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HISTOLOGICAL AND HISTOCHEMICAL ANALYSIS OF THE THYMUS IN TAILLESS AMPHIBIANS

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Literature contains numerous reports on the thymus of higher vertebrates while no detailed studies concerning this organ in lower vertebrates, such as the amphibians, have so far been made except for BARGMANN's monograph [12] on endocrine glands, which deals in some detail with the anatomy and histology of the amphibian thymus.

The thymus of the Anura, a glandular derivative of the first branchial arches, is of epithelial origin. It is a paired organ which arises by the proliferation of cells from the dorsal ends of the hyomandibular and branchial pouches. After full development, it is situated posterior to the tympanic membrane, behind the eye and ventral to the depressor mandibulae muscle. The organ measuring 3 mm by 1.5 mm, has an oval shape. It reaches its highest development in young individuals, and degenerates with advancing age as it does in higher vertebrates.

Since the thymus of amphibians has only been studied in its anatomical aspects, while its anatomical structure is hardly known, we have undertaken histological and histochemical investigations in the hope that our results might give rise to further research concerning the ontogenesis and phylogenesis of the thymus.

Material and method

Young, sexually mature frogs (Rana ridibunda, Rana esculenta, Bombina bombina) were used. The intention was to keep the animals under conditions as natural as possible, so they were kept in the open with freedom to stay both in and out of water. The investigations to be reported were performed between March and September, i. e. during the active phase of the animals.

Since the thymus is emptied rapidly under ether anaesthesia, it was deemed necessary to kill the animals by decapitation. The intensity of evacuation of the thymus during anaesthesia corresponds, according to the literature, to that observed after a starvation of 6 days [1]. The rapid evacuation of the thymus is well known as a symptom of the alarm reaction [10, 11].

A total of 150 animals was used so that 300 thymuses could be studied histologically and histochemically. Several fixing agents, such as Carnoy's fluid, calcium-formol and picric acid-alcohol were applied according to the method of staining, while fresh, frozen sections were used for certain histochemical tests. Methylbenzoate-paraffin served as embedding medium in every case. Serial sections 5 μ thick were made in 6 different planes (4 sections per plane) so as to be able to study the histology of the organ in all details.

The sections to be stained with Bartha's haematoxylin-eosin were fixed in Carnoy's fluid. Those to be dyed with azan were fixed in calcium-formol because Carnoy's fluid contains acetic acid which causes the epithelial reticulum of the thymus to shrink.

The presence of complex carbohydrates was tested by Schiff's periodic reaction (PAS) and also by the metachromasia following staining with toluidine blue. Fixing was performed with Carnoy's fluid in both cases.

Feulgen's reaction and methylgreen pyronine were employed for the detection of nucleic acids. The PAS reaction served as control. Fixing was performed with Carnoy's fluid.

Oil red 0 and the plasmal reaction were used for the study of lipids. Oil red 0 was dissolved in isopropyl alcohol. The material was fixed in calcium-formol and sectioned in the frozen state.

Of vitamins, we tested the presence of vitamin C. After mincing the thymus was kept without fixing in 5.7 and 10 per cent solutions of silver nitrate containing 5 per cent glacial acetic acid for 1 to 3 hours. It was then dehydrated, embedded in paraffin and sectioned.

Acid phosphatase was demonstrated according to Gomori, succinic acid dehydrogenase was tested with neotetrazonium chloride (NTC) in unfixed frozen sections.

Results

In view of the scarcity of data concerning the histological structure of the amphibian thymus, it was necessary to stain it first with haematoxylin-eosin in order to ascertain the basic structure so as to be able to evaluate subsequent results to be obtained with the various specific staining methods and histochemical procedures.

The thymus of the examined animals was found to be surrounded by a capsule of connective tissue. Both this capsule and the thymus itself contained numerous chromatophores. The organ consists of cortex and medulla, a pattern similar to the thymus of mammals. However, the similarity is restricted to the mere existence of these two components, while their arrangement, and so also the structure of the amphibian thymus, does not resemble that seen in mammals. While the mammalian thymus is lobated, no regular lobes divided by connective tissue were observed in amphibians. The capsule forms septa which project into the cortex and divide it into irregular lobules. The latter are bounded by septa on two sides only, while, on the third, the cortical portion passes into the medulla. Lobulation was observed over the entire cortex. The latter contained mostly thymocytes, while epithelial reticular cells seemed to constitute the bulk of the medulla. There was a certain number of thymocytes in the medullary substance which moreover contained a great number of large, strongly eosinophilic structures measuring from 20 to 30 μ in diameter; they corresponded to Hassall's corpuscles.

