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PROVOKED TISSUE REACTION OF THE THYMUS IN TISSUE CULTURE

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We have reported previously [1, 4—8.] that in conditions associated with tissue proliferation a characteristic, uniform tissue reaction takes place in the thymus. This reaction manifests itself with an increase in the number of Hassal's corpuscles, the appearance and accumulation in them of a PAS-positive substance, with an increase in the number of thymocytes, as well as with an intensive mast cell reaction. It has been found, for example, that in the thymus of the pregnant animal the PAS-positive thymocytes turned into mast cells showing toluidine metachromasia. Although we have already stated that all these observations point to the role of thymocytes in mast cell formation and that the emptying of the thymus is partly due to the conversion of thymocytes into mast cells, we still thought it necessary to verify our findings in tissue culture studies. For this reason in the experiments to be described below, we provoked the tissue reaction of the thymus by adding a PAS-positive, heparin-type substance to the tissue culture.

Methods

The observations were performed on 600 tissue cultures.

Tissues were explanted in Maximov cultures, in a hen plasma medium coagulated with chick embryo juice. The washing fluid was composed of one part of embryo juice, three parts of horse serum, six parts of Tyrode's solution and 150 U/ml penicillin. The cultures were washed at 3 day intervals.

Cultures showing the same rate of growth were selected for study. To the cultures we added 3 days after explantation heparin, treated in the following way. Heparin (Heparin pulvis Richter, 1 mg = 80 I. U.) dissolved in physiological saline (1 mg/ml) was ampouled, boiled for 1 hour, then placed into a water bath of 56° C for 48 hours. Thereby the heparin — used by us — lost its toluidine metachromasia and became PAS-positive. The Tyrode's solution in the cultures was replaced by the solution thus prepared. The control cultures were washed with the same fluid, except that these washing fluids contained no heparin.

The cultures were fixed in Carnoy 5, 24, 48 and 72 hours later. Serial sections were made from the 72-hour heparinized cultures, as well as from the control ones. The sections and the cultures were stained with Giemsa's stain and with toluidine blue.

Experimental

Cultures made of the thymus, lymph nodes, spleen and kidney of rat embryos (intrauterine, 15 to 19 days old), newborn and 1 month old rats were studied.

The behaviour of the heparinized thymus, lymph node and spleen cultures is shown in Tables I, II and III. (The heparin used was treated as specified above.) The crosses (+, ++ and +++) indicate the metachromatic cell count. Both the toluidine and the azur metachromasia appeared perinuclearly in the cells. No spontaneous metachromasia was noted in the control cultures. Although no planimetric measurements were made, it was observed that the heparinized thymus cultures grew more intensively than the control ones, whereas heparin had no effect on the growth of the spleen and lymph nodes.

In the embryonic thymus cultures endothelial tubes with numerous cells showing toluidine metachromasia around them, appeared 72 hours after heparinization. Similar endothelial tubes and cells were found in the control cultures, too, but these showed no evidence of metachromasia.

In order to prove that not every kind of epithelium was capable of producing from PAS-positive substance a substance showing toluidine metachromasia, we cultured renal epithelium and treated it with heparin (see above). The epithelium itself remained negative, but a few migrating cells of the mast cell type were found that showed toluidine metachromasia. The number of these cells showed no further increase.

In other experiments we used, instead of the heparin treated as specified, a solution containing 1 mg of glucuronic acid and 1 mg of glycosamine. No

Table I

*Behaviour of thymus cultures treated with heparin**

	In culture					In culture sections, metachromatic cells
	azur +		toluidine metachromasia		mast cells	
	epithelium	thymocyte	epithelium	thymocyte		
embryo 5h	+	—	—	—	—	
24	++	++	++	++	+	
48	++	++	++	+++	++	
72	++	+++	++	+++	++	+++
newborn 5h	—	—	—	—	—	
24	+	—	+	—	+	
48	++	+++	++	+++	++	
72	++	+++	++	+++	+++	+++
1 month 5h	—	—	—	—	—	
24	+	—	+	—	+	
48	+	+	+	+	+	
72	+	+	+	+	+	+

* (Heparin had been treated as specified in the text)

