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THE BEHAVIOUR OF THE THYMUS IN CONDITIONS ASSOCIATED WITH TISSUE PROLIFERATION

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The thymus has been classified both as a lymphatic organ and as an endocrine gland ; its function is still not clear. The studies on its role yielded conflicting results, depending in part on the species, sex and age of the experimental animals used, and in part on the methods employed in the investigations. There is no such evidence as would make it possible to discuss and study the thymus according to uniform points of view. The role of Hassal's corpuscles, the genesis of thymocytes and the evacuation of the thymus are problems all unclarified [13].

According to our investigations, morphological differences exist between the thymocytes and the lymphocytes produced in the lymph nodes, insofar as the thymocytes give a strongly positive alkaline phosphatase reaction, whereas the lymphocytes do not ; moreover, differences are demonstrable in the cell membrane, too [14, 16]. There is ample evidence in the literature indicating that the two kinds of cells differ in function. For example, many data show that plasma cells develop from lymphocytes, while, for instance, Shelin et al. [10] reported that such plasma cells are present even normally in the lymph nodes and spleen, but not in the thymus, in which they appear in small numbers only, even in response to experimental influences. According to Harris and Harris [7] lymph cells immunized *in vitro* produce antibodies, whereas the thymocytes do not, etc. It seems therefore that the two kinds of cells cannot be considered identical.

As to the other problem in our previous studies we observed that the corpuscles of Hassal secreted a PAS-positive substance corresponding to a neutral polysaccharide, and the corpuscle itself represented the active function of the thymus, developing from the epithelial reticulum of the thymus while secreting the above substance [9, 14, 15, 16, 17, 18].

The third problem is that of the evacuation of the thymus. The thymus is one of the organs that show fastest response in the organism's adaptation reactions ; it may be emptied so fast that it is virtually only the initial and end states that can precisely be determined morphologically. This may be one of the reasons why we do not know how and where the thymus empties

its cells. Some authors suppose that the thymocytes would enter the lymph circulation directly, whereas according to others (*e. g.* LEHNER, 8) they would be transformed into mast cells and would enter in that state the adjacent connective tissue, wherein their number would increase, for example in conditions associated with excitement [1]. At any rate, this problem is just as unsolved as is that of the thymus as a whole.

In the present experiments we have investigated all three problems. We think namely that the three problems are greatly interdependent and a solution may be found by such experiments, in which the three problems are studied on grounds of a uniform conception and by uniform methods. Just for this reason we have studied the thymus in conditions associated with tissue proliferation, because in such conditions the organs and their cells show themselves differently from what is seen in normal immunity.

First of all, we wished to determine the fate of the PAS-positive mucopolysaccharide we had found in earlier experiments. Furthermore, we followed up the fate of thymocytes in various conditions associated with cell proliferation, normal or pathological.

We have made observation on the thymus and other lymphatic organs of tumorous or tuberculous or pregnant rats and mice, as well as on those from rats in different stages of ontogenesis.

Methods

Ninety albino rats and 60 albino mice were used in the experiments. — Mice were inoculated with 0.3 ml of Ehrlich ascites tumour about 10 mm below and left from the umbilicus intraperitoneally. The tumour developed rapidly and the animals survived inoculation by 15 days on the average as determined in a special control group. Subcutaneous inoculations of the Ehrlich tumour were made in the back of mice, using 0.3 ml of ascites fluid. The tumour began grow at about 12 days and the average survival time was 46 days.

Rats were inoculated with Guérin tumour. These animals survived 42 days on the average. The tumour was inoculated subcutaneously, in the form of a suspension.

Other groups of rats were inoculated with 2 mg of Mycobacterium tbc. Ravenel (bovine type) grown in Sula's medium, suspended in physiologic NaCl solution. The inoculations were made intravenously, as described by FÖLDES [4]. The animals were killed 10 or 25 days later. By the end of that period many animals had already succumbed.

The thymus, usually the regional lymph node, as well as the spleen of 2 animals from each group were studied histologically, in all the above, as well as in the control groups. The specimens were fixed in Carnoy's fluid and embedded in paraffine. Histologic sections were stained with Giemsa, PAS, toluidine blue and cresyl-violet, respectively. Representative preparations from each group were stained with methylgreen-pyronine as well.

Experimental

A) *Studies on the thymus from rats in different stages of ontogenesis*

Fifteen to 19 days old rat foetuses, 10, 20, 50 and 150 g rats, as well as old rats weighing 250 g were studied in this group.