The epithelial reticulum consists of large, weakly staining cells; they are provided with processes, and their cytoplasm has a filamentous, foamy structure. These cells are to be found in the cortex as well, and the basic framework of the thymus evidently consists of epithelial reticulum also in the Anura. The medulla contains vessels, mostly capillaries.

Our observations have been grouped according to whether they concern the cortex, medulla or Hassall's corpuscles.

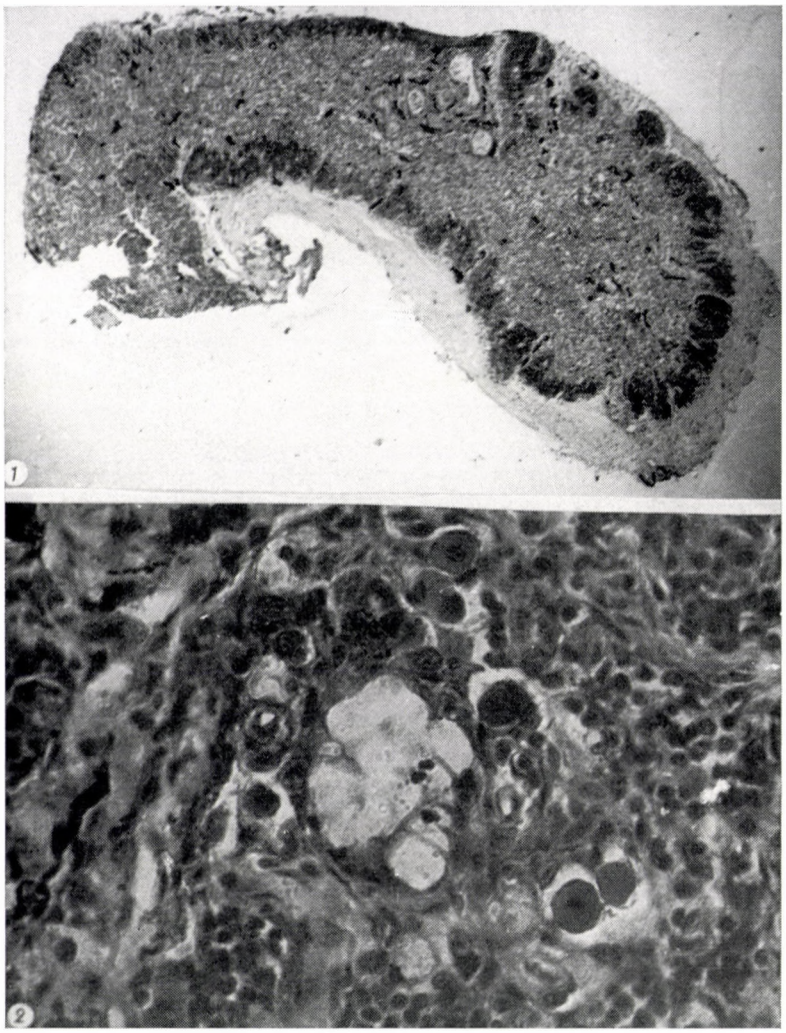

Fig. 1. Microscopical structure of the thymus in anuran amphibians, the cortex and medullar pattern is similar to that of mammalian thymus. ×40 (Low power magnification)
Fig. 2. Large eosinophilous cells containing nuclei rich in chromatin as well as a cysta surrounded by flattened epitheloid cells are to be seen. ×400

Cortex

This part of the thymus is richest in cells and constitutes about a third of the thymus tissue. The cells to be found here are small thymocytes measuring 2 to 3 μ in diameter. They are rich in chromatin. Stained with azan, the cortical substance as also the connective-tissue capsule and its septa revealed the presence of collagenous fibres which formed small bundles in the capsule. It was chiefly here that polysaccharides were observed in the thymocytes. Metachromatic staining with toluidine blue revealed a small number of violet-