Table II

Behaviour of heparinized lymph node cultures*

	In culture					In sections, metachromatic cells
	azur +		toluidine metachromasia		mast cells	
	epithelium	thymocyte	epithelium	thymocyte		
5h	—	—	—	—	—	
embryo 24	—	—	—	—	—	
48	—	±	—	±	—	
72	—	±	—	±	—	—
5h	—	—	—	—	—	
newborn 24	—	—	—	—	—	
48	—	±	—	±	—	
72	—	±	—	±	—	—
5h	—	—	—	—	—	
1 month 24	—	+	—	—	—	
48	—	++	—	—	—	
72	—	++	—	—	—	—

* (Heparin treated as specified in text)

Table III

Behaviour of heparinized spleen cultures*

	In culture					In sections, metachromatic cells
	azur +		toluidine metachromasia		mast cells	
	fibroblast	thymocyte	epithelium	thymocyte		
5h	—	—	—	—	—	
embryo 24	—	—	—	—	—	
48	—	±	—	—	—	
72	—	+	—	—	—	—
5h	—	—	—	—	—	
newborn 24	—	±	—	—	—	
48	—	±	—	—	—	
72	—	±	—	—	—	—
5h	—	—	—	—	—	
1 month 24	—	+	—	—	—	
48	—	+	—	—	—	
72	—	+	—	—	—	—

* (Heparin treated as specified in text)

metachromasia of any kind was observable in the thymus, or other cultures during the period of cultivation.

Discussion

Before discussing our results, let us briefly consider the role of heparin. We have pointed out when describing the methods that the heparin used had been pre-treated, decomposed. This was necessary for two reasons. One, as it is known mainly from the evidence published by HEILBRUNN, heparin inhibits mitosis, and as such damages tissue cultures, retarding their growth. Two (and this was the more decisive point), we wished to reproduce the experiments made in live animals, thus, we wanted to determine whether toluidine-metachromatic heparinocytes would be actually formed from PAS-positive thymocytes. Thus, heparin had to be broken down. It was found that as it being broken down and possibly when it is synthesized heparin passes into three forms, as examined by the three stains mentioned. The intact, active heparin stains metachromatically with toluidine blue and gives azur-positivity when stained with Giemsa's stain, and is PAS-negative. When slightly broken down, it loses the toluidine metachromasia, but still reacts positively on staining with azur, while in the markedly decomposed state it shows only PAS-positivity *(Fig. 14)*. These observations have made it possible properly to interpret the results of the above experiments, because it is obvious that if toluidine metachromasia develops in a tissue or cell treated with heparin, that reacts positively only to PAS, this will mean a synthesis of heparin, or of a heparin-type substance, from its metabolites.

The experiments have proved beyond doubt that in the thymus a substance showing toluidine metachromasia is formed from a PAS-positive substance. This takes place in the epithelial cells just as well as in the thymocytes. The process begins in the epithelial cells, but later it takes place also in the thymocytes. We have found such a process neither in the lymph nodes, nor in the reticulum cells of the spleen, nor in the renal epithelium, nor anywhere else, so that we may state that the phenomenon is characteristic of the epithelial reticulum of the thymus and of the thymocytes.

Our investigations showed further that regular cells, morphologically identical with mast cells, develop from the thymocytes and from the epithelial cells of the thymus reticulum. Thus, a difference can be noted between the thymocytes and lymphocytes, at least in the sense that in the thymus mast cells develop from the thymocytes, whereas this does not happen in the lymph nodes and spleen.

The fact that regular mast cells develop in thymus cultures makes it clear that the thymus must have a role in the genesis of mast cells. Thus,

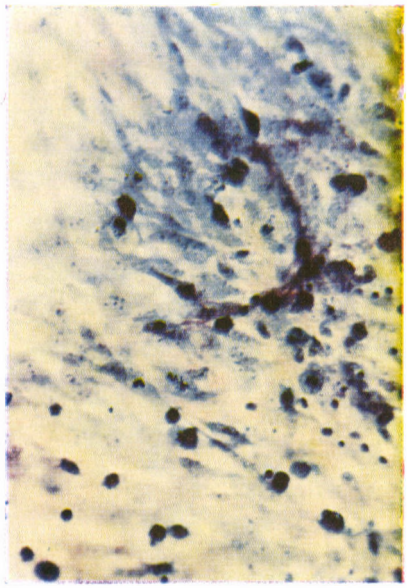

Fig. 1. Embryonic thymus culture 5 hours after heparin treatment. Appearance of azurpositive substance in epithelial plate. Giemsa. × 100

