THE GOLGI SUBSTANCE OF THE FEMALE GERM CELLS OF MACACUS RHESUS AND CANIS FAMILIARIS

H. K. YOSUFZAI

Department of Zoology, University of Dacca, Dacca

(Received August 12, 1961)

The Golgi substance of the female germ cells of *Macacus rhesus* and *Canis familiaris* was studied by silver, osmic and Sudan Black techniques. The Golgi elements in the young ovum of *Macacus rhesus* are situated at the juxta-nuclear position and are of polymorphic nature. This juxta-nuclear stage gives rise to yolk-nucleus and peri-nuclear stages. When the oocytes further grow, the Golgi elements disperse and leave a clear area in the region of their original concentration. In the final stages the Golgi elements are seen scattered throughout the cytoplasm. In the case of *Canis familiaris* the Golgi elements in very young oocytes are situated at the juxta-nuclear position. Next stages are the yolk-nucleus, semi-perinuclear and the perinuclear stages. In fully mature oocytes of *Canis familiaris* the Golgi elements are seen scattered throughout the cytoplasm. The Golgi elements are of polymorphic nature in the female germ cells of *Macacus rhesus* and *Canis familiaris* and the classical methods reveal correct picture of the Golgi elements, which is identical to that as revealed by Sudan Black. The investigation clearly demonstrates that there is no hard and fast rule regarding the shape of the Golgi elements in germ cells. The author holds the view that there is no justification whatsoever for changing the name of the Golgi bodies to "Lipochondria" as suggested by Baker.

1. Introduction

The abundance of mammalian types in Pakistan affords a vast field for cytological research. For some reason or other, not much work has yet been done from the modern cytological point of view on the Golgi elements of the female germ cells of mammals.

As is well-known, the study of the Golgi elements in animal cells has given rise to extensive discussion resulting in controversies. About half a century has passed since the 'discovery' of the Golgi elements, yet we still lack an acceptable view about this important structure of the cell. The views held by various cytologists regarding shape, existence, nature and origin of this important cytoplasmic structure vary considerably. Particularly, during the past fifteen years a new controversy has arisen regarding the very existence of this cell organ. Baker,[2–5] Palade and Claude,[28] and Thomas[30–32] deny the existence of Golgi elements as revealed by the classical methods.

Several workers have studied and described the Golgi elements in the female germ cells of mammals, but much of the evidence is conflicting and there is no general agreement regarding the origin, nature and ultimate fate of this structure in the female germ cells of mammals. It was with a view to throw more light on the recent controversy regarding the Golgi elements that the author studied the behaviour of Golgi elements in the female germ cells of *Macacus rhesus* and *Canis familiaris*.

2. Material and Technique

For the present investigation two animals were selected from the mammalian group - *Macacus rhesus* and *Canis familiaris*. The ovaries of animals of ages ranging from six months to three years were examined and studied. Vivisection not being possible, chloroform was used to anaesthise the animals and the ovaries were removed before the actual death of the animal. In all cases, the ovaries were immediately removed from the body of the animal and were cut into several small pieces in normal salt solution. These pieces were immediately transferred to the fixative.

Among non-osmic fixatives, Da Fano and Cajal proved to be very successful both in the case of dog and monkey. The pieces of ovaries were kept at 0°C. about 30 hours in Da Fano and almost the same time in Cajal, changing the fixative after every 12 hours. The material after fixation was washed for 10 minutes in distilled water and kept in 1.5% silver nitrate solution from 72 to 80 hours. The change of silver nitrate was given after every 18 hours. It was noticed that Formalin was responsible for the shrinkage of the cytoplasm so the quantity of Formalin was reduced from 15% to 10%.

The material was then washed three to four times in distilled water and transferred to the reducer where it was allowed to remain for about half the time taken for silver impregnation. The reducer solution was made up as follows:

Hydroquinone	1.5 g.
Distilled water	100 ml.
Neutral Formalin	10 ml.
Sodium sulphite	0.5 g.

The material was then washed in distilled water, dehydrated and embedded. Sections were cut from 5μ to 6μ thick. The toned as well as the

untoned slides were studied mostly without staining. The toning was done by 0.2% gold chloride for half a minute, then slides were washed in distilled water for about a minute and finally treated with hypo solution for three minutes. A few toned slides were stained with Benda's Safranin and Light green.