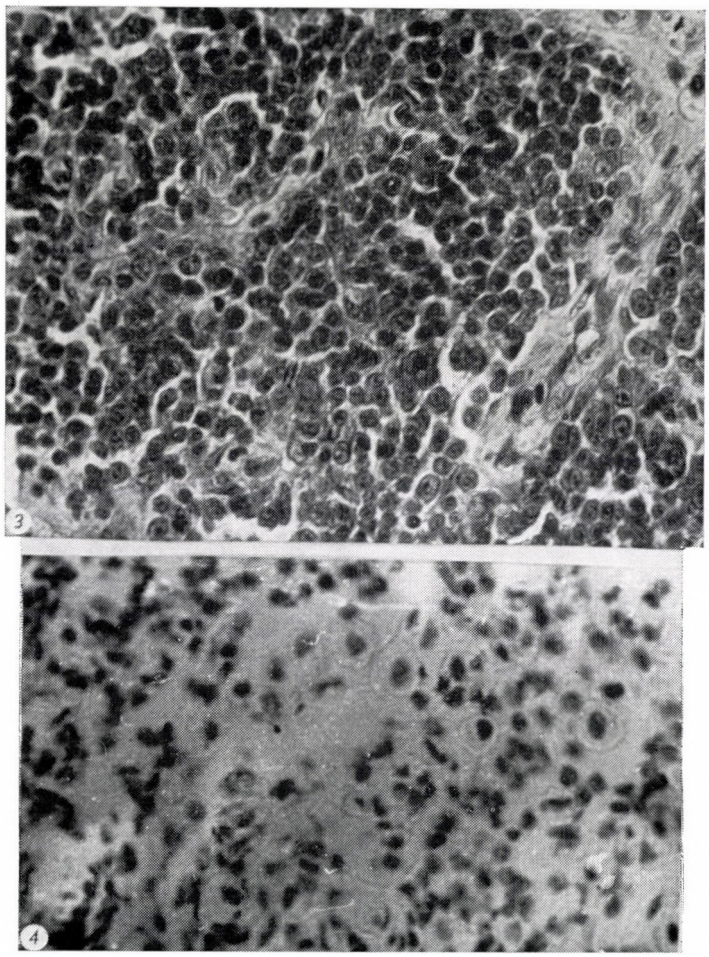

Fig. 3. Thymus cortex with a septum of connective tissue without forming of regular lobes; thymocytes, lymphocytes and cells of the epithelial reticulum are to be seen, all rich in RNA. ×200 —

Fig. 4. Feulgen reaction in the medulla of thymus in anura. ×200. —

reddish cells in the connective-tissue capsule. Their colouration was diffuse, and they resembled mast cells as seen in the subcapsular zone of the thymus of higher vertebrates. The thymocytes and some epithelial reticular cells showed RNA activity.

Pyroninophilia was weaker than in the medulla. The cells took both methyl green and pyronine. We observed pyroninophilia in the nucleolus and nuclear membrane of the epithelial cells as also in the cell membrane of the thymocytes. It follows that, in the cortical substance, RNA is contained in the thymocytes, lymphocytes and the epithelial reticular cells alike.

The cells of the connective-tissue septa, their cell membrane and nuclear membranes in particular, gave a positive acid phosphatase reaction.