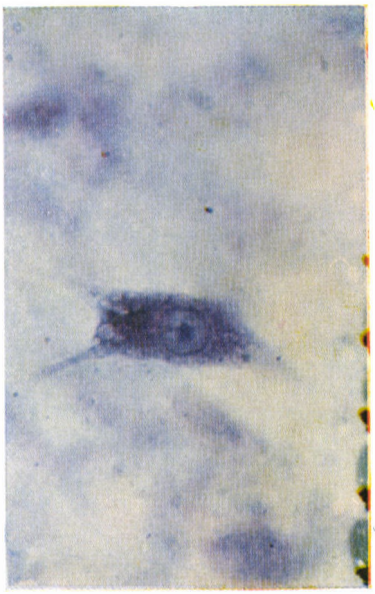

Fig. 2. Thymus from a rat aged 13 to 15 days. Appearance of culture 24 hours after heparin treatment. Mast cell in the culture. Giemsa. × 200

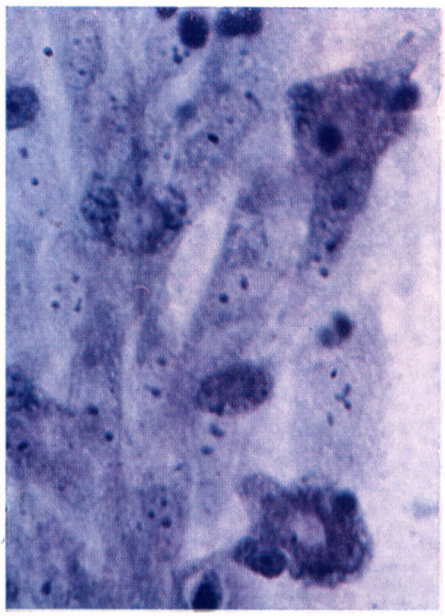

Fig. 3. Culture of thymus from a 15-day old rat embryo 48 hours after heparin treatment. Perinuclear accumulation of metachromatic substance in epithelial cells. Giemsa. × 100

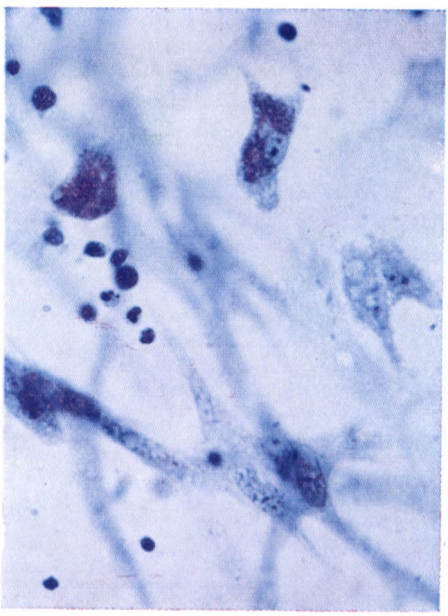

Fig. 4. The same as shown in *Fig. 3.* Metachromatic mast cells, with nuclei characteristic of thymocytes in one of them. Giemsa. × 100

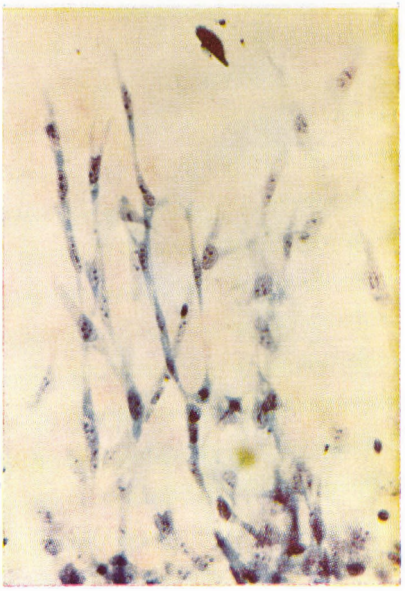

Fig. 5. 15-day old embryonic control culture 5 days after explantation. Metachromasia is not visible anywhere. Growth is less marked and less epithelium-like than in the heparinized cultures. Giemsa. × 100