Among the osmic preparations for the demonstration of Golgi bodies, Ludford's latest modifications gave fairly satisfactory results though it did not prove successful in case of young eggs. The osmic acid has very poor penetrating power and is not a good fixative for the mammalian ovary.

Very small pieces of ovary were fixed in equal volumes, 1% osmic acid and saturated solution of corrosive sublimate in physiological salt solution (Mann's fluid) for 24 hours, then washed for half an hour with distilled water. The material was transferred to 2% osmic acid in which it was kept from 4 to 7-day. After 7 days osmication, fat was observed. Before this period no result could be obtained. The longer treatment of the materials with osmic acid made it too brittle to give any satisfactory results. The osmication was carried out at room temperature and during winter at 35°C. The material after osmication was washed for 24 hours in distilled water, then dehydrated and embedded. Sections were cut from 5μ to 6μ thick. The bleaching was done according to Henneguy's method with potassium permanganate (1%) followed by 4% oxalic acid. In the case of monkey the fat was very resistant and took about 48 hours to dissolve in turpentine. In the case of dog it took only 12 hours.

Sudan Black B was employed for the demonstration of the Golgi elements in the female germ cells of *Macacus rhesus* and *Canis familiaris*. The procedure followed was that recommended by Gatenby and Moussa.[18] Certain minor modifications were found necessary to meet the different nature of the tissue. The method and technique were the same as employed during the study of spermatogenesis and oogenesis of *Fasciola hepatica*.[34,35] Small pieces of ovaries were fixed in Flemming without acetic acid for 3 hours, washed in running water for 24 hours and transferred to Gurr's gum syrup for 20 hours. Sections from 15μ to 20 μ in thickness were cut by the freezing method and subsequently washed for 10 minutes in running water. The sections were then transferred to tubes containing 50% alcohol for 3 minutes and 70% alcohol for 2 minutes. Sections were stained in different strengths of Sudan Black B solution; one part of filtered saturated solution of Sudan Black and two parts of 70% alcohol gave excellent results. The sections were left in the stain overnight, and were differentiated in 50% alcohol for one minute, washed in distilled water and mounted in Farrant's medium. Some of the preparations were made according to Baker's 'formal Sudan Black technique.'[2]

3. Observations

Macacus rhesus.—The Golgi bodies in the case of young oocytes fixed in Da Fano and Cajal fixatives appear in the form of a compact mass at the juxta-nuclear position. The oocytes do not carry any follicle cells at this stage. The nature of Golgi elements is difficult to ascertain due to the compact nature of the apparatus (Fig. 1). In the next stage the Golgi elements are observed in the yolk nucleus of Balbiani area. The yolk nucleus is a circular ball carrying Golgi elements and a central vesicle with a centrosome (Fig. 2).

The Golgi bodies are seen in the partial perinuclear stage when the egg has two to three layers of follicle cells. The Golgi bodies start dispersing from their original position. The Golgi elements are in the form of granules, comma-shaped structure and of various shapes showing that they are polymorphic in nature (Fig. 3). The next stage is the one when the Golgi mass extends round the nucleus forming a perinuclear ring. The Golgi elements in the original juxta-nuclear position form a fairly thick mass (Figs. 4, 5 and 6).

As the oocyte further grows, the Golgi elements disperse and leave a clear area in the region of their original concentration. It may be regarded as an archoplasmic area which is said to be homologous with the yolk nucleus of Balbiani. In this clear zone a centrosome is also visible at times (Fig. 7). The Golgi bodies are now seen scattered round the nucleus and also round the archoplasmic area. In the more advanced oocytes the Golgi bodies of polymorphic nature are seen scattered throughout the cytoplasm (Fig. 8).

Canis familiaris.—The Golgi apparatus appears for the first time in very young oocytes at the juxta-nuclear position. Such oocytes do not possess follicle cells (Figs. 9 and 10). The Golgi elements from the very beginning are in the form of granules, vesicles, or comma-shaped bodies. In a few young oocytes, the author observed Golgi bodies aggregated in the form of two patches, either on the same side or at opposite poles.

In a little advanced oocyte the juxta-nuclear mass of Golgi bodies is arranged in the region which may be called yolk-nucleus area. This yolk - nucleus is a centre of great activity, as later

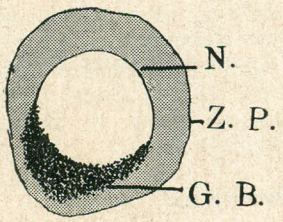


Fig. 1.—Young oocyte showing Golgi bodies in form of a compact mass in the juxta-nuclear position. Cajal toned.

