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NEW CONTRIBUTIONS TO THE HEPARIN-AFFINITY OF THE THYMUS. I

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As it has been described [1, 2, 3, 5, 7, 8, 9, 10], in conditions associated with tissue proliferation the rat and the mouse thymus produces an uniform tissue reaction, consisting of the appearance of a PAS-positive substance, its accumulation in the corpuscles of Hassall and in the thymocytes, as well as of an increased formation of mast cells. The PAS-positive substance added to a thymus tissue culture in the form of heat-treated, modified heparin is synthesized as a metachromatic substance in the cells some of which correspond to regular mast cells. This finding was looked upon as proving that while the cells of the lymph nodes and of the spleen are unable to take up the components of the metachromatic substance or to synthesize its complete form, the thymus produces the substance in large quantities.

These experiments have left several problems unanswered, among them the affinity of the thymus to heparin substances, the elective nature of this affinity, and whether this function requires the presence of the whole organ or single thymus cells, too, are capable of the function.

Methods

Observations were made in 200 tissue cultures of the Maximow type. The materials tested originated from rats ranging in age from newborn to three days. Specimens of thymus, liver, spleen and lymph nodes were explanted on coagulated chicken plasma and 48 hours later washed with a mixture of horse serum, chicken embryo juice, Tyrode's solution (3 : 1 : 6) for one hour. Twenty-four hours later, *i. e.* 72 hours after explantation, the specimens were washed with the above-described mixture to which this time was added 1 mg/ml of heparin (Heparin pulvis, Richter, 80 I. U.) kept previously in a water bath at 56° C for 48 hours and boiled for 4 hours. (The heparin thus treated loses its metachromasia but retains its PAS-positivity.) Subsequently the cultures were washed at 48-hour intervals with heparin-free washing fluid. The first fixation of thymus tissue was made 72 hours, of other organs 96 hours, after washing with heparin; then fixation was continued at 48 hour intervals for 10 days and at 24 hour intervals for another 10 days. The cultures were stained with toluidine blue, some *in toto*, others in serial sections.

Results

Thymus

Seventy-two hours after washing with heparin many metachromatic cells are visible partly in the central area and partly at the periphery; a few scattered

ones in other parts. The cells nearer to the centre are similar to epithelial cells, while those at the periphery have nuclei like the thymocytes, but in a swollen cytoplasm. The mother piece is vital, necroses are not visible in it *(Fig. 1)*.

In the migration zone almost every cell, either thymocyte or epithelial cell, contains heparin; mitoses are frequent and the intracellular granulation is dense. At the periphery of the migration zone there are less metachromatic cells than near the centre. The cultures grow more intensely than the controls *(Fig. 2)*.

In the 5-day culture many cells have migrated from the mother piece, though about half of this is still dense. The cytoplasm of metachromatic cells continues to increase.

In the *in toto* cultures mitotic cells abound, corresponding to the intense rate of growth. At the periphery there are few metachromatic cells, even less than in the younger cultures, but masses of them are seen around the mother piece.

In the 7-day cultures the mother piece is filled with metachromatic cells and the central area is delimited from the periphery by a very thin zone composed of non-metachromatic cells. The migration zone contains great numbers of metachromatic cells, most of them with thymocyte-like nuclei. An interesting feature *(see Fig. 3)* is the way in which metachromatic cells are leaving the tissue.

Ten days after washing with heparin the picture is similar to that outlined above, except that the size of the mother piece is definitely reduced; the number of cells in the migration zone is practically unchanged *(Figs. 4, 5)*.

The 11-day-old preparation is quite remarkable in that the mother piece is composed of several small islets with many metachromatic cells. In the migration zone a considerable number of heparin-containing cells is still present, though the cells stain paler, lighter *(Figs. 6, 7, 8)*.

In the 18-day-old mother piece continuity is almost completely lost. Most of the epithelial cells still present are metachromatic, and some polynuclear cells are visible between the islets. There are metachromatic cells in the zone of migration *(Fig. 9)*.

Twenty days following heparin treatment very few cells are in the mother piece, many of them metachromatic. Metachromatic cells occur also in the migration zone.

