, and, as far as I am aware, his experiments have not been repeated.

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