MORPHOLOGICAL STUDIES OF MITOCHONDRIA AND GOLGI APPARATUS IN THE GLYCOGEN-FREE AND GLYCOGEN-LADEN OVA IN THE RAT OVARIES

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Introduction

Since the presence of glycogen-free and glycogen-laden ova in the rat ovaries had been reported by the writer (2), the fluctuation in their number was subsequently observed during the estrous cycle, pregnancy and lactation, and further it was experimentally investigated under various dietary conditions (4 and 5). The results were that the number of glycogen-laden ova in the secondary and Graafian follicles decreases during pregnancy and lactation, while it is constant under various dietary conditions, but that an opposite situation happens with glycogen-free ova ; that is, their number increases during pregnancy and lactation, and it is affected by various dietary conditions. In those studies, however, the morphological features of mitochondria and Golgi apparatus were not investigated comparatively between the glycogen-free and glycogen-laden ova.

The present investigation dealt with the morphology of mitochondria and of Golgi apparatus in the glycogen-free and glycogen-laden ova of the rat ovaries.

Materials and Methods

Non-pregnant rats which possessed the normal estrous cycle were used to observe glycogen-laden ova, while pregnant rats were used for glycoge-free ova, because it is known by a previous investigation (5) that glycogen-laden ova are contained more in the ovaries of non-pregnant rats, whereas glycogen-free ova are abundantly contained in the ovaries of pregnant rats.

The ovaries from these animals were immediately fixed in Champy's fluid or Regaud's fluid to demonstrate mitochondria, in cadmiun-formalin or uranium-

formalin or cobalt-formalin for Golgi apparatus. The ovaries thus treated were embedded in paraffin, and cut serially at 3 to 4μ.

For the demonstration of mitochondria, the sections were stained mainly by Heidenhain's iron-hematoxylin method, and partially by Altman's method. For the Golgi apparatus the sections were treated mainly by Aoyama's method, and partially by Cajal's method or Da Fano's method.

To examine whether the ova stained by the methods to demonstrate mitochondria and Golgi apparatus were the ones which contained glycogen, the sections were further stained by the PAS method after the microscopical observation. On the other hand, to determine the exact distribution of glycogen in the ova, tissues treated by the freezing-drying technique were stained by the PAS method. The identification of glycogen was made by means of the salivary test at 37°C in an incubator.

Results

In the primordial follicle, the ovum, which possessed a spherical nucleus containing small chromatin granules and a large nucleolus, is separated from the adjacent interstitial tissue by a layer of flattened follicular cells. A small amount of mitochondria appear in the form of granules uniformly throughout the cytoplasm of the ovum, and also a small amount of it in the form of fine granules in the flattened follicular cells (Fig. 1). The Golgi apparatus is not found anywhere in the cytoplasm of the ovum, whereas it appears in the form of granular mass in the proximity of the nucleus of the follicular cells (Fig. 2). None of the ova in the primordial follicles contain glycogen.

In the primary follicle, the ovum increases in size, and its nucleus enlarges, and the follicular cells which were flat in the preceding stage also perform a progressive development getting thicker and becoming cuboidal in shape. The mitochondrial granules in the ovum increase in number being distributed evenly throughout the cytoplasm. The Golgi apparatus does not yet appear. The mitochondrial granules in the follicular cells also increase in number, and the Golgi apparatus forms a network of rodlets. The ovum in a follicle at this stage contains almost no glycogen.

In the secondary and Graafian follicles, the ovum continues to increase in size, becoming surrounded by zona pellucida which gradually becomes thicker. A large amount of mitochondrial granules are found in the perinuclear region (Fig. 3). For the first time, at this stage, the Golgi apparatus appears abundantly in the form of granules in the perinuclear region, and some Golgi apparatus is also distributed adjacent to the zona pellucida (Fig. 4). Sometimes, Golgi granules around the nucleus come together, exhibiting clumps (Fig. 5). In the follicular cells at these last two stages, a moderate amount of mitochondrial granules are found and the Golgi apparatus takes a form of massed granules.

When the ovum is nearly full grown in the enlarged Graafian follicles, the mitochondrial and Golgi granules spread throughout the whole cytoplasm, though they do not increase in their number (Figs. 7 and 8). Some ova at these last stages contain no glycogen, while others contain a small to a large amount of it. In those glycogen-laden ova, the glycogen deposition occurs at almost the same region as the mitochondria and Golgi apparatus do; that is, in the early stage of the growth of the ovum, a small to a large amount of glycogen appears primarily in the perinuclear region, along with the further growth of it, it comes to appear throughout the whole cytoplasm (Figs. 6, 9, 10 and 11). As for the genetic features of mitochondria and of Golgi apparatus, however, little differences are found between the glycogen-free and glycogen-laden ova.