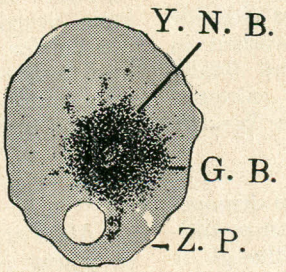


Fig. 2.—Young oocyte showing yolk-nucleus of Balbiani with a central visicle containing a granule. Cajal toned.

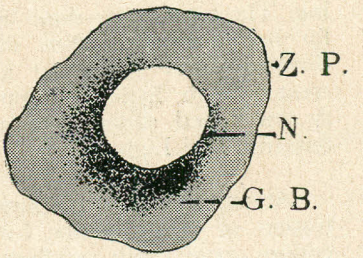


Fig. 3.—Young oocyte showing partial peri-nucle.r ring Cajal toned.

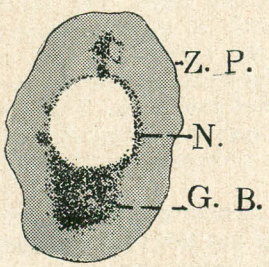


Fig. 4.—Oocyte showing concentration of Golgi bodies in the yolk-nucleus of Balbiani area and the peri-nuclear ring is in the process of formation. Da Fano toned.

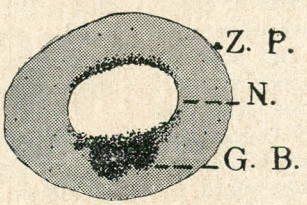


Fig. 5.—The oocyte shows concentration of Golgi bodies in yolk-nucleus of Balbiani and peri-nuclear regions. Da Fano toned.

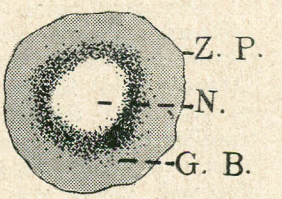


Fig. 6.—Oocyte showing peri-nuclear ring of Golgi elements. Cajal toned.

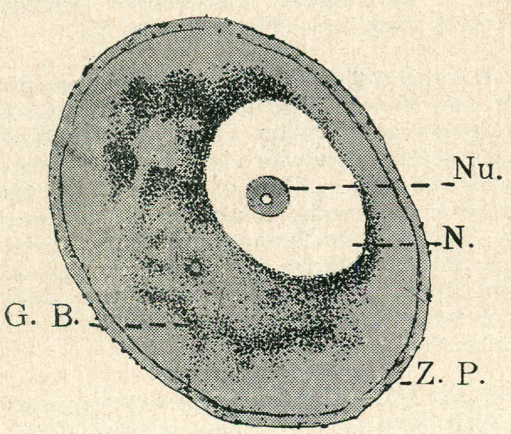


Fig. 7.—Oocyte showing archoplasmic area. Golgi bodies are seen round the nucleus and the archoplasmic area. Cajal toned.

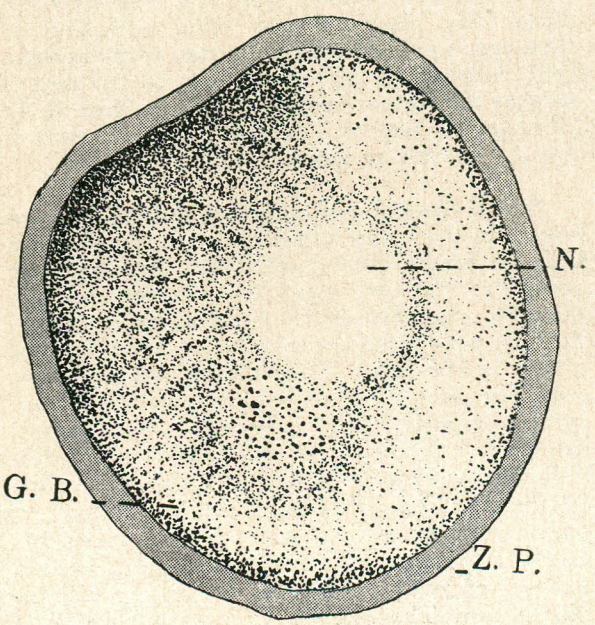
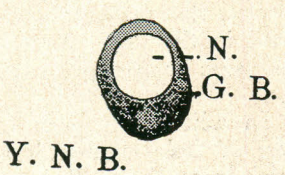


Fig. 8.—A mature ovum showing dispersed condition of Golgi bodies. Da Fano toned.