In the thymus from the *15 days old rat foetus* very few PAS-positive cells were visible. The PAS-positive substance tended to occur between the

epithelial elements, as a product of cell secretion. Corpuscles of Hassal were not visible. Epithelial elements preponderated over lymphocytes and were to be found among them, in the form of big islets. Mast cells were not found.

In the *18 to 19 days old rat foetus* an equilibrium existed between the epithelial and thymocyte matter. PAS-positive substance was present as in the former stage, it seldom occurred intracellularly. Regular mast cells were found in the thymus. Between the epithelial elements, mainly in the peripheral lobules, thymocyte-like cells appeared, with more or less perinuclear granulation showing azur or toluidine metachromasia in the cytoplasm.

In the *10 g rat*, big, degeneration-free corpuscles of Hassal were seen. In the thymus cavities filled with mesenchyma appeared at sites ; the mast cells were in these, as well as in the capsule. The corpuscles of Hassal contained PAS-positive substance. In some cases mast cells, even in the thymus itself, were found at many sites. In the peripheral lobe of the thymus epithelial-gland-like tissue was seen, the picture being similar to that found in the thymus of the 18 to 19 days old foetus ; between the epithelial cells thymocyte-like cells appeared, with perinuclear granulation showing azur and toluidine metachromasia in the cytoplasm *(Fig. 1)*.

In the *20 g rat* some slight PAS-positivity and a few mast cells were found. There were much less epithelial islets than in the 10 g animal.

In the *50 g rat* there was a pure lymphatic thymus with very few epithelial elements, very little PAS-positive substance and a slight increase in the mast cell count.

In the *150 g rat* the PAS-positive substance was increased. The epithelial elements were more marked than in the 50 g rat. As to the mast cells, there was not much difference from the 50 g rat. The number of corpuscles of Hassal was small, though higher than in the 50 g rats.

In *old rats weighing 250 g* the number of both the thymocytes and the epithelial elements decreased markedly. Big corpuscles of Hassal, filled with PAS-positive substance were visible. The mast cell count was low ; the mast cells were hypergranulated, and disintegrated at many sites. Some of them showed marked vacuolisation.

B) *Studies on the thymus and lymphatic organs from tumorous animals*

1. Rats inoculated with Guérin tumour. Until the tumour "took", changes in the thymus were slight. At the time of "taking" the PAS-positive substance increased in the corpuscles of Hassal and numerous PAS-positive thymocytes appeared. These had an extremely swollen cytoplasm, as a result of the accumulation of the PAS-positive substance in them. With the advance in the growth of the tumour, large numbers of mast cells appeared in the capsule, connective tissue trabecules and the cortical layer of the thymus *(Fig. 2,*

Fig. 3). The process was found to be parallel with the decrease in the number of thymocytes. In older rats, in which the tumour did not take there also appeared PAS-positive cells *(Fig. 4)*. In general, the more advanced the tumorous invasion, the greater the increase in the number of PAS-positive cells in the thymus. In the lymph nodes and spleen, there were only a very few mast cells and occasional PAS-positive cells. In the final stage of the tumour the lymph nodes (especially the regional ones) were filled with metastases, but even then the number of PAS-positive cells was quite small.

2. *Mice inoculated intraperitoneally with Ehrlich ascites tumour.* Three days after intraperitoneal inoculation, large numbers of PAS-positive thymocytes appeared in the thymus. In these cells the nuclei were sometimes in the centre, sometimes excentric. The cells were situated in the cortex, but first of all in the periphery of the lobules. Numerous PAS-positive cells occurred around the corpuscles of Hassal as well. The corpuscles contained a great amount of PAS-positive substance, were cavernous at many sites, forming glandular lumenlike structures filled with much PAS-positive substance *(Fig. 5)*. All these PAS-positive areas showed neither azur granulation, nor metachromasia on staining with toluidine. In some cells, however, mainly in those of the medulla, metachromatic granules were visible. In the capsule of the thymus the number of mast cells was greatly increased. No characteristic changes were detected in the lymph nodes and spleen. At that phase gross examination revealed neither ascites, nor any other change in the animals.

Seven days after intraperitoneal inoculation the picture was still about the same, except that the increase of PAS-positive substance in the cyst-like structures was even more marked and the number of PAS-positive thymocytes had increased. The peripheral lobes of the thymus had begun to become empty. No characteristic changes were visible in the lymph nodes and spleen. The tumour was palpable in the abdomen, the amount of ascites fluid had increased and a small solid tumour appeared at the site of the injection.