In the atretic follicles, the ova exhibit various kinds of degenerating processes as have already been reported by the writer (3). The mitochondrial and Golgi granules which are distributed at random throughout the cytoplasm of these atretic ova gradually decrease in number during degeneration, and finally they disappear.

Discussion

In a previous paper (2), the writer reported that in the cytoplasm of the rat ovum glycogen was found in a crescent shape when the tissue was fixed with 95 per cent alcohol. In the present investigation, the ovaries were treated by the freezing-drying technique as it was necessary to determine the exact distribution of glycogen in the ovum, to observe the regional relations between the glycogen and mitochondria or Golgi apparatus. The results were as follows; glycogen granules were located only in the perinuclear region in a young ovum, while they were distributed evenly throughout the whole cytoplasm in a grown one. These facts show that the transposition of glycogen in the ovum which takes place during the application of such fixative as 95 per cent alcohol can be avoided by the application of the freezing-drying technique.

It is generally considered that mitochondria in a cell contain certain enzymes of Kreb's oxidation cycle and fatty acid oxidase, and that they undoubtedly reflect the activity of the cell by their distribution in it and by their changes in form and number (6). It is demonstrated, for example, that mitochondria in the liver are reduced in inanition (1).

In this investigation, it was found that mitochondria abundantly appear in the form of granules in the perinuclear region of the ovum in the secondary follicle, showing a high metabolic activity, whereas when the ovum is nearly full grown in the enlarged Graafian follicle, the mitochondrial granules are distributed evenly throughout the cytoplasm but they do not increase in

number, showing a low metabolic activity.

Golgi apparatus has been thought by many investigators to play an important role in cellular activities. It is also considered that in the female germ cells the Golgi apparatus takes part directly or indirectly in the formation of the fatty yolk during oogenesis (7). But the role of Golgi apparatus on the glycogen accumulation is almost unknown.

In this investigation, it was found that Golgi apparatus is located at the perinuclear region of the ovum in the secondary follicle, and afterwards when the ovum is nearly full grown it also spread throughout the whole cytopasm just as mitochondria.

In spite of the distribution of mitochondria and Golgi apparatus coinciding with that of glycogen during the growth of the ovum as described above, no differences were found in the morphology of mitochondria and Golgi apparatus between the glycogen-free and glycogen-laden ova. This result seems to demonstrate a fact that there is no direct relation, in the rat ova, between the morphology of mitochondria and of Golgi apparatus and the accumulation of glycogen.

Acknowledgment

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Summary

The results obtained in this investigation are summarized as follows:

In the ovum in the primordial follicle, a moderate amount of mitochondrial granules appear throughout the cytoplasm; they increase in number in the primary follicle. But Golgi apparatus is not found in the ovum in the primordial or primary follicle. In the ovum in the secondary and Graafian follicles, a large amount of mitochondrial and Golgi granules are located in the perinuclear region. When the ovum is nearly full grown in an enlarged Graafian follicle, the mitochondrial and Golgi granules spread throughout the whole cytoplasm, but they do not increase in number. In an atretic ovum, mitochondrial and Golgi granules, which at first are distributed at random throughout the cytoplasm, decrease gradually in number during the degeneration, and they finally disappear.

Ova in the primordial and primary follicles contain no glycogen; while in the secondary and Graafian follicles, both glycogen-free and glycogen-laden ova appear. In those glycogen-laden ova, the deposition of glycogen occurs at almost the same region as that of mitochondria and Golgi apparatus; that is, it appears at the perinuclear region during the early growth of the ovum, and evenly spreads out in a nearly full grown ovum. As for the morphological

features of these cellular elements, little differences are found between the glycogen-free and glycogen-laden ova.

References

1) Bourne, G. (1951). Cytology and Cell Physiology, p. 232, Oxford Univ. Press, London.
2) Ishida, K. (1952). Tohoku J. Agr. Res., **3**, 39.
3) ,, (1953). Ibid., **4**, 29.
4) ,, (1959). Ibid., **9**, 127.
5) ,, (1959). Arch. hist. jap., **16**, 69.
6) Maximow, A. A. and W. Bloom (1952). Textbook of Histology, p. 14, W.B. Saunders Co., Philadelphia and London.
7) Nath, V. and R. M. Dhawan (1954). Res. Bull. Panjab Univ., Nos. 59-60, p. 153.

Plate 1

Explanation of Figures

Fig. 1. Ovum in the primordial follicle. Heidenhain's iron-hematoxylin stain. ×1000.
A small amount of mitochondria are seen in the cytoplasm.

Fig. 2. Ovum in the primordial follicle. Aoyama's method. ×1000.
Golgi apparatus is not seen in the cytoplasm.

