# ADRENERGIC INNERVATION AND DISTRIBUTION OF CHOLINESTERASE IN THE HUMAN OVARY

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It is shown by the methods of Falck and Hillarp and of Karnovsky that the adrenergic innervation and the distribution and activity of cholinesterase in the ovaries of women aged from 20-45 years are variable, evidently on account of highly complex cyclic processes in this organ. The innervation is rich, the generative elements of the ovary (follicles and corpora lutea) being surrounded by dense nerve plexuses, and as they undergo regression the number of nerve fibers decreases.

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Histochemical investigations of the autonomic nervous system in various organs and tissues has received considerable attention and literature on this subject is extensive. However, the innervation of the human ovary remains almost completely unstudied by means of histochemical methods, as is mentioned in a recent publication [10].

The first part of this investigation was devoted to the study of the internal innervation of the ovary using the usual neurohistological methods [3-6]. To obtain a complete picture of the innervation of the human ovary, a combination of neurohistological and the latest histochemical methods is essential.

The object of the present study was to determine the adrenergic innervation and distribution and activity of cholinesterase in the human ovary.

## EXPERIMENTAL METHOD

Altogether 25 ovaries of normal structure, confirmed macroscopically and microscopically, from women of childbearing age (from 20-45 years) were investigated.

To determine catecholamines the histochemical fluorescence method of Falck and Hillarp [7-9], modified by Govyrin [1] was used. Sections of the ovaries were cut to a thickness of 15-20 $\mu$ and freeze-dried. The dried sections were kept for 1 h in formaldehyde vapor at 80°C, and then embedded in polystyrene and studied with the ML-2 luminescence microscope. The postganglionic sympathetic structures, especially their terminal portions, were readily distinguished by their high concentration of catecholamines from their characteristic bright-green fluorescence, given by condensation products of catecholamines with formaldehyde.

To detect the distribution and activity of cholinesterase, Karnovsky's method [11] was used. By this method both true cholinesterase and pseudocholinesterase can be determined by the use of the specific substrates acetylthiocholine iodide and butyrylthiocholine iodide; pH of buffer 6.0.

## EXPERIMENTAL RESULTS

The adrenergic innervation is rich in all parts of the ovary. In the hilum of the ovary, among the loose connective tissue consisting of collagen, elastic, and a few bundles of smooth muscle fibers and large numbers of blood vessels, there are numerous thin and thick nerve trunks and nerve fibers. They lie mainly in the adventitial layer of the blood vessels, and also on the surface of the muscular layer of the arteries. The nerve trunks form very extensive small-looped plexuses; a similar picture was seen also in sections stained with methylene blue, although the results obtained by the method of Falck and Hillarp show con-

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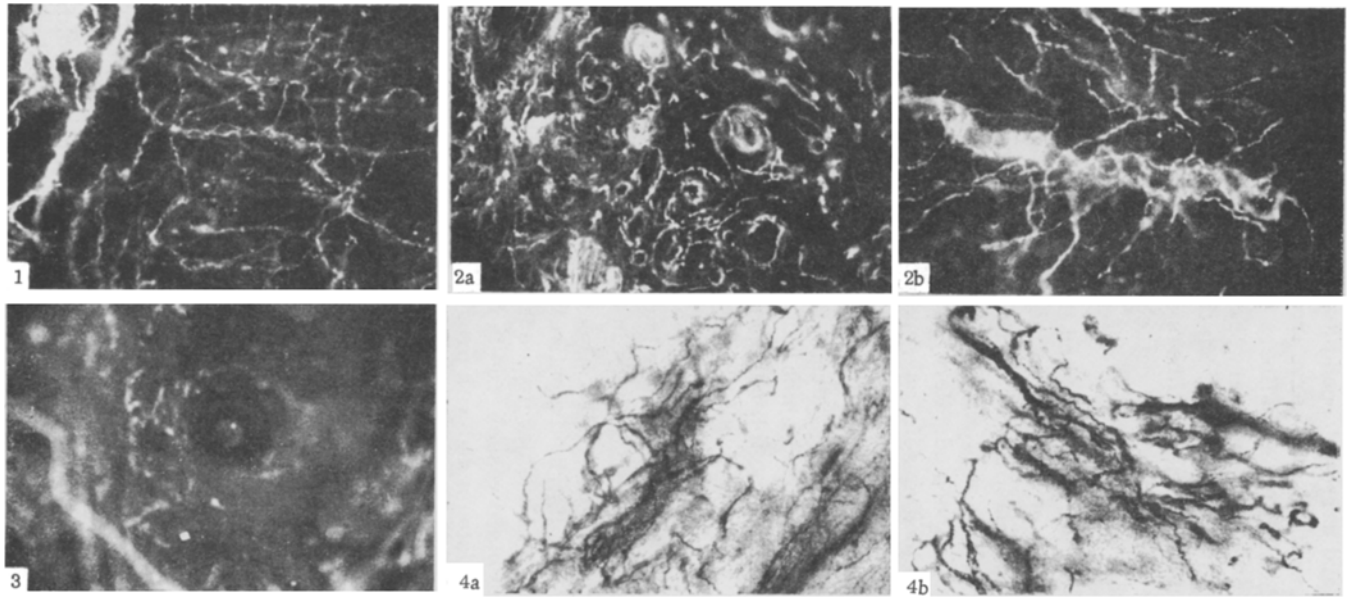


Fig. 1. Ovary of woman aged 30 years. Adrenergic nerve plexus located at the border between the hilum and medullary layer of the ovary, 70×.