Eleven days after intraperitoneal inoculation the thymus could hardly be recognized. When stained with PAS, the whole organ was flame-red, all the cells visible being practically PAS-positive *(Fig. 6)*. Enormously distended cysts filled with PAS-positive substance were seen. The structure of the whole thymus was markedly loosened. Regular mast cells were visible in both the cortex and the medulla, but first of all between the PAS-positive thymocytes. In the peripheral lobes there was no thymus tissue, but the number of mast cells was increased *(Fig. 7)*. In the sinuses of lymph nodes tumour cells were found. The cellular pattern of the spleen was poor. The ascites caused a marked distension of the abdomen, the mesentery was infiltrated by masses of small tumours. The solid tumour was the size of a pea.

Fifteen days after intraperitoneal inoculation in about 50 per cent of the animals no thymus was found, the organ had been completely emptied [5].

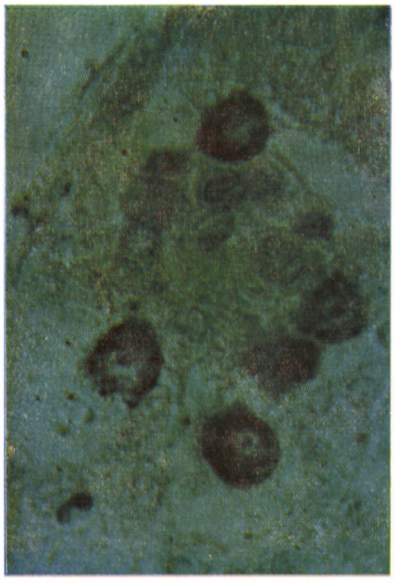

Fig. 1. Thymus from a rat weighing 10 g. Big thymocytes, with perinuclear metachromatic granules in the cytoplasm. Toluidine blue. × 500

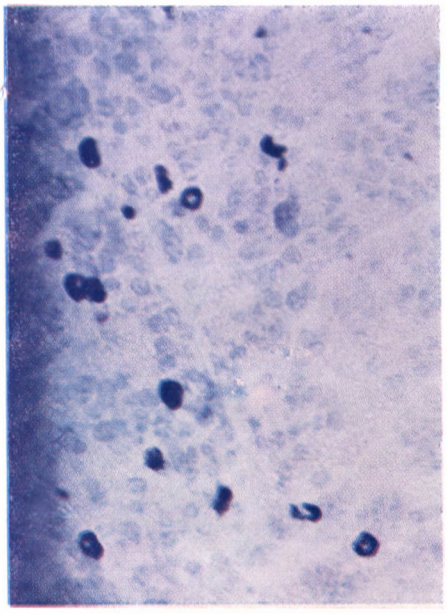

Fig. 2. Thymus from a rat with Guérin tumour. Metachromatic substance accumulating in thymocytes. Toluidine blue. × 100

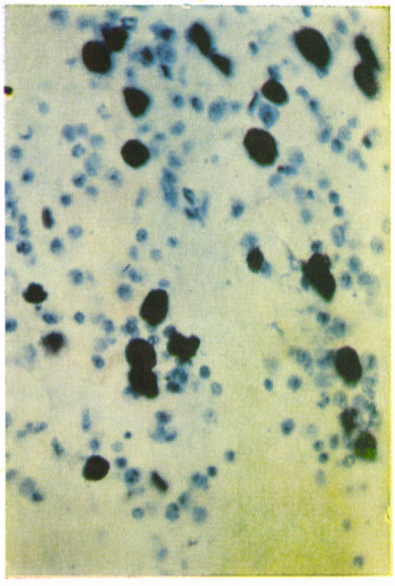

Fig. 3. Thymus from a rat with Guérin tumour, 25 days after subcutaneous inoculation. Numerous mast cells in the parenchyma of the thymus. Giemsa. × 200

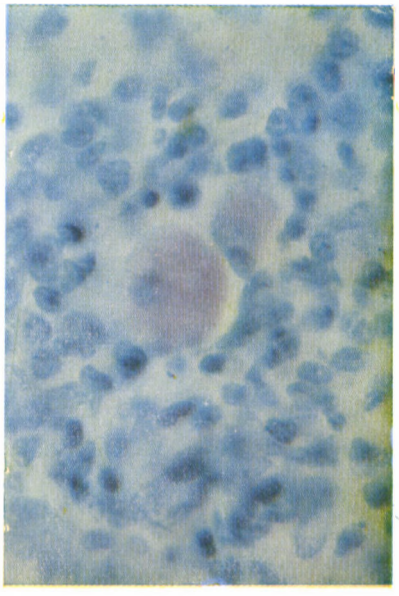

Fig. 4. Thymus from an old rat weighing 250 g, 42 days after the subcutaneous inoculation of Guérin tumour. The tumour did not take. Big PAS-positive cells visible. PAS ; oil immersion