Fig. 3. Ovum in the small Graafian follicle. Heidenhain's iron-hematoxylin stain. ×200.
A large amount of mitochondrial granules are seen in the perinuclear region.

Fig. 4. Ovum in the small Graafian follicle. Aoyama's method. ×400.
Golgi granules are seen abundantly in the perinuclear region, and some of them are distributed adjacent to the zona pellucida.

Fig. 5. Ovum in the small Graafian follicle. Aoyama's method. ×400.
Golgi granules are seen in the perinuclear region, exhibiting clumps.

Fig. 6. Ovum in the small Graafian follicle. PAS stain after Aoyama's method. ×400.
Glycogen granules are seen in the perinuclear region.

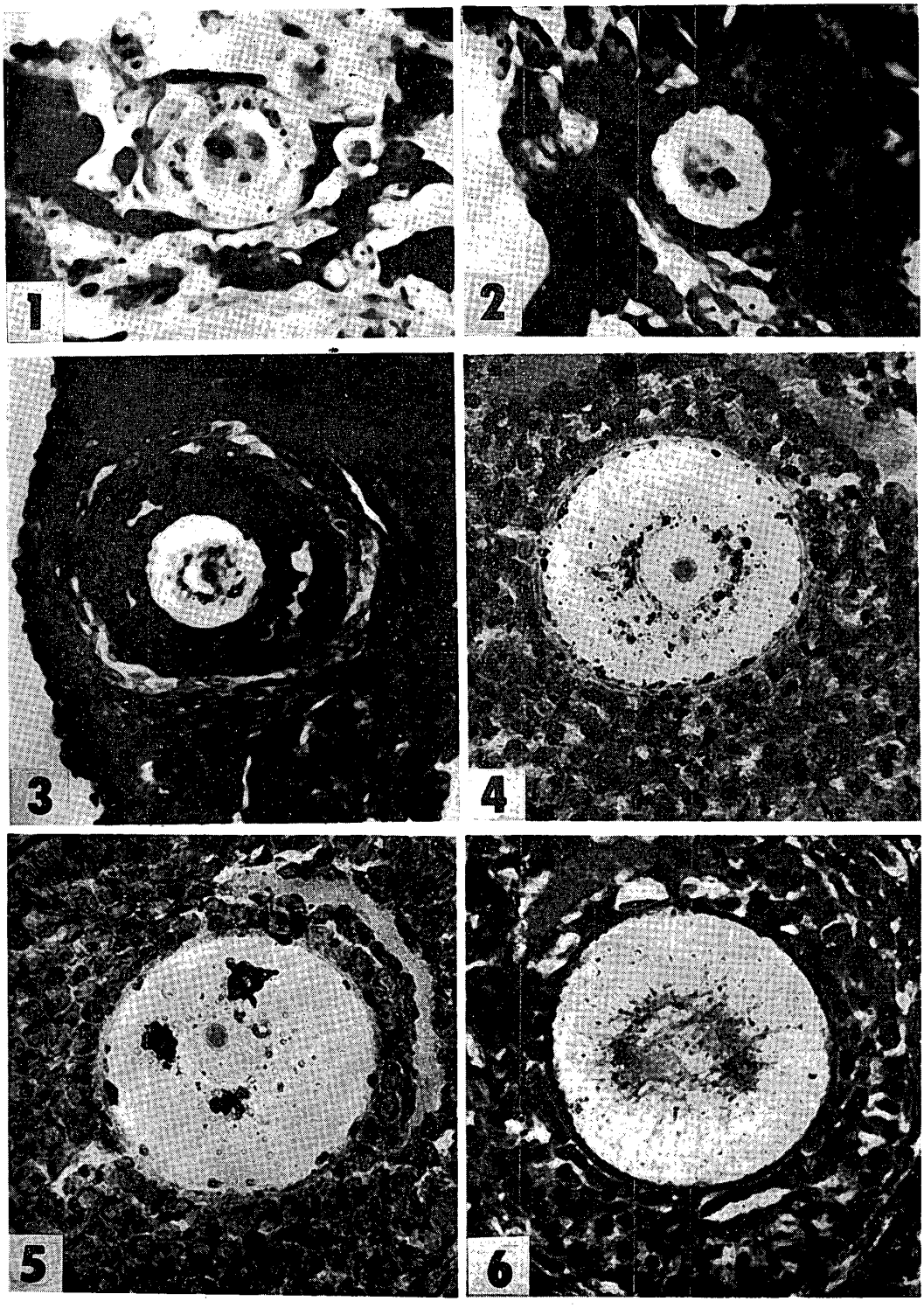
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Plate 2