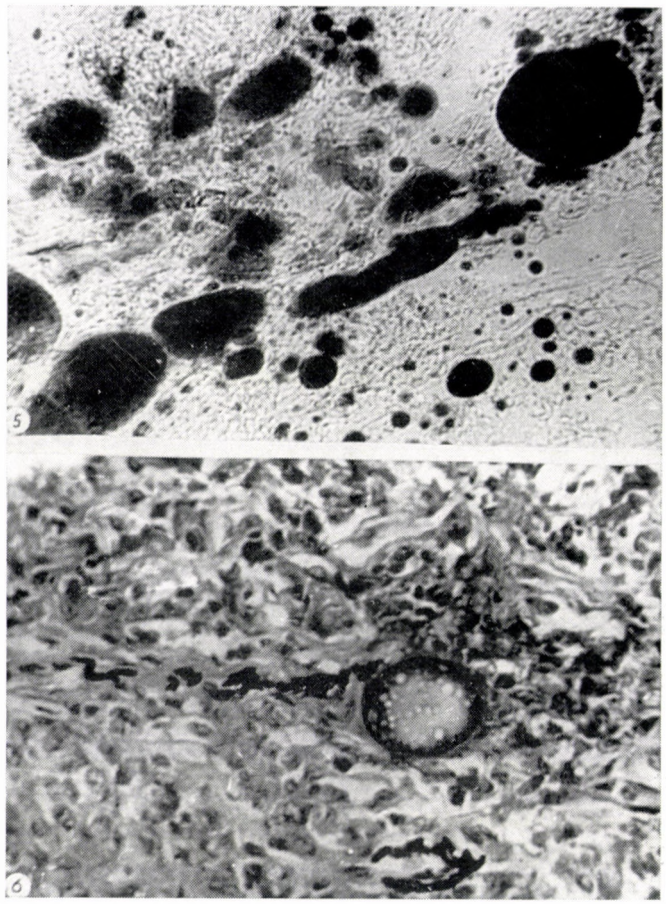

Fig. 5. Droplets and granules of lipids in the medulla; oil red O staining. ×200. — *Fig. 6.* Hassall's corpuscles filled with PAS positive substance and vacuoles. ×400.

Medulla

This area contains most of the epithelial reticular cells of the thymus, furthermore Hassall's corpuscles, and a few thymocytes. Histochemical reactions were usually more pronounced than in the cortex. Oil red 0 revealed the presence of unevenly distributed lipids. Sections from the middle plane of the organ seemed to be richest in lipids. They appeared in the form of droplets and granules. Freshly frozen sections were used for the reaction: since their thickness varied between 10 and 15 μ, it was not possible to demonstrate the lipids in the cells themselves.

The epithelial reticular cells gave a positive plasmal reaction which seemed to be homogeneous over the entire medullary substance, a phenomenon indicative of the presence of plasmalogen.

A positive PAS reaction indicative of the presence of polysaccharides, was observed in Hassall's corpuscles and around them in certain cells of the epithelial reticulum.

The medullary substance exhibited a vigorous nucleic acid activity. The nuclei of the epithelial reticular cells and the nucleoli of the thymocytes showed intensive pyroninophilia.

Hassall's corpuscles

Their structure is essentially the same as in higher vertebrates: a central, so-called active, part is surrounded by a capsule of endothelioid cells. Under the microscope, this capsule is seen to surround structure consisting of a single and sometimes of several cells. Their nucleus is granular and abounds in chromatin. The central, active part of Hassall's corpuscle has, thus, a cellular structure in contrast to the organ in higher vertebrates. Its size is about 20 to 30 μ. The endothelioid capsule consists of 2 to 3 cells. Azan stain revealed collagenous fibres between them. The central part of Hassall's corpuscles contained much DNA. The cytoplasm was bright red in colour and the nucleus greenish-blue in preparations stained with methylgreen pyronine. The nuclear membrane and the nucleolus in the central — *i. e.* the enzymatically and histochemically active — part of Hassall's corpuscles gave a positive phosphatase reaction.

Beside Hassall's corpuscles a small number of cysts filled with PAS-positive substance was found. Whether they represent a cystic degeneration of Hassall's corpuscles or, on the contrary, the incipient stage of their formation (as claimed by TÖRŐ and AROS — 11) has yet to be clarified up. Cysts in amphibians are not lined by columnar epithelium; they have an epithelium of flat cells and sometimes contain colloid.

All reactions for the demonstration of vitamin C were negative so that the thymus of amphibians seems to contain no such compound.

Discussion

Histological and histochemical examinations have proved the thymus of Anura to be similar to the corresponding organ of higher vertebrates in some, and dissimilar in other respects. Although morphologically the Hassall's corpuscles of Anura differ from those of higher vertebrates, they show hardly any difference in histochemical reactions. TSCHASOWNIKOW [6] after X-ray irradiation observed the development of Hassall's corpuscles which had a central part consisting of but one or two cells. He regarded this phenomenon as a symptom of degeneration induced by the irradiation. We, too, succeeded in observing such structures and suggest that this type of Hassall's corpuscle

does not indicate degeneration but is characteristic of the phylogenetically low amphibians.