Liver

Ninety-six hours following heparin treatment some of the few epithelial cells growing out from the liver tissue show slight metachromasia. In the mother piece there are definitely metachromatic epithelial cells. The central necrosis is very marked.

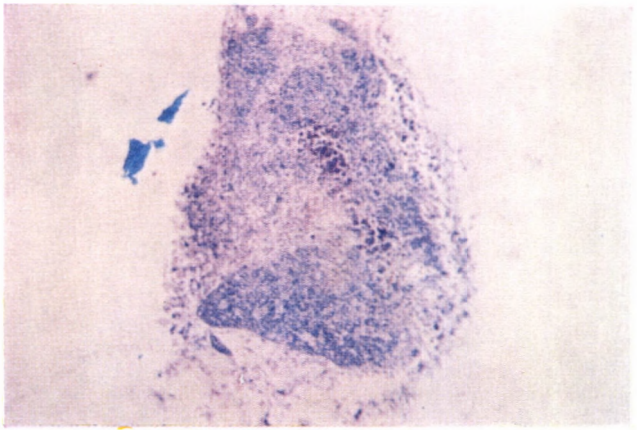

Fig. 1. Mother piece, three days after treatment. In the marginal zone cells staining blue with toluidine, in the centre metachromatic cells. Toluidine blue, ×60

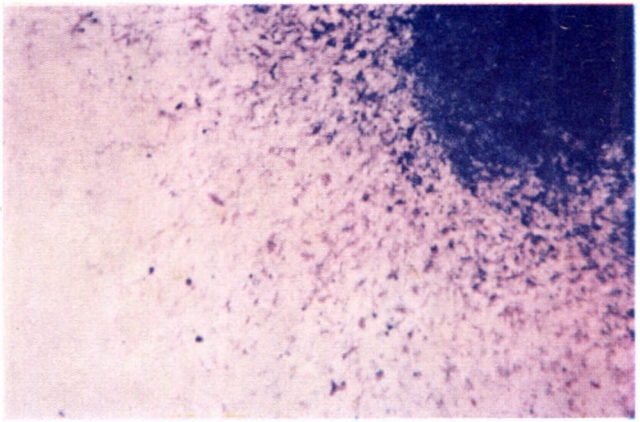

Fig. 2. Same as in *Fig. 1.* Culture stained *in toto.* Many metachromatic cells in the zone of migration. Toluidine blue, ×60

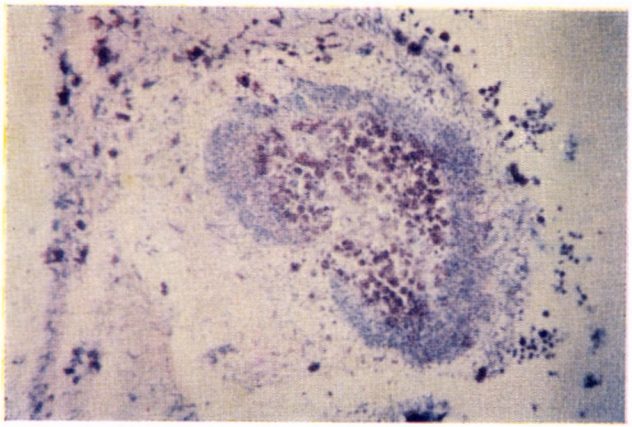

Fig. 3. Seven days after treatment: abundant metachromasia. The picture is remarkable because it appears as if the metachromatic cells would be flowing out into the migration zone through a gate formed by non-metachromatic cells. Toluidine blue, ×60

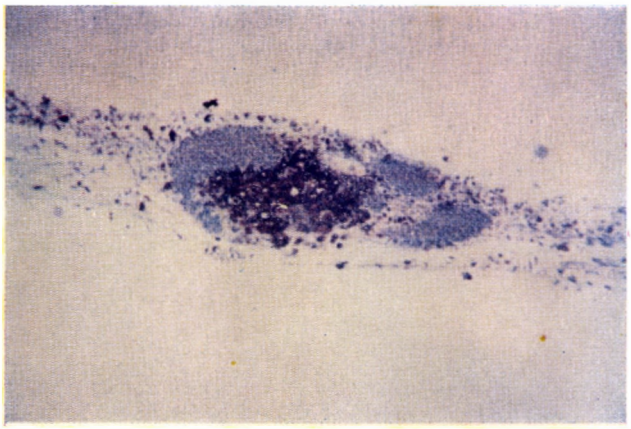

Fig. 4. Section of a culture, 10 days after treatment. The central area is filled with metachromatic cells, at the periphery no metachromatic cells are visible. Toluidine blue, ×60