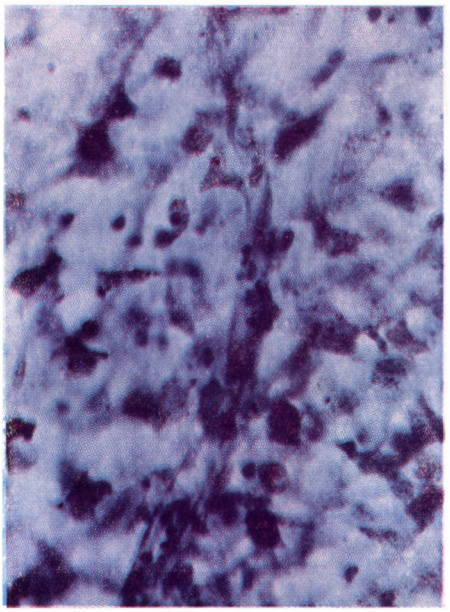

Fig. 6. Appearance of embryonic thymus culture, 72 hours after heparinization. Development of endothelial tube, with many metachromatic cells around it. Giemsa. × 100

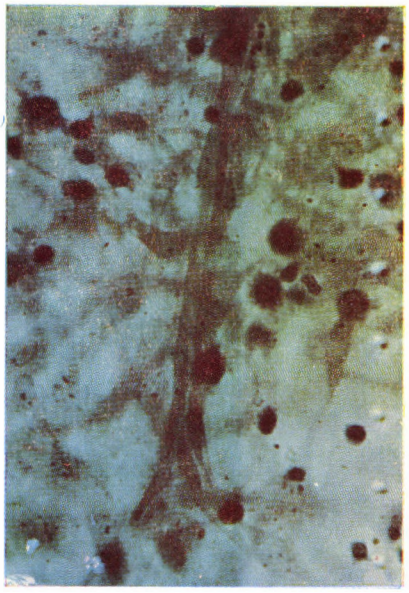

Fig. 7. 6-day old control thymus culture. Even around the endothelial tube there are no metachromatic cells. Giemsa. × 100

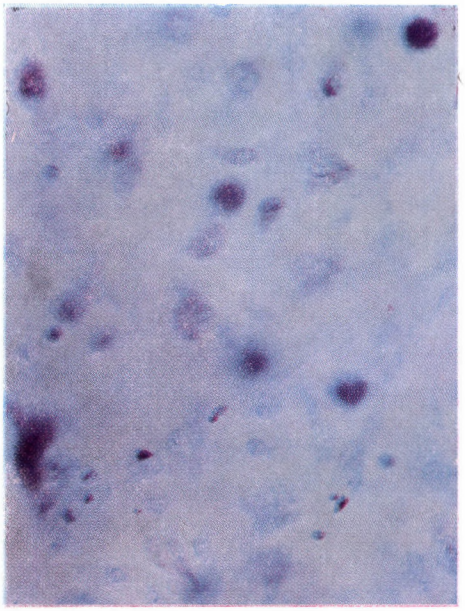

Fig. 8. Thymus culture from newborn rat, 72 hours after heparin treatment. Many metachromatic cells with nuclei characteristic of thymocytes. Toluidine blue. × 200

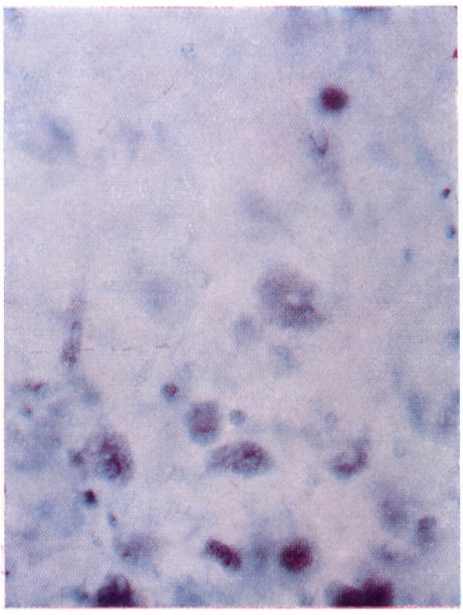

Fig. 9. The same as Fig. 8. Mastcell-like cells, a few of them still resembling thymocytes. Toluidine blue. × 200