Fig. 9.—Young oocyte showing Golgi apparatus at the juxta-nulcear position. Da Fano toned.



Abbreviations: G. B., Golgi bodies; N., Nucleus; Nu., Nucleolus; Y.N.B., Yolk-nucleus of Balbiani; Z. P., Zona pellucida.
Scale: 2 cm. = 0.01 mm.

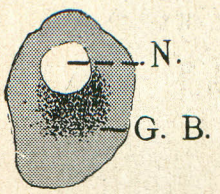


Fig. 10.—Young oocyte showing Golgi elements in the yolk-nucleus of Balbiani area. Cajal toned.

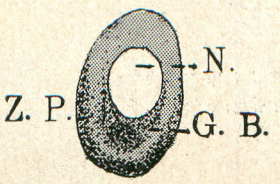


Fig. 11.—Oocyte in which Golgi bodies lie around and away from the yolk-nucleus area. Archoplasm is seen in the middle of the Y. N. B. area. Cajal toned.

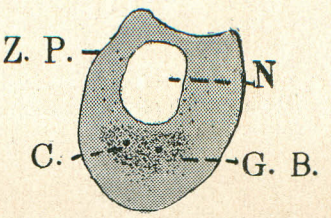


Fig. 12.—Oocyte showing archoplasm in which the Golgi elements and two centrosmes are seen. Cajal toned.

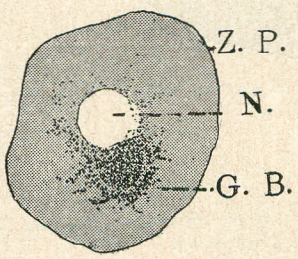


Fig. 13.—Oocyte showing archoplasmic area filled with Golgi bodies. Cajal toned.

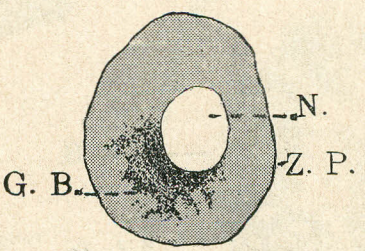


Fig. 14.—Oocyte showing dispersal of Golgi elements from the yolk-nucleus area. Cajal toned.

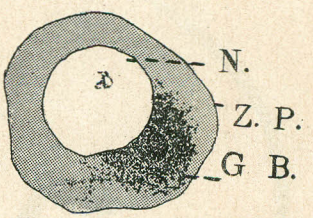
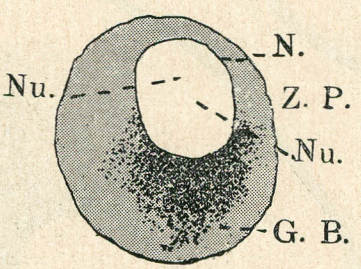


Fig. 15.—Oocyte showing further dispersal of Golgi elements from the yolk-nucleus area. Da Fano toned.

Fig. 16.—Golgi bodies are seen dispersing from yolk-nucleus area. Da Fano toned.



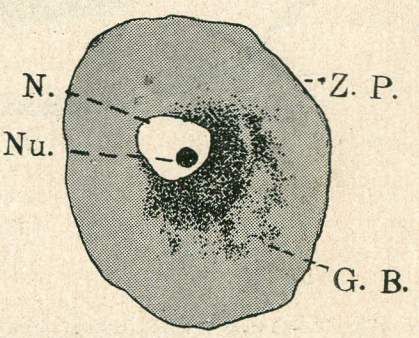


Fig. 17.—Oocyte showing that the Golgi elements have started surroundig the nucleus. Archoplasm of thicker consistency is seen. Cajal toned.

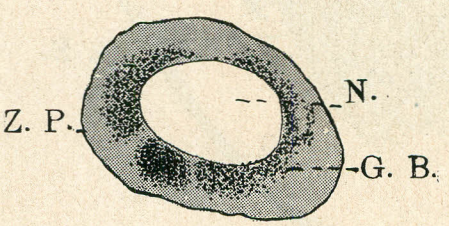


Fig. 18.—Oocyte showing Golgi elements. Da Fano toned.