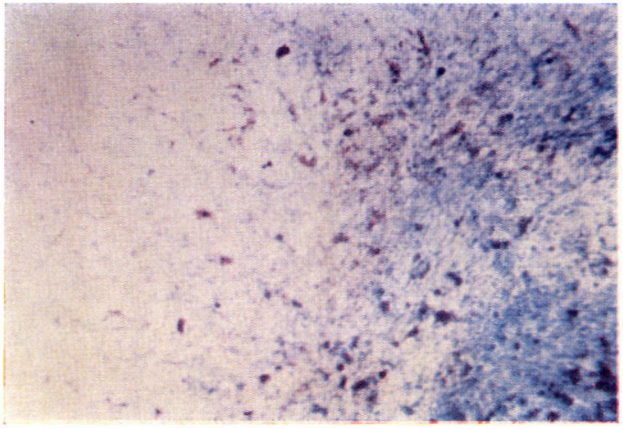

Fig. 5. The same, in an *in toto* preparation. Large numbers of metachromatic cells in the migration zone around the mother piece. Toluidine blue, ×60

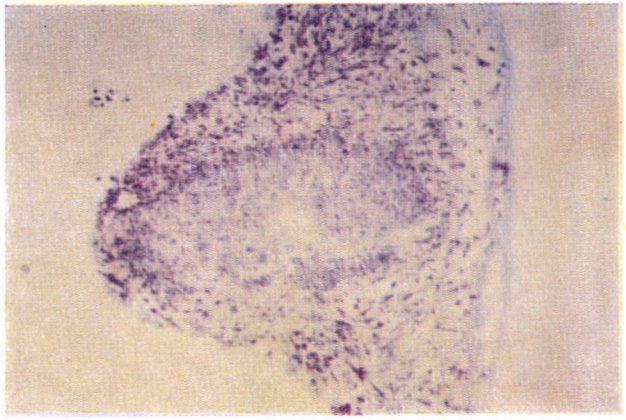

Fig. 6. Section from a culture, made on the 12th day. The mother piece is poor in cells, but many of them are metachromatic. Toluidine blue, ×60

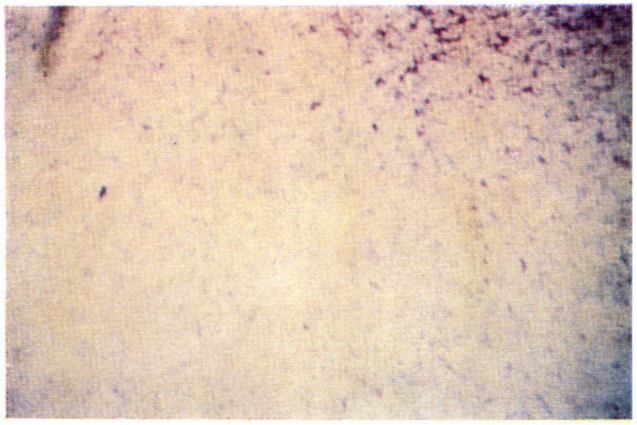

Fig. 7. The same, in an *in toto* preparation. There are no metachromatic cells in the peripheral part of the migration zone. Toluidine blue, ×60

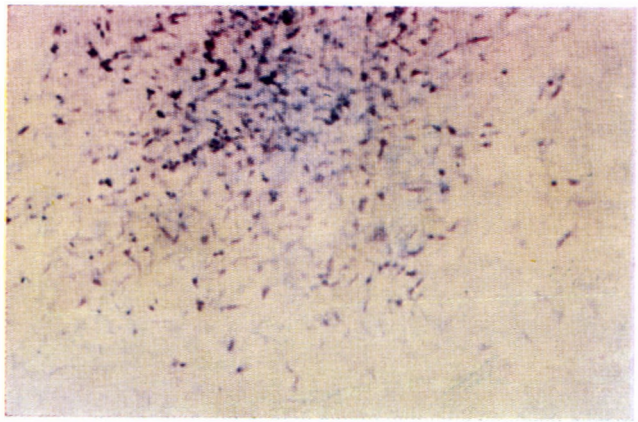

Fig. 8. 16 days after treatment. Many big, metachromatic cells in the migration zone. Toluidine blue, ×60