Explanation of Figures

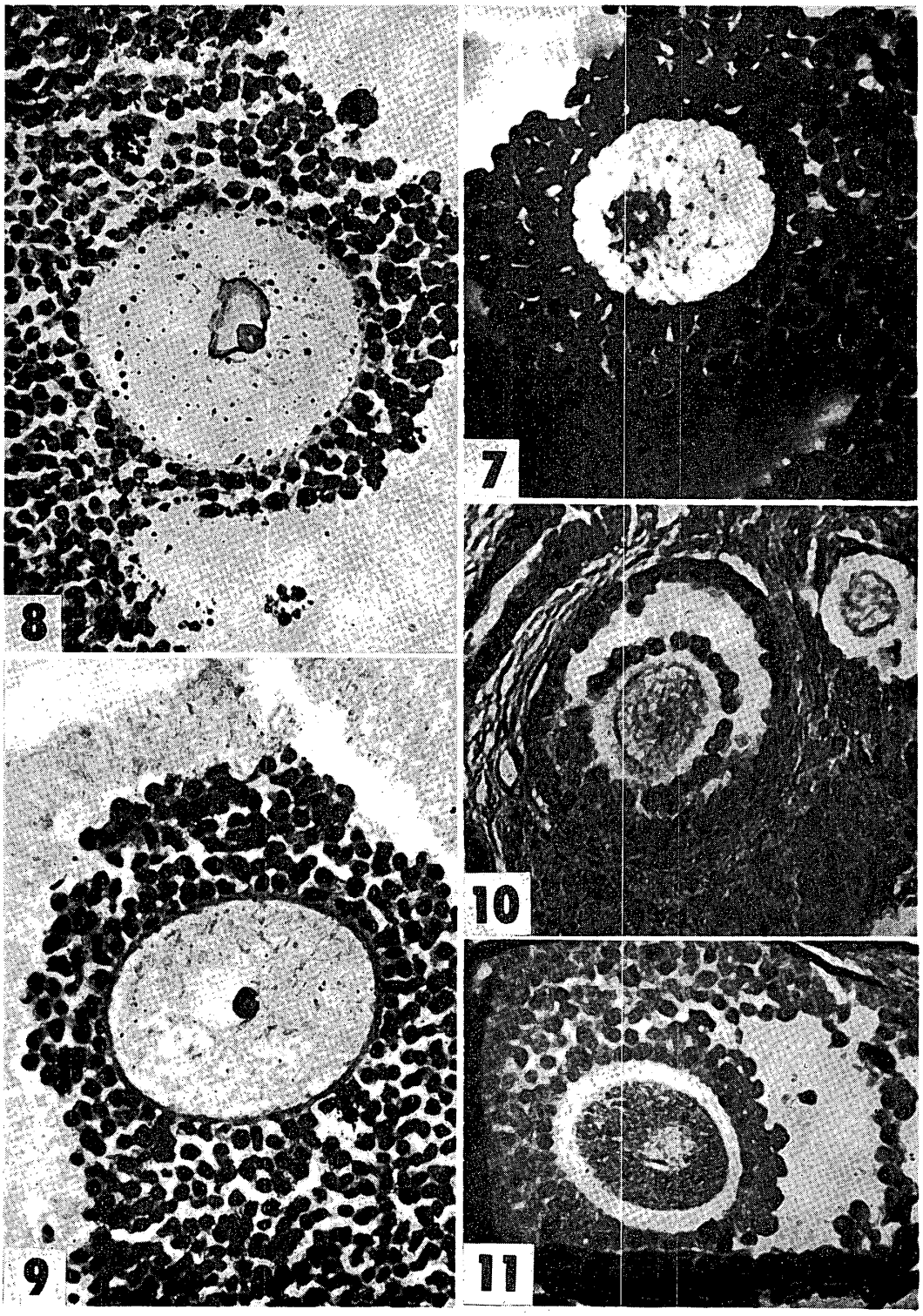
Fig. 7. Ovum in the Graafian follicle. Heidenhain's iron-hematoxylin stain ×300.
Mitochondrial granules are seen throughout the cytoplasm.

Fig. 8. Ovum in the Graafian follicle. Aoyama's method. ×400.
Golgi granules are seen throughout the whole cytoplasm.

Fig. 9. Ovum in the Graafian follicle. PAS stain after Aoyama's method ×400.
Glycogen granules are seen throughout the whole cytoplasm.

Fig. 10. Ovum in the small Graafian follicle. PAS stain after freezing-drying technique. ×300.
Glycogen granules are seen in the perinuclear region.

Fig. 11. Ovum in the Graafian follicle. PAS stain after freezing-drying techinque. ×300.
Glycogen granules are seen throughout the whole cytoplasm.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