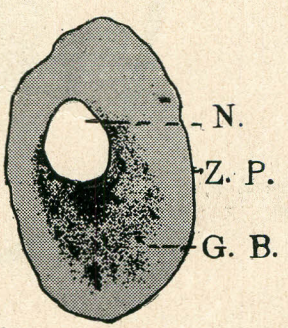
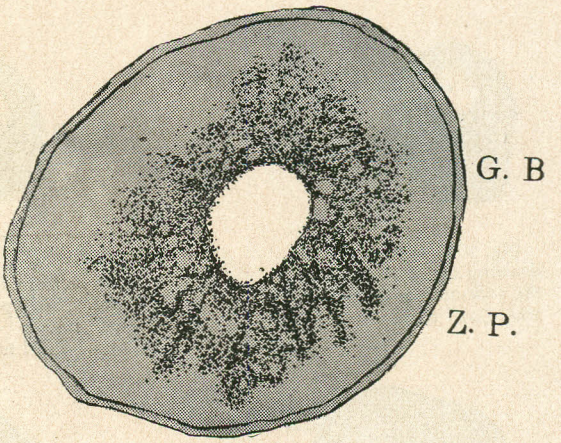


Fig. 19.—Oocyte showing a wide band of Golgi elements round the nucleus. Cajal toned.

Abbreviations; C., Centrosome; G. B., Golgi bodies; N., Nucleus; Nu., Nucleolus; Z. P., Zona pellucida. Scale: 2 c.m. = 0.01 m.m.

Fig. 20.—Oocyte showing medullary ring of Golgi bodies. The migration of Golgi bodies is not uniform on all sides of the nucleus. Da Fano toned.



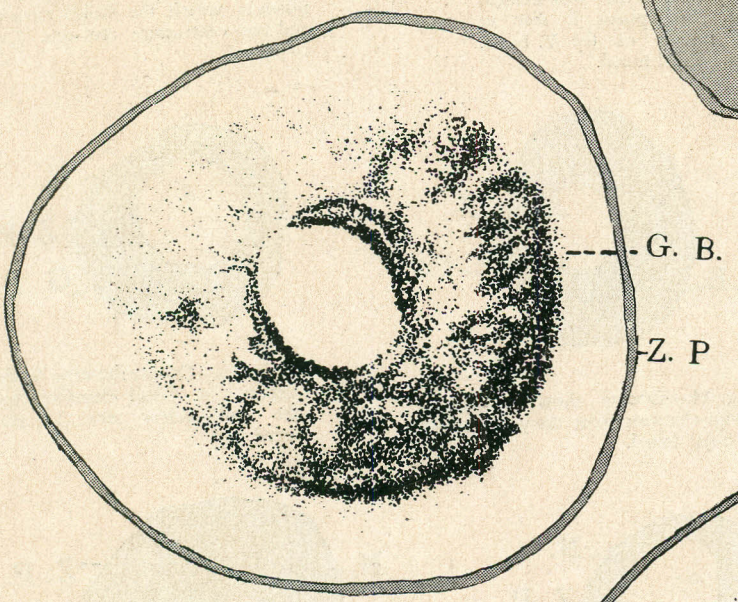
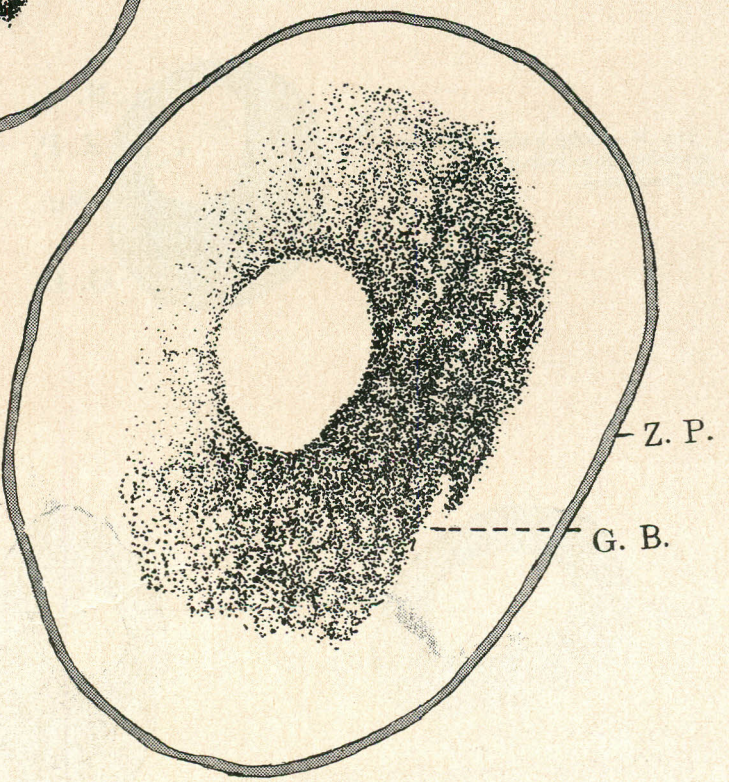