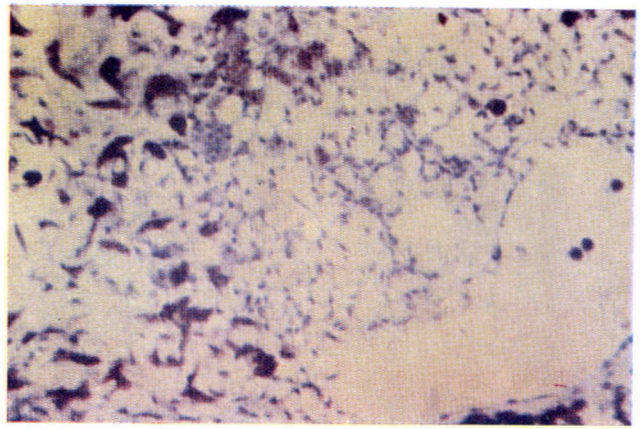

Fig. 9. 18 days after treatment. There are still many metachromatic cells in the section. Near the centre there is giant cell syncytium. Toluidine blue, ×60

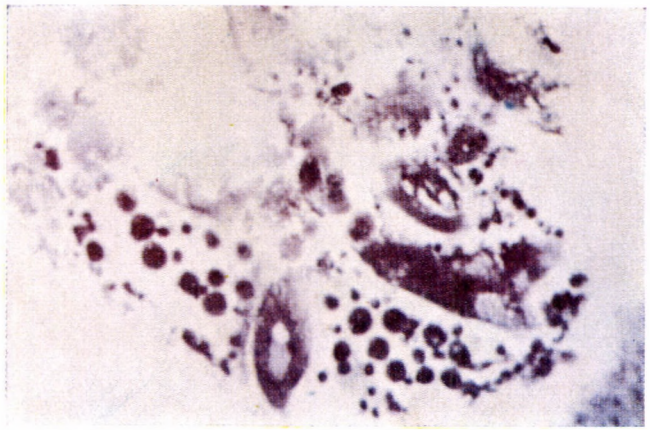

Fig. 10. Section made from a liver culture, 12 days after treatment. Greatly swollen liver cells, many rounded forms. Toluidine blue, ×60

Six days after treatment a few big, metachromatic cells appear in the zone of migration. Large numbers of big metachromatic cells are visible in the mother piece; these are swollen and abound in heparin. The majority occurs in the central area, the marginal zone is composed of heparin-free cells.

Nine days after treatment metachromatic cells are no longer detectable in the zone of migration, but many granules are found in the epithelial cells of the mother piece. Later, some multinucleated cells, giant cells and many degenerated forms appear *(Fig. 10)*. No other cytological change takes place until the end of the culture period. The number of cells in the mother piece continues to decrease and no metachromatic cells are visible in the zone of migration.

Spleen

Six days after heparin treatment do the first metachromatic cells appear in the zone of migration. At 9 days 1 or 2 cells appear, and no further increase in their number takes place until the end of the culturing period. Before the 6th day the mother piece contains no metachromatic cells; in the 9-day specimen 1 or 2 fibrocyte-like cells occur, showing slight metachromasia; these cells do not increase in number until the end of the period of cultivation.

Lymph node

Ninety-six hours and 6 days following treatment a few lymphoblast-like, round, metachromatic cells appear in the migration zone, next to the mother piece. At 9 days a few apparently viable metachromatic cells are visible; these are not mast cells, being different from them in both size and shape. Their number increases slightly until the 12th day, but there is no further increase during cultivation. Metachromatic material is found occasionally in some cells of the mother piece.