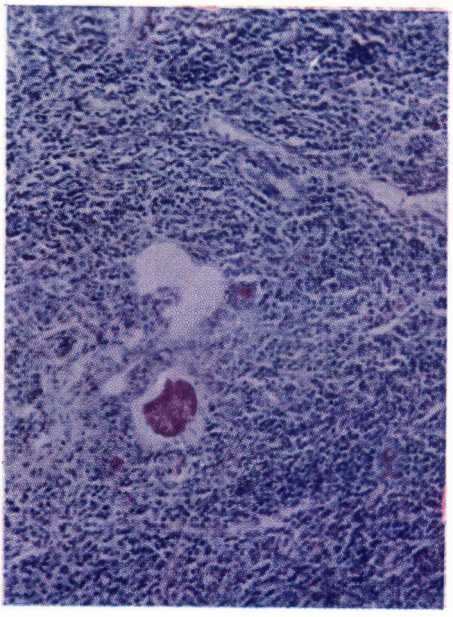

Fig. 5. Mouse thymus, 3 days after the intraperitoneal inoculation of Ehrlich ascites tumour. A cavity lined with epithelium, showing cyst-like distension, is filled with PAS-positive substance. PAS. × 100

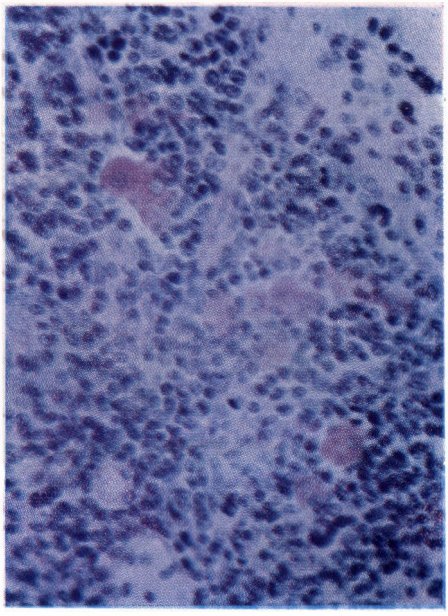

Fig. 6. Mouse thymus, 11 days after the intraperitoneal inoculation with Ehrlich ascites tumour. Many PAS-positive cells. PAS. × 100

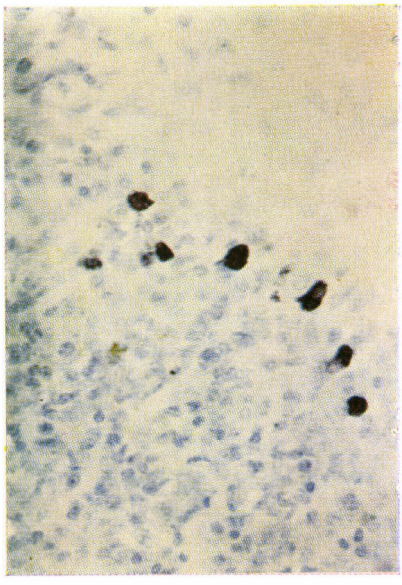

Fig. 7. Mouse thymus, 11 days after the intraperitoneal inoculation with Ehrlich ascites tumour. Mast cells. Cresyl violet. × 100