It should be noted that a Hassall's corpuscle consisting of a single cell remains unicellular during the whole time of the animals development. (The single cell represents here the central, active, part of the corpuscle.) It is known from observations in vitro of the thymus of higher vertebrates, *e. g.* guinea pigs or mice, that at a certain phase in the development of Hassall's corpuscles their central part consists of but a single cell.

Accumulation of PAS-positive matter in Hassall's corpuscles goes hand in hand with an extremely weak enzymatic activity of the thymus. TÖRŐ [5] claims that the peculiar concentric onion-leaf pattern of the central part of Hassall's corpuscles develops from the PAS-positive substances in the cells. Such a concentric pattern has not been observed in the Hassall's corpuscles of the amphibian thymus. It should be remembered that the epithelial potency of the thymus is not the same in Anura as in vertebrates of the higher orders. The thymus arises from the fourth and fifth branchial pouches in the former, and from the third and fourth in the latter.

Strong PAS-positivity, observable in Hassall's corpuscles and in the cells of the cortex indicates the presence of a considerable amount of polysaccharides. Judging from the digestibility by saliva, the PAS-positivity is due to the presence of glycogen. It serves as a reserve to be consumed by the organism when food is scarce. Hence the amphibian thymus can be regarded as a centre of polysaccharide metabolism, a storehouse of carbohydrate.

Though fairly active, the medulla of the amphibian thymus does not exhibit the multiplicity of RNA and DNA observable in the corresponding organ of rats or mice. This was illustrated by the uniform intensity of methyl-green pyronine staining, and a phenomenon indicative of the weak nucleic-acid metabolism in the thymus of Anura.

Enzymatic activity was likewise weaker than in the thymus of mammals. This shows that the functions of the epithelial reticular cells are not as many-fold as in mammals [8], a fact in accord with the phylogenic rank of amphibians [10]. It is, on the other hand, noteworthy that the amphibian thymus is so active as regards storage of glycogen. The epithelial reticular cells are incapable of synthetizing DNA so that, in this respect, amphibian metabolism is essentially different from that of the mammals, a phenomenon associated with the poikilothermia of amphibians.

Summary

Sections prepared from the thymus of tailless amphibians have been subjected to histological and histochemical analyses. It was found that all principal morphological structures observable in the thymus of higher vertebrates, such as cortex and medulla, Hassall's corpuscles and epithelial reticulum, were likewise present in the amphibian thymus; the histochemical reactions were practically identical with those obtained in higher vertebrates.

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HISTOLOGISCHE UND HISTOCHEMISCHE UNTERSUCHUNG DER THYMUSDRÜSE VON FROSCHLURCHEN

(Rana ridibunda, Rana esculenta, Bombina bombina)

E. KAPA

An Thymusdrüsenschnitten von Froschlurchen wurden histologische und histochemische Untersuchungen durchgeführt und festgestellt, daß die wichtigeren morphologischen Einheiten des Thymus von höheren Wirbeltieren: die Mark- und Rindensubstanz, die Hassallkörperchen sowie das Epithelretikulum auch im Thymus der Froschlurche nachgewiesen werden können. Die Ergebnisse der histochemischen Reaktionen waren mit denen der höheren Wirbeltiere annähernd identisch.

ГИСТОЛОГИЧЕСКОЕ И ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ЗОБНОЙ ЖЕЛЕЗЫ БЕСХВОСТНЫХ ЗЕМНОВОДНЫХ

(Rana ridibunda, Rana esculenta, Bombina bombina)

Э. КАПА

Автор на срезах зобной железы бесхвостных земноводных проводил гистологические и гистохимические исследования. Он установил, что основные морфологические единицы зобной железы высших позвоночных, мозговое и корковое вещество, гассалевские тельца и эпителиальная сеточка, обнаруживаются также в зобной железе бесхвостых земноводных. Результаты гистохимических реакций приблизительно одинаковы с результатами, полученными у высших позвоночных.

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