Fig. 2. Ovary of woman aged 21 years; medullary layer. a) Blood vessels of different calibers, surrounded by adrenergic nerve fibers, 70×; b) artery surrounded by a thick network of adrenergic nerve fibers, 140×.

Fig. 3. Ovary of woman aged 21 years. Cortical layer, repening follicle around which run adrenergic nerve fibers, 280×.

Fig. 4. Ovary of woman aged 30 years. Medullary layer. a) Nerve plexus. Nerve fibers with high acetylcholinesterase (ACE) activity. Karnovsky's method, 116×; b) nerve ending of plexiform type. High ACE activity. Karnovsky's method, 240×.

clusively that these nerve fibers are adrenergic. One such nerve plexus located at the border between the hilum and the medullary layer of the ovary can be seen in Fig. 1, in which the thin nerve trunks and nerve fibers run sometimes more deeply, sometimes more superficially, interweaving with each other and forming a dense plexus.

The medullary layer, occupying the central part of the ovary, is richly supplied with blood vessels. Arteries and veins twist and ramify in it, forming anastomoses. Adrenergic nerve fibers give a clear reaction for catecholamines form both wide- and narrow-looped plexuses in this layer. The relationships between the blood vessels and adrenergic nerve fibers and nerve trunks are particularly clearly marked in this layer. A specimen in which blood vessels of different calibers can be seen, surrounded by nerve fibers, is illustrated in Fig. 2a; part of the medullary layer of the ovary through which runs an artery surrounded by a dense network of adrenergic nerve fibers is shown in Fig. 2b; these fibers ramify and interweave with each other to form plexuses. This rich supply of adrenergic fibers and their close connection with the blood vessels are evidently explained by the important role of catecholamines in the neurohumoral regulation of this endocrine gland.

The cortical layer of the ovary has a complex structure, and it includes the generative elements of the organ; the stroma differs from the usual connective tissue in the presence of numerous spindle-shaped cells lying among the collagen fibers and the few elastic fibers. This layer of the ovary has a rich adrenergic innervation, and the view of Jacobowitz and co-workers [10] that the human ovary contains only a moderate number of adrenergic fibers cannot be accepted. Here, just as in other parts of the ovary, adrenergic nerve fibers run along the course of the blood vessels and separately from them, forming plexuses of different degrees of complexity. Nerve trunks ramify and surround the follicles in different stages of development and regression. As the follicle ripens, the number of nerve fibers increases. Characteristically, numerous vessels run around the follicles, with nerve fibers in their adventitia. A section through the cortical layer of the ovary with a ripening follicle around which run adrenergic nerve fibers is shown in Fig. 3. No fibers could be found among the cells of the follicular epithelium. A moderate number of adrenergic fibers was observed in the stretic follicles (cystic form), mainly along the course of the blood vessels. In the obliterative form of atresia of the follicles, solitary nerve fibers were seen.

The adrenergic innervation of the corpora lutea was studied at different stages of development and regression, and also during pregnancy. Adrenergic nerve fibers in the corpora lutea are localized mainly in the adventitia of the blood vessels. In the stages of proliferation and, in particular, vascularization, the number of nerve fibers is increased and they are distributed entirely along the vessels; no nerve fibers could be seen actually among the lutein cells. With the development of the corpus luteum its central cavity becomes filled with connective tissue in which blood vessels run in different directions, surrounded by thin nerve fibers. During regression of the corpus luteum and with progressive hyalinization, fewer and fewer adrenergic nerve fibers are seen.

In the true corpora lutea, by comparison with the corpus luteum of menstruation, in the early periods of pregnancy the structure of the nervous apparatus is largely preserved, although during pregnancy the reaction of the corpora lutea to catecholamines is weakened.

No chromaffin cells were found in the human ovaries.

The human ovary is thus characterized by a rich adrenergic innervation, which undergoes morphological and functional changes in relation to the cyclic processes taking place in this organ.

The distribution and activity of cholinesterase in the nerve structures of the human ovary were studied by means of Karnovsky's thiocholine method. It was found that all layers of the human ovary contain nerve fibers possessing cholinesterase activity, and that the nerve fibers possessing high activity of this enzyme are distributed unevenly: in some places they form dense plexuses, while in others only solitary nerve fibers are present. A section through the medullary layer of the ovary, containing a nerve plexus in which the nerve fibers exhibit high cholinesterase activity, is illustrated in Fig. 4a. A second distinctive feature is that sections incubated concurrently with butyrylthiocholine did not give a reaction, i.e., the results suggest that specific cholinesterase is predominant in the nerve structures of this organ.

However, the cholinergic nerves in the ovary were fewer in number than the adrenergic, in agreement with Jacobowitz's findings [10].

The generative elements of the ovary, the follicles and corpora lutea, were surrounded by dense nerve plexuses with well-marked enzyme activity. During regression of the follicles and corpora lutea the number of nerve fibers decreased. Solitary nerve fibers ran among the theca-lutein cells. Cholinesterase activity in the nerve fibers was much weaker in the corpora lutea during pregnancy. Nerve endings are found in all part of the ovary, but more frequently in the cortical and medullary layers.

Receptors of plexiform type, similar in structure to the receptors described by Plechkova [2] in the uterus, urinary bladder, and other organs, were found. A nerve ending of plexiform type with very high enzyme activity, located in the connective tissue of the medullary layer of the ovary, is illustrated in Fig. 4b.

Histochemical methods of investigation thus greatly extend our knowledge of the nerve structure of the ovary. Existing experimental data were confirmed, showing that the human ovary possessed both adrenergic and cholinergic innervation. The enzyme reactions in the nerve tissue of the ovary are marked by their variability, evidently the result of highly complex cyclic processes.

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