Transport of Mast Cells by the Blood Circulation

Mast cells appear in a very great number in the subcutaneous connective tissue and peritoneal fluid of rats[1]. In our previous experiments[2–5] we have established – and it has been confirmed also by other authors[1,6–8] – that mast cells form, primarily through the transformation of lymphoid elements[4,9], in the thymus and lymph nodes. The proportion of young mast cells is decreased in the peritoneal fluid after the extirpation of the thymus[5] which infers that the mast cell formation capacity of the thymus influences also the periphery. It is, however, not clear whether the removal of the thymus effects a decrease in the peritoneal fluid of such lymphoid cells as are capable of transformation also on the periphery, or whether it brings about a cessation in the outflow of mature mast cells from thymus to periphery. COMBS, LAGUNOFF and BENDITT[10] as well as we ourselves[5] could establish in the peritoneal fluid young lymphoid mast cells, which we have described previously; thus their transformation into mature mast cells seems to be probable. In spite of the possibility that mature mast cells might also become transported to the periphery – particularly if necessitated by a rapid reaction – cannot be discarded. We do not, however, know what could be the route of such transportation. Cells can reach other regions of the organism from the thymus and lymph nodes primarily through the blood flow and lymphatic vessels. The presence of tissue mast cells in the blood of the rat has so far not been reported[1]. We presume that they are present in such a low number that they have so far escaped the attention of the investigators. Thus we attempted to raise the number of mast cells possibly present in the blood with the aid of ligatures, and partly by medicinal treatment, in order to make possible their detection.

We injected a rinsing solution containing 10 ml 0.7% NaCl and 1.5% sodium citrate into the abdominal cavity of rats. After a 3 min pause – during which the rats were anaesthetized with ether – we opened the abdominal cavity and collected the rinsing fluid, dropped it on slides and let it dry at room temperature. Continuing the cutaneous incision of the median laparatomy – made in the interest of the removal of the rinsing fluid – on the thorax, we detected and prepared the axillary veins running below the pectoral musculature and the jugular veins before their entrance into the thoracic cavity, slipping surgical seams under them. In the exposed abdominal cavity the inferior vena cava was detected at the point where it leaves the upper margin of the liver and a loose loop placed on it. Then the diaphragm was transected in a semi-circle and the thoracic cavity exposed. At this point the preplaced seams were severed and the veins ligated. After a period of about 20 sec, a few drops of blood were drawn with a syringe from the right part of the heart. Thus we succeeded in obtaining this blood containing cells ensuing primarily from the thymus and the thoracic duct. Smears were made and these, as well as the peritoneal preparates, were stained after Carnoy fixation with iron-alum-alcianblue-safranin, according to Balogh's modification[11] of the acidic mucopolysaccharide staining of SPICER[12].

For the purpose of quantitative counts, the blood was drawn up in a melanger-pipette used for white blood cell counts, was mixed in 1:100 proportion with Türk solution containing a few drops of saturated solution of toluidine blue and in a few minutes the mast cells, which appeared in purple colour, were counted in the hemacytometer. For the evaluation of smears mast cells were counted in 1500 visual fields in 600-fold magnification.

In another experimental group each rat received 5 mg/100 g body weight cortisone (Adreson-Organon) for 6 days. On the eighth day, examinations were performed in the above described manner. In all experiments 24 male Wistar CB rats of 170–200 g weight were used.

The results of the experiments (Table I) show that tissue mast cells can be found in the blood although only in a small number. The prolonged cortisone treatment, however, increased the number of mast cells in the blood as it did in the lymphatic organs and peritoneal fluid[5]. This increase is about the tenfold of the original value, if we regard the values obtained in the hemacytometer; however, it is substantially greater – about 45-fold – if we examine the smears. This difference can be explained by the fact that in the hemacytometer only such mast cells can be recognized which supravitally absorb toluidine-blue, whereas the alcian-blue-safranin method – which is suitable also for the staining of the juvenile forms – gives a better result[5,11]. The mast cells found in the blood conform morphologically those which are found also in the peritoneal fluid and the developmental forms are also present (Figures 1, 2, 3). For the appraisal of the developmental forms, the observations of COMBS et al.[10] and the classification which proved to be suitable in our previous studies at the evaluation of the results was taken into consideration.

Table I. Quantity of mast cells in the blood

	Smears/ 1500 visual fields	Blood cell/mm³
Control	0.6	6.5
Cortisone treated	27.2	67.4
Significance	$p < 0.01$	$p < 0.01$

Table II. The proportion of mast cells in the blood and peritoneal fluid in percent

	Lymphoid	Mixed	Mature	Destroyed
Blood of cortisone treated rats	8.1	18.4	45.5	28.0
Peritoneal fluid of cortisone treated rats	6.2	12.0	11.0	70.8
Peritoneal fluid of controls	30.5	36.2	32.6	0.7

[1] H. SELYE, *The Mast Cells* (Butterworths, Philadelphia 1965).
[2] G. CSABA, I. TÖRÖ, T. ÁCS and F. I. KISS, Acta morph. hung. *9*, 187 (1960).
[3] G. CSABA, I. TÖRÖ and K. MOLD, Acta Anat. *48*, 114 (1962).
[4] G. CSABA and I. OLÁH, Acta biol. hung. *19*, 347 (1968).
[5] G. CSABA, L. SURJÁN JR., J. FISCHER, J. KISS, I. TÖRÖ JR., Acta biol. hung., in press (1969).
[6] H. GINSBURG, Ann. N.Y. Acad. Sci. *103*, 20 (1963).
[7] H. GINSBURG and L. SACHS, J. natn. Cancer Inst. *31*, 1 (1963).
[8] R. KLEIN, C. r. Soc. Biol. *157*, 718 (1963).
[9] G. CSABA, J. KISS and I. OLÁH, Acta. biol. hung., in press (1969).
[10] J. W. COMBS, D. LAGUNOFF and E. P. BENDITT, J. Cell Biol. *25*, 577 (1965).
[11] G. CSABA, Acta biol. hung., in press (1969).
[12] S. S. SPICER, J. Histochem. Cytochem. *8*, 18 (1960).