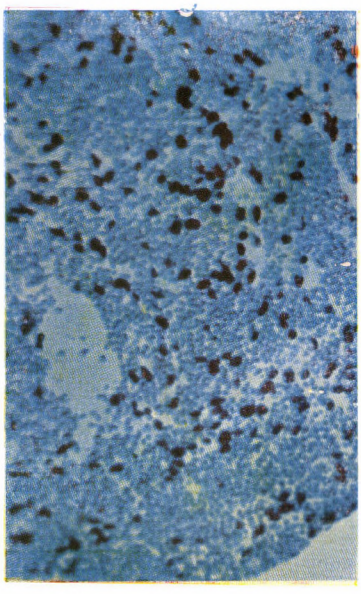

Fig. 8. Thymus from rat, 18 to 19th day of pregnancy. Mast cells Giemsa. × 100

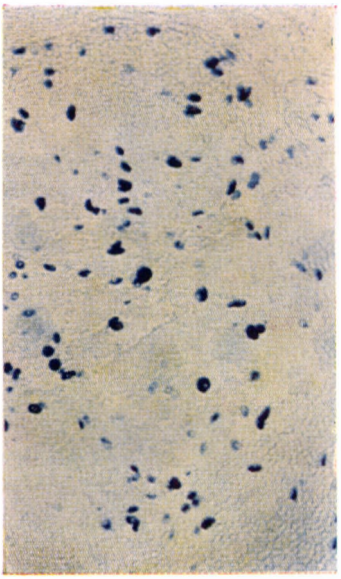

Fig. 9. Thymus from pregnant (18—19 days) rat. The thymocytes contain more or less of the metachromatic substance. Mast-cell-like cells. Toluidine blue. × 100

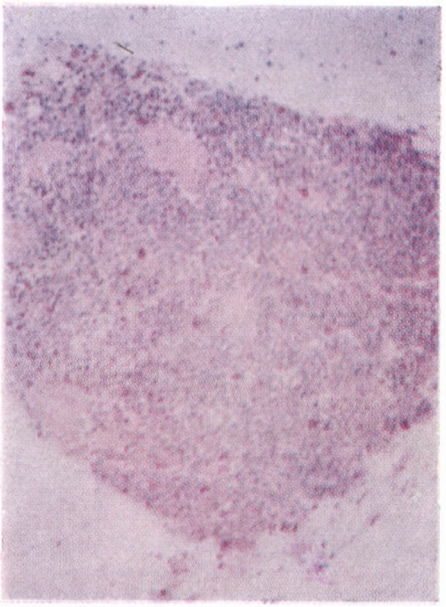

Fig. 10. The same as in *Fig. 9.* Many PAS-positive thymocytes. PAS. × 100

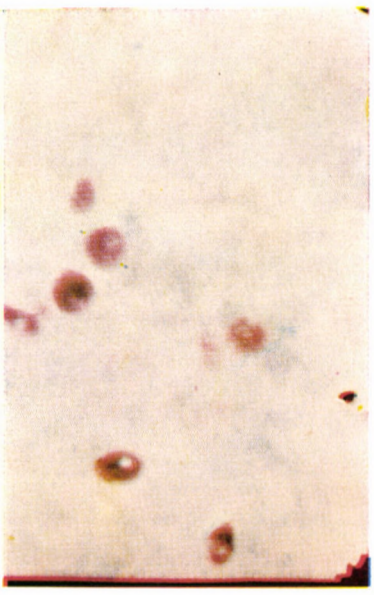

Fig. 11. Thymus from pregnant (18—19 days) rat. Metachromatic granules in the cells. Mast cells. Toluidine blue. × 300

Fig. 12. Same as in *Fig. 11* from another area. Metachromatic granules and some fully developed mast cells are visible. Toluidine blue. × 300

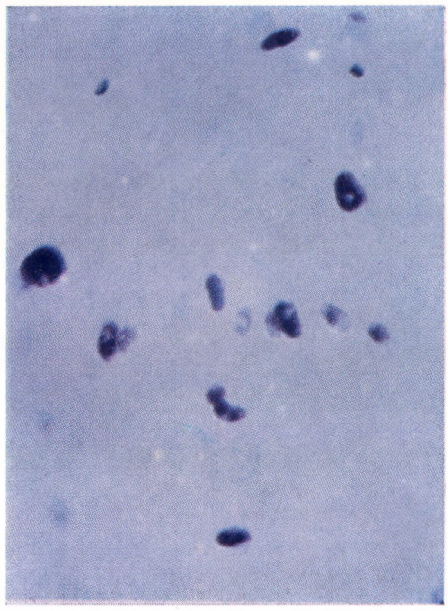

Fig. 13. Same as in *Fig. 12* Enlarged thymocytes filled with metachromatic granules and regular mast cells are visible. Toluidine blue. × 150