Fig. 21.—Oocyte showing migration of Golgi elements. Cajal toned.

Fig. 22.—Oocyte showing uniform migration of Golgi elements. Cajal toned.



Abbreviations: G. B., Golgi bodies; Z. P, Zona pellucida. Scale 2 cm. = 0.01 mm.

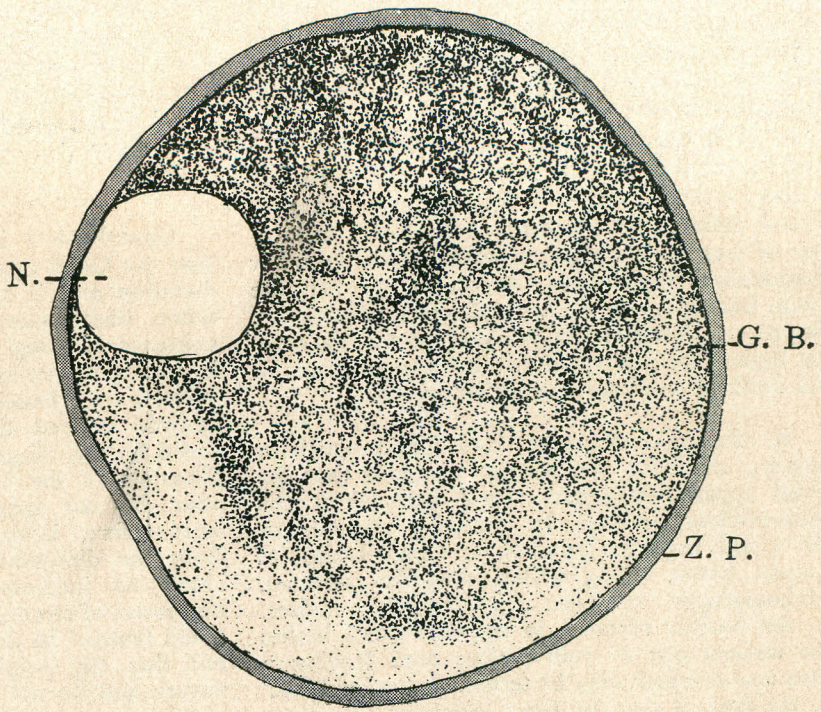


Fig. 23.—Oocyte showing dispersed condition of Golgi bodies. Cajal toned.

Abbreviations: G. B., Golgi bodies; N., Nucleus; Z. P., Zona pellucida. Scale: 2 cm. = 0.01 mm.

stages show that this area is responsible for the origin and dispersal of Golgi elements (Fig. 11). In some oocytes, the Golgi bodies lie around and away from the yolk-nucleus area leaving a centre of protoplasm of thicker consistency which may be termed archoplasm (Figs. 12, 13 and 14). As the oocyte grows further, it is observed that the Golgi bodies increase in number, and they arrange themselves round the nucleus, forming a semi-perinuclear ring (Figs. 15, 16 and 17). This semi-perinuclear ring of Golgi elements furhter extends itself round the nucleus giving rise to the perinuclear stage (Figs. 18 and 19). The Golgi bodies now disperse and occupy the medullary region. In some cases the migration is uniform on all sides of the nucleus (Figs. 20, 21 and 22). In other cases the migration is particularly active at one pole of the nucleus, thus giving rise to an interesting stage of Golgi dispersal. At one side of the nucleus the Golgi bodies are seen travelling towards the medullary region. The perinuclear concentration of Golgi bodies seems to last for some time after which it may disintegrate and disappear.