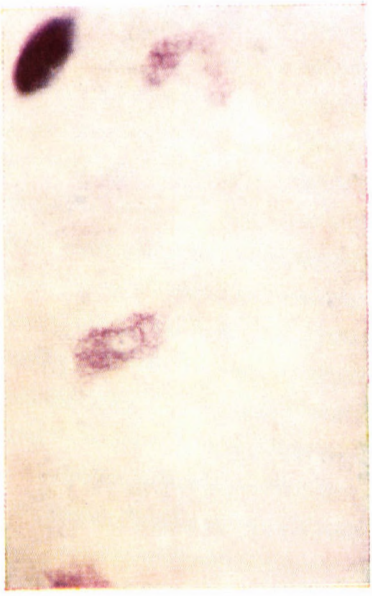

Fig. 10. The same as *Fig. 9.* Regular mast cells have developed. Toluidine blue. × 200

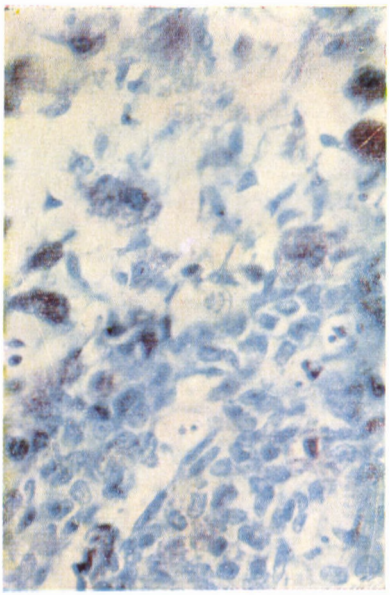

Fig. 11. Section of embryonic thymus culture 72 hours after heparin treatment. Big mastcell-like metachromatic cells at the periphery. Giemsa. × 200

Fig. 12. Embryonic spleen culture 72 hours after heparin treatment. Small, lymphocyte-like cells containing metachromatic substance. Giemsa. × 100

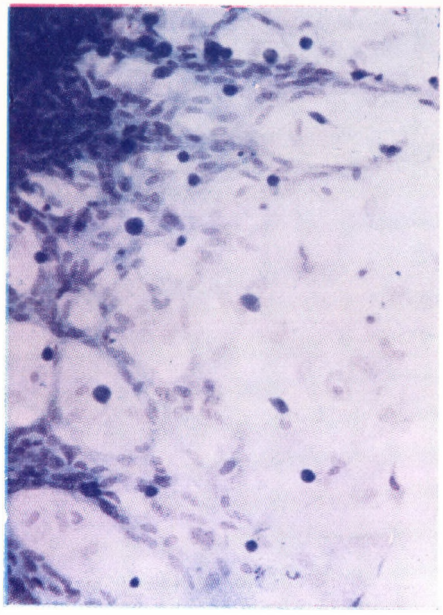

Fig. 13. Embryonic lymph node culture 72 hours after heparin treatment. Azur metachromatic substance in the cytoplasm of a few lymphocyte-like cells. Giemsa. × 100

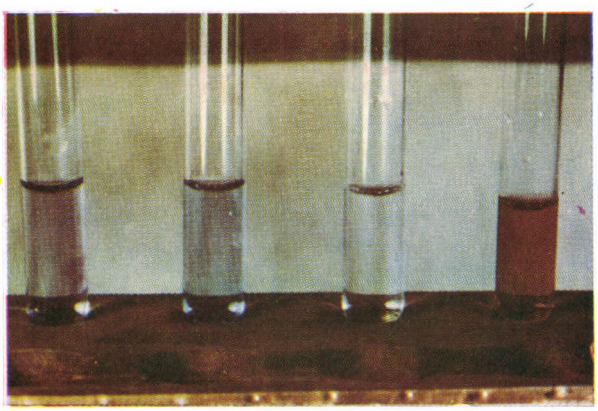

Fig. 14. Full-value and decomposed heparin stained with toluidine blue and PAS. Tubes 1 and 2 : intact and decomposed heparin stained with toluidine blue. Tubes 3 and 4 : intact and decomposed heparin stained with PAS

the experiments strongly supported our observations made *in vivo* that thymocytes develop first into PAS-positive cells and later into mast cells showing toluidine metachromasia. Likewise, our experiments corroborate the results obtained by LEHNER.