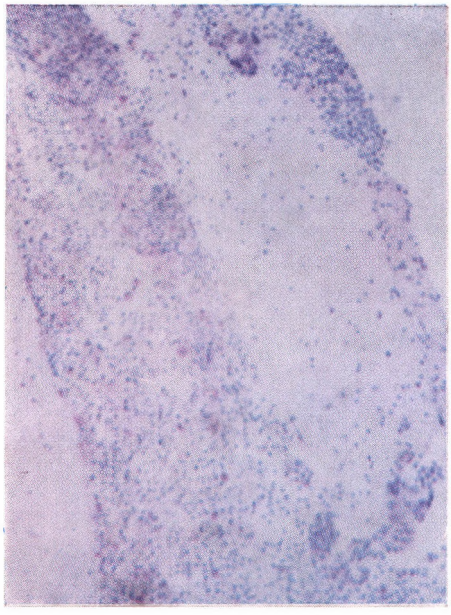

Fig. 14. The same as in *Fig. 13* stained with PAS. The thymocytes are filled with PAS-positive substance. PAS. × 100

Even where it could be found, only a few lobes displayed an extremely loosened thymus tissue. The number of PAS-positive cells decreased, but the cysts filled with PAS-positive substance were still present. Metastases were found in the lymph nodes and in some cases also in the spleen. Most of the animals were dying.

3. *Mice inoculated subcutaneously with Ehrlich ascites tumour.* The picture was comparable to that seen in the rats inoculated subcutaneously with Guérin tumour. PAS-positive corpuscles of Hassal and PAS-positive cells occurred. In the capsule and connective tissue of the thymus, and later also in its parenchyma, an increased number of mast cells appeared.

C) *Rats intravenously infected with tuberculosis*

Ten days after inoculation with Mycobacterium the thymus was reaction-free. At that time tubercles began to appear in the lungs, together with a strong plasma cell reaction. No mast cells were found around the tubercles, and their number in the thymus was as usual. PAS-positive cells were not found and the corpuscles of Hassal contained normal amounts of PAS-positive substance.

Twenty-five days after infection, when the animals were near to death, a few PAS-positive cells were visible in the thymus and at sites the corpuscles of Hassal were filled with PAS-positive substance. In the connective tissue of the thymus the number of mast cells was significantly increased and such cells occurred at many sites in the thymus, mainly in the cortex. The thymus had not emptied. In the lung, tubercles had developed, a regular lymphocytic-plasmocytic barrier was also visible. Langhans-type giant cells appeared in great numbers, but mast cells were not visible anywhere.

D) *Pregnant rats*

Almost without exception, the thymus glands from pregnant rats contained a great amount of PAS-positive thymocytes. The peripheral lobes of the thymus were emptying and in such lobes the PAS-positive substance in the cytoplasm of the thymocytes was especially well observable, owing to the reduced number of cells present. In the same lobules (on staining the consecutive sections with different stains) these PAS-positive cells showed also azur and toluidine metachromasia *(Fig. 8—Fig. 14)*. This is clearly visible in the figures. The toluidine-stained preparation shows that most of these cells are well-defined mast cells. Considerably increased numbers of mast cells were visible in the parenchyma and the connective tissue of the thymus, first of all in the zone of PAS-positive thymocytes. Likewise, corpuscles of Hassal filled with PAS-positive substance appeared. No change whatsoever was detectable in the lymph nodes and spleen.

Discussion

Analysing this variable material, which has been selected according to a given point of view, it is clear that in the conditions examined the thymus showed an almost identical behaviour, at the same time when the lymphatic tissue and the spleen displayed quite slight reactions, which were different from that shown by the thymus. The thymus of mice inoculated intraperitoneally with the Ehrlich ascites tumour exhibited the most characteristic pattern. This was obviously due to the fact that this was the only kind of lesion that developed rapidly and killed the animals within about 2 weeks. However, the phenomena encountered after inoculation with the Ehrlich tumour occurred also in the other conditions, though in the latter they developed less rapidly. In their course, depending on the quality of the process, one or another of the changes found in the animals with ascites was in the foreground, but all of the variants could be detected.

What are these phenomena composed of? As it has been seen, a PAS-positive substance appears in the corpuscles of Hassal, these corpuscles increase in number and size, and in some cases are converted to cyst or gland-like structures [3]. The patterns bear a close resemblance to what we may call a glandular transformation of the thymus. At the same time, PAS-positive substance is accumulating in the thymocytes, which tend to occur mainly at the periphery of the lobes of the thymus. Meanwhile, the number of mast cells increase in the connective tissue of the thymus. The uniformity of these changes in every one of the benign or malignant conditions associated with tissue proliferation indicates that what we are dealing with is a tissue reaction of the thymus, which is uniform and characteristic of that organ. Considering, further, that in immunization processes the thymus remains practically inactive while the spleen and lymph nodes are highly active, whereas in the present experiments a hyperfunction of the thymus was associated with a relative inactivaty of the lymph nodes and spleen, the role of the thymus prevails especially in processes associated with some dysharmony of tissues. This appears to be the first and probably most important conclusion to be drawn from our experiments.