Discussion

According to the results thymus tissue has an extremely marked affinity to the heparinoid substance tested. A single washing with the heat-treated heparin sufficed to cause the appearance of metachromatic cells in both the migration zone and the mother piece; these cells are still present as late as 20 days after treatment. If we compare this evidence with that obtained for the spleen and lymph nodes, we seem to stand on firm ground when stating that of the three it is only the thymus that can synthesize heparin. This finding suggests that the thymus may possess this specific function also in the living organism.

The situation is different in the case of the liver. The parenchymal cells of this organ seem to be capable of synthesizing heparin. But while in the

thymus this function is associated with the formation of mast cells, this does not happen in the liver, as suggested by the fact that the cells accumulate heparin, undergo excessive swelling, form many giant cells and yet there are practically no mobile metachromatic cells in the zone of migration. The morphological picture is normal insofar as it is taken for proved that the liver can synthesize heparin.

The thymus seems therefore to be the only organ capable of producing full value mast cells while the spleen and the lymph nodes are practically incapable of that function. The liver seems to synthesize heparin, but this finds no expression in the migration of mobile cells; the cells are storing this material, as it is known also from the literature, the spleen and the lymph nodes produce plasma cells but no mast cells [4, 6].

Metachromasia was the strongest in the cells of the thymus mother piece and in the zone of migration, next to the mother piece. The farther from the mother piece the cells are migrating, the more they lose of their metachromasia; those lying most peripherally show no metachromasia at all. This may have two explanations. As heparin is added just once to the cultures, the cells migrating outward may lose their heparin content and, gaining access to no further heparin, gradually lose their metachromasia. The other possibility is that the epithelial cells of the mother piece have taken up the components of heparin, synthesize heparin of full value and give it over to the easily migrating cells. In this case the process would be as has been suggested on the basis of our experiment *in vivo*. As in our earlier tissue cultures treated with heparin at 48-hour intervals metachromatic cells were found exclusively near the mother piece, the second possibility is what probably occurs. It seems therefore that heparin synthesis requires that the thymus should not act as a special tissue and isolated cells are capable of synthesizing heparin.

Summary

The effect of a single treatment with heparin on tissue cultures of rat thymus, liver, spleen and lymph nodes has been studied. Thymus has been found to have the strongest affinity and a single treatment heparin ensured continuous migration of metachromatic cells for 20 days. Thymus tissue is essential for the synthesis of heparin, independent cells are not capable of that function. The liver, too, synthesizes heparin, but produces no mast cells. The spleen und lymph nodes play no role in the synthesis of heparin.

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О СРОДСТВЕ ЗОБНОЙ ЖЕЛЕЗЫ К ГЕПАРИНУ. I

ДЬ. ЧАБА, И. ТЕРЕ и Е. КАПА

Исследовалось действие однократной обработки гепарином на тканевые культуры зобной железы, печени, селезенки и лимфатических узлов крыс. Было установлено, что из исследованных тканей наибольшее сродство выявляет зобная железа и однократное промывание гепарином обеспечивает в течение 20 дней непрерывную миграцию метахроматических клеток. Далее было установлено, что к синтезу гепарина необходима ткань зобной железы, одиночные клетки неспособны синтетизировать гепарина. Печень также способна синтетизировать гепарин, однако, образования тучных клеток в печени не происходит. В синтезе гепарина селезенка и лимфатические узлы не имеют значения.

NEUERE BEITRÄGE ZUR HEPARIN-AFFINITÄT DES THYMUS. I

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Die Wirkung einmaliger Heparinbehandlung auf Gewebekulturen aus Thymus, Leber, Milz, und Lymphknoten von Ratten wurde untersucht und festgestellt, dass von den untersuchten Geweben die Thymusdrüse über die stärkste Affinität verfügt. Eine einmalige Behandlung mit Heparin gewährleistet 20 Tage hindurch eine ununterbrochene Migration der metachromatischen Zellen. Es wurde ferner festgestellt, dass zur Heparinsynthese Thymusgewebe erforderlich ist, isolierte Zellen sind dazu nicht fähig. Die Leber kann gleichfalls Heparin synthetisieren, doch erfolgt in der Leber keine Mastzellenbildung. Die Milz und die Lymphdrüsen kommen hinsichtlich der Heparinsynthese nicht in Betracht.

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