The Golgi bodies may be granular, vesicular, comma-shaped, or may form irregular masses. In the fully mature ovum the Golgi elements are not res ricted only to the medullary region. The whole cell gets packed with the Golgi bodies (Fig. 23).

4. Discussion

In 1898 the famous Italian neurologist, Camillo Golgi,[19] discovered the "apparato reticulare interno." Since then this has been termed after his name as 'Golgi apparatus.' The real significance regarding the nature and function of Golgi elements is still a baffling problem to cytologists.

Weigl[33] studying the oogenesis of certain mammals has shown that the Golgi apparatus is of complex nature. In 1913 Rio Hortega[29] reported that the Golgi apparatus in the early oocytes of the guinea-pig and rabbit is at the juxta-nuclear position in the form of net-work. Cattaneo,[11] in the young oocytes of bat, guinea-pig and rabbit, observed Golgi apparatus in the form of net-work situated at one pole of the nucleus. Gatenby[13] observed Golgi bodies in the early stages of the oocytes of *Saccocirus* in the form of curved dictyosomes placed in the juxta-nuclear archoplasmic region.

Gatenby and Woodger[14] believe that the mammalian Golgi apparatus 'consists of numerous semilunar plates or rods and not of branched straight bodies as drawn by Hortega.' Nihoul[24] describes Golgi apparatus consisting of grains or batonnets in the form of compact mass observable at one pole of the nucleus in the young oocytes of rabbit. According to Gressen[20] 'in the mouse,

however, the Golgi bodies are fairly evenly distributed throughout the cell; furthermore, the individual Golgi elements appear as rods and granules and do not form a mesh work or masses of entangled filaments as described for the bat, guinea-pig and rabbit.' Clement[12] in *Sciurus palmarum*, observed in early oocytes the Golgi apparatus in the form of a small juxta-nuclear mass of a close net-work, which later increases and fragments. At a still later stage due to further fragmentation discrete Golgi elements are produced. Aykroyd[1] mentions that the Golgi apparatus of human oocytes resembles those in other mammalian germ cells. According to her the Golgi bodies are in the form of rods and granules. Beams and King[7] while working on the developing eggs of guinea-pig observed Golgi elements in the form of irregular masses or granules.

In the animals under investigation both in monkey and dog the author observed that the Golgi appartus to begin with is situated at the juxta-nuclear position. In the case of monkey, the Golgi appartus consists of Golgi bodies of various forms, i.e. granules, dust-like particles, comma-shaped structure and irregular masses. As the oocytes advance in age the Golgi bodies are thrown out of yolk-nucleus area leaving a clear space, which may be termed as 'archoplasm.' In the case of dog the Golgi elements of various shapes are seen packed in the juxta-nuclear position. Later on the Golgi bodies start migrating from this position and an 'archoplasm' free from Golgi elements becomes visible.

Regarding the Golgi apparatus, the author is now in a position to say that the net-work as described by Weigl,[33] Rio Hortega,[29] Cattaneo,[11] and Clement[12] are nothing but artefacts due to excessive precipitation of silver or osmium. In the light of his observation the author believes that the Golgi elements are of polymorphic nature in the female germ cells of mammals.

According to Parat,[25-27] the Golgi bodies are nothing but vacuome. The classical Golgi apparatus according to him is the result of the excessive precipitation of the silver and osmium inside the vacuoles and in the narrow spaces between them.

Baker[2-5] claims that silver and osmic methods are unreliable for the study of Golgi elements. In 1944 Baker studied the Golgi elements by means of his 'formal-sudan-black technique' and contended that the Golgi apparatus consists of 'dense lipoid-containing substance, diffuse lipoid containing substance,' 'neutral-red vaculoes' and 'the Golgi product.' In 1949 Baker modified his previous conception of the structure of the Golgi elements and reported 'that neutral red, especially when used at higher concentrations than that suggested on page 296, gives rise to pink or red vacuoles where none existed before.' Baker in 1951 stated that 'the classical Golgi apparatus consists of particles of osmium hydroxide or of silver deposited in or on various objects in various cells.' Palade and Claude,[28] Thomas[30-32] and Marshall[22] deny the existence of Golgi elements in cells.