In all the above experiments, all of them conditions associated with tissue proliferation, the lymph cells in the thymus accumulated a PAS-positive substance, whereas those in the spleen and lymph nodes did not. This difference between the two kinds of cells is due not to a difference in the environment, but to the fact proved by us earlier, that the thymocytes differ in nature from the lymphocytes. This has been confirmed also by the observation that with the development of a malignant tumour the blood level of PAS-positive substances is increased [6, 11, 19], and yet the lymphocytes in the spleen and lymph nodes do not take up those substances.

Thus, the second important conclusion is that thymocytes are capable of taking up PAS-positive substances and differ in this respect from the lymphocytes in the lymph nodes and spleen.

Furthermore, the experiments have confirmed our earlier statements as regards the corpuscles of Hassal, notably that these corpuscles are active, functioning structures, which increase in number when the thymus is exposed to specific strain, and which produce PAS-positive substances. The gland-like structure of the corpuscles of Hassal is likewise indicative of active function. On the basis of our earlier investigations, the corpuscles of Hassal were considered to be the gland-representants of the thymus, in which the secretion is released with the development of the corpuscles. Still, unlike with the endocrine glands, the secretion is not given over directly into the blood serum, but is taken up by adjacent thymocytes. This is suggested by our observation that large numbers of thymocytes filled with PAS-positive substance were found around the PAS-positive corpuscles of Hassal. As regards the secretive mechanism, in this question we agree with BOMSKOW [2]. On the other hand, we shall discuss later how we think transport takes place and what conclusions our experiments permit in this respect. At any rate, it seems clear that the corpuscles of Hassal develop first of all in connexion with active thymus function and turn into gland-like structures producing a specific product.

A further problem of importance is that of the evacuation of the thymus. We have found that it is invariably the peripheral lobes of the thymus that become empty, and the central ones follow suit. This, however, fails to explain the mechanism of emptying, all that it implies is that the thymus is capable of segmental function. A close relationship must exist between the emptying of the thymus and the appearance of mast cells. When namely the number of PAS-positive cells is increasing in the peripheral parts of the lobes of the thymus, mast cells appear in large numbers in the connective tissue of the organ. Later, when PAS-positivity increases, many mast cells appear in the parenchyma of the thymus as well. An even more convincing proof, however, is the fact that in the thymus of pregnant rats the same cells could be seen in the PAS-positive and in the toluidine-metachromatic state, indicating that the two kinds of cell are in fact transformation products. These two forms of mast cells (PAS-positive and the one showing toluidine metachromasia) had been known before, but our experiments have proved with a high degree of certainty that mast cells are formed first of all from thymocytes. The process takes place not only in pathological conditions, but also in normal ones, *e. g.* in pregnancy. Studies on normal animals often revealed metachromatic granules in the thymus of young rats of 10 g, as a new corroboration of our view. We are fully aware of the need for further evidence, especially such as may be obtained in tissue culture studies ; such investigations are in progress.

We do not claim that mast cells would appear in the thymus in every case of tissue destruction and proliferation, or that their development would always meet peripheral requirements. While the mast cells form a barrier around malignant growths, they do not appear around tubercles. This, however, does not yet alter the fact that the release of mast cells plays an important role in the reactivity of the thymus.

When discussing the problem of thymocytes and mast cells, we must consider the nature of the PAS-positive substance as well. In contrast to the data reported by SMITH and THOMAS [12], that material is not glycogen, not being digested by diastase. According to our earlier investigations, it is a mucopolysaccharide, which in this state may be neutral, but later it becomes more acid and sulphated, thus obtaining its metachromasia. The process takes place intracellularly in the parenchyma and in the connective tissue of the thymus. Further studies are required to reveal the details of this process.

It is possible that this PAS-positive substance is the hormone, or a component of the hormone, of the thymus and that the thymus is a special endocrine gland, whose secretion is carried away by cell transport. Further studies will have to decide this problem. It is noteworthy that in tuberculous rats the reaction of the thymus was late, observable only when the second specimen was taken, *i. e.* at a time when tissue destructions predominate, not the processes of immunization. The same occurs in pregnancy, in which state one cannot speak about the presence of foreign material or about a pathological condition, yet the hyperactivity of the thymus is elicited by tissue destruction.

