## GOLGI COMPLEX AND MITOCHONDRIA IN THE SECRETORY CYCLE OF THE CHROMAFFIN CELL IN THE RENEWAL PHASE

N. L. Yushkevich UDC 612.459.014.21

The role of the Colgi complex, the mitochondria, and the other cell structures in the secretory process has been the subject of investigation for many years. The changes in the organoids of the gland cell during the period of active function are extensively described in the literature [1, 4, 5, 8-10, 12, 14, 15], but there is no information about their changes in the chromaffin cells. In the extensive study undertaken by Bennet [7] on cats, no significant morphological changes were found in the mitochondria of the chromaffin cells, nor was any conclusion drawn regarding their participation in the secretory process. This applies still more to the Golgi complex, described by Bennet as a clump of rigid shape lying next to the nucleus.

In a previous investigation the author [6], studied the dynamics of the structure of the Golgi complex and mito-chondria in the chromaffin cells of the adrenal in rats during the phase of active secretion. In this paper, the changes are described in the same organoids—the Golgi complex and mitochondria—during the active renewal of the catecholamines, and an interpretion of these changes is given. A parallel investigation was made of the same material by É. Kh. Priimak [3], using the electron microscope.

The gland cells are known to function asychronously. This is inconvenient when observations are made on the trend of changes in the organoids. By stimulating the process of secretion by insulin, maximal emptying of the gland is obtained as a result of the massive outpouring of adrenalin, and as a result the phases of secretion are brought closer together. Administration of glucose neutralizes the action of insulin and brings about the large scale renewal and accumulation of the catecholamines.

## EXPERIMENTAL METHOD

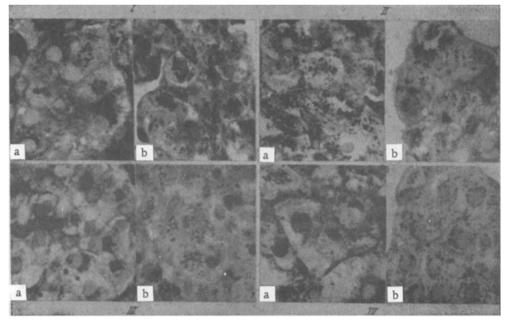
Experiments were carried out on male rats weighing 150-200 g. The animals were given a subcutaneous injection of insulin in a dose of 15-20 units/100 g body weight. When signs of shock appeared (exophthalmos, convulsions, etc.), the rats were given two or more subcutaneous injections of 1-2 ml of 10% glucose to bring them out of the state of shock. The animals were sacrificed at various times after injection of insulin: 9, 12, 24, and 48 h, and 2, 3, and 6 days. The adrenals were fixed in a mixture of potassium bichromate (histochemical reaction for adrenalin and noradrenalin) and potassium chromate (for detection of total catecholamines); in a 10% solution of potassium iodate [11], (to detect noradrenalin); in Champy's fluid with treatment by the Kopsh-Kolachev and Da Fano methods (to detect the Golgi complex); and in the fluids of Régaud and Colster, followed by staining by Altman's method (to detect the mitochondria).

## EXPERIMENTAL RESULTS

Histochemical reactions for adrenalin and noradrenalin were used as controls for determining the phases of secretion at different times of the experiment. The chromatin reaction remained at the "shock" level 9 and 12 h after injection of the insulin [6], i.e., nearly all the cells containing adrenalin had expelled their content and did not give a positive histochemical reaction.

Insulin has no stimulant action on the secretion of noradrenalin, and the "noradrenalin" cells retained their whole range of colors from pale yellow to bright yellow-brownish throughout the experiment. After 24 h the positive chromatin reaction gradually increased, and on the 2nd and 3rd days it was found in all the new "adrenalin" cells, while after 6 days the whole medulla of the adrenal stained just as intensively as in the control rats.

A. N. Severtsov Institute of Morphology of Animals, Academy of Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 12, pp. 105-107, December, 1966. Original article submitted March 26, 1965.



Changes in organoids of rat adrenal chromaffin cells in the phase of renewal of catecholamines, 9 (I), 12 (II), 24 (III) h and 3 days (IV) after injection of insulin. a) Golgi complex (fixation in Champy's fluid with treatment by the Kopsh-Kolachev method, counter staining of the nuclei with hemalum); b) mitochondria. Objective 60x, ocular 10x.

After 9 and 12 h (see figure Ia, and IIa) changes similar to those in the animals in a state of shock were observed in the Golgi complex; progressive hypertrophy of the complex, with loosening and spread of its elements over the cytoplasm. After 24 h (see figure, IIIa) the Golgi elements scattered throughout the cytoplasm seemed to be drawn toward the nucleus. This state of the organoid persisted on the 2nd and 3rd days (see figure, IVa). Later cells were found in which the Golgi structures were condensed and grouped close to the nucleus. On the 6th day the zone of the Golgi complex in most of the "adrenalin" cells was much smaller and looked like a small basket or cap, formed from the osmiophilic bands and lying next to the nucleus. This form was typical for the cells of the control gland.