The genesis of these mast cells is a problem *in se*. We have namely found that if a mixture of glucuronic acid and glycosamine is added to the cultures, no metachromatic cells are formed. If we consider this finding in the light of the evidence that in response to the subcutaneous injection of glycosamine regular mast cells appear in guinea pigs, we feel justified in suggesting that whereas in that case the material responsible for metachromasia was available, in the tissue culture it was not present. As far as the structure of heparin is known, the attachment of sulphate in the ester seems to be the factor to be considered in the first place ; the cells can synthesize heparin probably only in its presence.

The fact that thymus cultures grew more intensively with decomposed heparin than without it suggests that this substance may be essential for the growth of the thymus.

In the thymus the decrease in the number of epithelial elements was associated with a decrease in the mast cell producing ability, whereas as the same time an increased number of metachromatic cells appeared, chiefly in the lymph nodes. This may suggest that a considerable percentage of the thymus cells has already been transferred to the lymph nodes, or it may imply that at the time of the involution of the thymus the organism starts compensatory processes in other organs. At any rate, these cells are not of full value, as it is indicated also by the fact that they are not toluidine-metachromatic, but show merely azur-positivity, as the same time when the cells in the thymus show a regular toluidine metachromasia.

Summary

In tissue cultures a tissue reaction has been provoked that had been observed on the thymus *in vivo*. Decomposed heparin (one which reacts positively only to PAS) was added to cultures of thymus, lymph nodes and spleen from rats of different ages and it has been found that in the epithelial reticulum of the thymus and in the thymocytes a metachromatic substance had been synthesized and regular mast cells had been formed. In the spleen and lymph nodes only very slight number of rudimentary cells showed metachromasia. The experiments corroborated the evidence obtained *in vivo* for the tissue reaction of the thymus.

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ИСКУССТВЕННО ВЫЗВАННАЯ ТКАНЕВАЯ РЕАКЦИЯ ЗОБНОЙ ЖЕЛЕЗЫ В ТКАНЕВЫХ КУЛЬТУРАХ

ДЬ. ЧАБА, И. ТЁРЁ и Э. КАПА

В своих опытах авторы искусственно вызывали в тканевых культурах наблюдаемую ими прижизненно тканевую реакцию зобной железы. К тканевым культурам зобной железы, лимфатических узлов и селезенки крыс различного возраста они добавили расщепленный — исключительно PAS положительный — гепарин и установили, что в эпителиальной сеточке зобной железы и в тимоцитах синтетизируется метахроматическое вещество и образуются регулярные тучные клетки. Появление метахроматических клеток наблюдается в селезенке и в лимфатических узлах только в форме зачаточных клеток в совершенно минимальном количестве. Проведенными опытами авторам удалось подкрепить сделанные прижизненно наблюдения тканевых реакциий зобной железы.

PROVOZIERTE GEWEBSREAKTION DES THYMUS IN GEWEBSKULTUREN

GY. CSABA, I. TÖRŐ und E. KAPA

In Gewebskulturen wurde die in vivo beobachtete Gewebsreaktion der Thymusdrüse provoziert. Zu Gewebskulturen des Thymus, des Lymphdrüse und Milz von Ratten verschiedenen Alters wurde gespaltenes — ausschliesslich PAS-positives — Heparin zugefügt und festgestellt, dass im Epithelretikulum der Thymusdrüse und in den Thymozyten metachromatischer Stoff synthetisiert wird und reguläre Mastzellen entstehen. Das Erscheinen der metachromatischen Zellen kann in der Milz in den Lymphdrüsen nur bei einer ganz minimalen Anzahl von rudimentären Zellen beobachtet werden. Durch diese Versuche gelang die in vivo gemachten Beobachtungen im Zusammenhang mit der Gewebsreaktion der Thymusdrüse zu bestätigen.

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