In earlier investigations we had shown that the thymus would respond to various effects by the same reaction, the one manifesting itself with the evacuation of thymocytes, the appearance of Hassal's corpuscles and the secretion of a neutral mucopolysaccharide. The present results add to this that in conditions associated with tissue destruction, *i. e.* when the harmony of the tissues is disturbed, not only the PAS-positive mucopolysaccharides appear and the number of the corpuscles of Hassal increases, but an increase occurs also in the number of PAS-positive thymocytes and mast cells, as a representation of an active function of the thymus.

Summary

The tissue reaction of the thymus has been studied in animal experiments, in conditions associated with tissue proliferation. It has been found that malignant tumours, advanced proliferative tuberculosis and pregnancy all elicited a hyperfunction of the thymus, manifesting itself with an increase in the number of Hassal's corpuscles, the appearance of large quantities of a PAS-positive material in them, the appearance of PAS-positive thymocytes, as well as with a marked mast cell reaction. It is suggested that all these features are in correlation with one another, that the corpuscles of Hassal have an active, gland-like function

and that the thymocytes are apparently transformed into mast cells. As a difference between thymocytes and lymphocytes, it is pointed out that in the above-mentioned conditions the thymocytes contain PAS-positive substance, whereas the lymphocytes of the lymph nodes and spleen do not. The opinion is expressed that the function of the thymus becomes manifest first of all in the state of tissue dysharmony, in contrast to other lymphatic organs, which take part in the defense against injury caused by foreign substances.

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ПОВЕДЕНИЕ ЗОБНОЙ ЖЕЛЕЗЫ В СОСТОЯНИЯХ, СОПРОВОЖДЕННЫХ РАЗРАСТАНИЕМ ТКАНИ

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Авторы исследовали в опытах на животных тканевую реакцию зобной железы при различных состояниях, сопровождаемых разрастанием ткани. Они установили, что прогрессирующая, пролиферативная стадия опухолей и туберкулеза, как и беременность вызывают гиперфункцию зобной железы, проявляющуюся в размножении гассалевских телец, появлением в них большого количества перйодная-кислата-шиф (PAS) положительного вещества и перйодная-кислата-шиф (PAS) положительных тимоцитов, как и сильной тучноклеточной реакции. Все перечисленные явления авторы приводят в взаимную связь и приходят к тому заключению, что гассалевские тельца обладают активной, железе подобной функцией, причем тимоциты предположительно преобразовываются в тучные клетки. Они устанавливают, что разница между тимоцитами и лимфоцитами заключается в том, что в вышеприведенных состояниях тимоциты содержат перйодная-кислата-шиф (PAS) положительное вещество, а в лимфоцитах лимфатических узлов и селезенки такого вещества не имеется. По мнению авторов, функция зобной железы проявляется, прежде всего, при состояниях тканевой дисгармонии, в противоположность другим лимфатическим органам, участвующим в предотвращении повреждений, причиненных чужеродными веществами.

DAS VERHALTEN DES THYMUS BEI MIT GEWEBSWUCHERUNG EINHERGEHENDEN ZUSTÄNDEN

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Es wurde in Tierversuchen die Gewebsreaktion der Thymusdrüse bei verschiedenen mit Gewebswucherungen einhergehenden Zuständen untersucht und festgestellt, dass das progressive, proliferative Stadium von Geschwülsten und Tuberkulose, sowie Schwangerschaft eine Hyperfunktion des Thymus auslösen, die sich in der Vermehrung der Hassalschen Körperchen, im Erscheinen grosser Mengen von PAS-positiver Substanz und PAS-positiven Thymozyten in letzteren, sowie in einer starken mastzelligen Reaktion manifestiert. Diese Erscheinungen werden miteinander in Verbindung gebracht und die Schlussfolgerung gezogen, dass die Hassalschen Körperchen über eine aktive, drüsenartige Funktion verfügen und dass sich die Thymozyten vermutlich in Mastzellen umwandeln. Als Unterschied zwischen den Thymozyten und Lymphozyten wird festgestellt, dass die Thymozyten in Verbindung mit den obenangeführten Zuständen eine PAS-positive Substanz enthalten, während die Lymphozyten der Lymphdrüsen und der Milz keinen solchen Stoff aufweisen. Verfasser sind der Meinung, dass die Funktion des Thymus sich in erster Reihe in den Zuständen der Gewebsdysharmonie manifestiert, im Gegensatz zu den übrigen Lymphorganen, die an der Abwehr der durch Fremdkörper verursachten Schädigungen teilnehmen.

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