Many mitochondria were present in the chromaffin cells 9 and 12 h after injection of insulin (see figure Ib, and IIb), and they varied in size. Large, round mitochondria were commonest, scattered irregularly throughout the cytoplasm of the chromaffin cells. Similar changes were observed in the animals in a state of shock [6]. After 24 h (see figure IIIa), the number of mitochondria was somewhat smaller. They were distributed more or less uniformly throughout the cytoplasm. After 2 and 3 days (see figure IVb), the mitochondria were smaller and fewer in number. They were often grouped around the nucleus. After 6 days the shape and distribution of the mitochondria were close to normal, they were smaller, more varied in shape (round, shaped like threads or rods), and they were situated mainly around the nucleus.

The results of the biochemical estimation of adrenalin in the rat adrenals [13] showed that 9 h after injection of insulin the maximal emptying of the gland takes place. The low level of adrenalin persits until 48 h, after which its content rises appreciably and is fully restored by the 6th day.

According to the author's observation, adrenalin synthesis does not cease during shock [6], and continues 9 and 12 h after injection of insulin, as shown by the histochemical reaction indicating the presence of a few, weakly stained "adrenalin" cells at these times. This is confirmed by the wide dispersion of numerous tiny vesicles with catecholamines during electron-microscopic investigation of these cells [2]. The state of the organoids also supports the continuing synthesis of adrenalin: the increase in the number and volume of the mitochondria and the hypertrophy and spread of the Golgi elements throughout the cytoplasm. The hypertrophy of the Golgi apparatus, observed particularly clearly in the present experiment after a period of 12 h, may be explained on the basis of the most widely held view of the significance of the Golgi structures, namely, to accumulate newly formed products in the cell [5, 9, 12]. The intensive hormone production requires a wider distribution of the structural components condensing secretory products on or into themselves. The increase in the number and size of the mitochondria after 9 h and 12 h by comparison with these indices in the "shock" state of the glands can be explained on the basis of

modern views [14] of the increasing demand by the "adrenalin" cells for chemical energy for the resynthesis of adrenalin. The decrease in this demand when sufficient secretion has accumulated in the cell leads to a decrease in the number and size of the mitochondria in the subsequent period of the experiment.

Complete morphological and functional recovery of the gland occurs after 6 days.

This investigation thus showed that during the active renewal of adrenalin in the adrenal medulla of the rat, taking place after stimulation of secretion of the gland by insulin, changes are observed in the Golgi complex and mitochondria. The changes in the organoids take the form of loosening of their structure, hypertrophy and wide-spread fluttering of the Golgi elements throughout the cytoplasm, and by an increase in the number and size of the mitochondria occupying the greater part of the cytoplasm.

## LITERATURE CITED

- 1. B. V. Kedrovskii, Uspekhi Sovr. Biol., 23, No. 3, 375 (1947).
- 2. É. Kh. Priimak, Doklady Akad. Nauk SSSR, 162, No. 5, 1171 (1965).
- 3. É. Kh. Priimak, Doklady Akad. Nauk SSSR, 166, No. 6, 1443 (1966).
- 4. I. B. Tokin, Arkh. Anat. Gistol., Émbriol., No. 12, 3 (1963).
- 5. R. V. Khesin, Biochemistry of Cytoplasm [in Russian], Moscow (1960).
- 6. N. L. Yushkevich, Byull. Éksp. Biol., No. 3, 108 (1966).
- 7. H. Bennet, St. Am. J. Anat., 69 (1941), p. 333.
- 8. A. B. Dalton, in the book: The Cell, New York, 2 (1961), p. 603.
- 9. M. G. Farguhar, Trans. New York Acad. Sci., 23 (1961), p. 346.
- 10. L. Herman and P. I. Fitzgerald, Trans. New York Acad. Sci., 23 (1961), p. 332.
- 11. N. A. Hillarp and B. Höckfelt, Acta. Physiol. Scand., 30 (1953), p. 55.
- 12. G. Hirsch, Naturwissenschaften, 47 (1960), p. 25.
- 13. B. Höckfelt, Naturwissenschaften, 25 (1951), p. 92.
- 14. O. Lindberg and L. Ernster, in the book: Problems in Cytophysiology [Russian translation], Moscow (1957), p. 111.
- 15. A. B. Novikoff, in the book: The Cell, New York, 2 (1961), p. 299.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. Some or all of this periodical literature may well be available in English translation. A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.