Gatenby[15-17] in a series of papers showed that the Golgi apparatus exists as a definite cell-inclusion and is entirely different from the vacuome. Bhattacharya and Das[8] demonstrated the existence of Golgi bodies and the vacuome side by side in the young oocytes of pigeon. Gatenby, Moussa and Dosekan[18] in 1949 stated: 'R. H. Brown[9] showed that the neutral red vacuoles could be centrifuged away from the argentophile filaments of the Golgi apparatus, which thereafter did not appear different from what they were before; lastly the approach to the subject from the direction of the germ cells appeared clearly to indicate that there were two different categories of structures - neutral red globules and Golgi bodies.' In the female germ cells of monkey and dog the Golgi elements are polymorphic in nature and do not possess vacuoles. It is surprising that Baker considered that neutral red vacuoles are actually part of the Golgi apparatus.

Recently Gresson[21] reviewed the literature on the Golgi substance and states that 'Baker, Thomas [30] and Marshall[22] quite rightly stress the importance of examining living material, but in their papers there is a remarkable lack of reference to work with the phase-contact microscope that has produced evidence contrary to their beliefs on the nature of the Golgi material.'

In the light of the present investigation the author is convinced that the Golgi elements of the female germ cells are polymorphic in nature and have no connection with vacuoles. The picture as revealed by silver and osmic methods is identical with that obtained by Sudan Black B. He therefore holds his previous view[34,35] that the classical methods often give a true picture of Golgi elements, their form and disposition in the living cytoplasm, and there is no ustification whatsoever for changing the name of the Golgi bodies to 'Lipochondria' as suggested by Baker.[4]

References

1. O. E. Aykroyd, Z. zellforsch. u. Mikroskop. Anat. 27. Band, 5, Heft. (1937).
2. J. R. Baker, Quart. J. Microscop. Sci.,

85, (1944).
3. Ibid., Quart. J. Microscop. Sci., 90 (1949).
4. Ibid., Proc. Linnean Soc. London, 162, (1950).
5. Ibid., Nature., 168, (1951).
6. H. W. Beams, Anat. Record, 45, (1930).
7. H. W. Beams and R. L. King, Cytologia., 8, (1938).
8. D. R. Bhattacharya and R. S. Das, Nature, Novr. 2, (1929).
9. R. H. Bowen, Anat. Record, 32, (1926).
10. R. H. Bowen, Anat. Record, 35, (1927).
11. D. Cattaneo, Arch. Ital. Anat. Embriol., 12, (1914).
12. R. Clement, Alld. Univ. Studies., (1933).
13. J. B. Gatenby, Quart. J. Micr. Sci., 66,(1922).
14. J. B. Gatenby and J. H. Woodger, J Roy. Microscop. Soc., (1920).
15. J. B. Gatenby, Proc. Roy. Soc. London, 104, (1929).
16. J. B. Gatenby, Am. J. Anat., 48, (1931).
17. J. B. Gatenby, Am. J. Anat., 51, (1932).
18. J. B. Gatenby T. A. A. Moussa, and F. Dosekun, La Cellule., 53, facs. 1, (1949).
19. C. Golgi, Bull. Soc. Med. Pavia, (1898).
20. R. A. R. Gressen, Quart. J. Microscop. Sci., 75, (1933).
21. Ibid., La Cellule., 54, (1952).
22. A. J. Marshall, Science Progress, 157, (1952).
23. V. Nath, Z. Zellforsch., 13, (1931).
24. J. Nihoul, La Cellule., 37, (1927).
25. M. Parat, LK. C. R. Soc. Biol., 96, (1927).
26. Ibid., LK. C. R. Soc. Biol., 96, (1927).
27. Ibid., Assoc. Anatomistes Prague (2-4 avril), (1928).
28. G. E. Palade and A. Claude, J. Morphol., 85, (1949).
29. P. Rio Hortega, Trab. Lab. Invest. Biolog., tom II., (1913).
30. O. L. Thomas, Quart. J. Microscop. Sci., 88, (1947).
31. Ibid., Quart. J. Microscop. Sci., 89, (1948).
32. Ibid., J. Comp. Neurol., 95, (1951).
33. R. Weigl, Bull. Intern. Acad. Sci. Cracovie., B., (1912).
34. H. K. Yosufzai, La Cellule., 55 (1952).
35. Ibid., La Cellule., 55, (1953).