As a result of cortisone treatment, besides the rise of the number of mast cells in the peritoneal fluid, a decided shift to the right was observable (Table II); this confirms our previous findings[5]. This shift to the right with concomitant appearance of damaged mast cells was found also in the blood picture (Table II; Figures 4 and 5). However, here the ratio of the damaged mast cells is much less significant than among the peritoneal mast cells. Due to the smallness of the total mast cell number, the ratio of the developmental forms could not be established in the blood of cortisone-treated animals.

Thus, upon the basis of the experiments, it may be established that the developmental forms of mast cells and mature mast cells are present in the blood of rats and upon the effect of cortisone treatment they increase which, in the light of our experimental system, renders it probable that the mast cells formed in the thymus and lymph nodes entered the circulation. Thus the transportation of mast cells from the site of generation to the periphery via the circulation seems to be reasonable. Besides, numerous signs[5] infer that mast cells can differentiate in loco from other, primarily from lymphatic, elements; the presence of quite young mast cell-forms in the peritoneal fluid also supports this assumption.

Fig. 1 Fig. 3

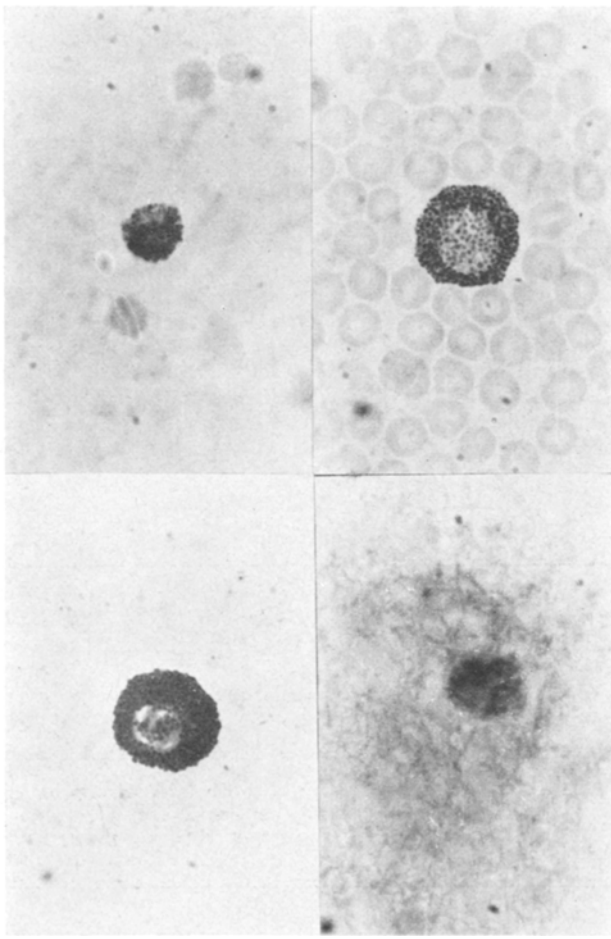

Fig. 2 Fig. 4

Fig. 1. Young mast cell of mixed granulation in the blood. Alcian-blue-safrain, oil immersion. × 900.

Fig. 2. Safranin positive mast cell in the blood. Alcian-blue-safranin, oil immersion. × 900.

Fig. 3. Safranin positive mast cell in the blood. Alcian-blue-safranin, oil immersion. × 900.

Fig. 4. Damaged mast cell in the blood. The halo of the cell is alcian-blue positive, oil immersion. × 900.

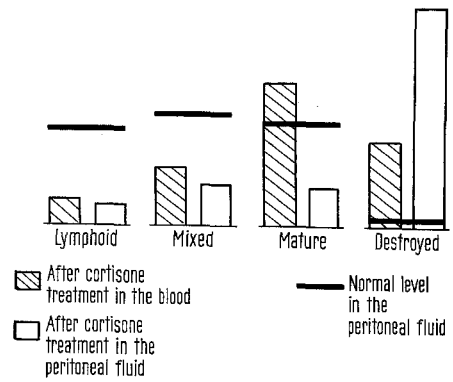

Fig. 5. Effect of cortisone on mast cell proportion. Columns show the proportion of the blood and peritoneal fluid. Dark lines mark values in the peritoneal fluid of untreated animals. Normal blood values are not shown as, due to low values, the proportion could not be established.

Zusammenfassung. Untersuchungen der Gewebemastzellen im Rattenblut ergaben eine bedeutende Zunahme der Mastzellen nach Behandlung mit Cortison. Ein Transport der Mastzellen von lymphatischen Organen über den Blutweg in die Peripherie wie auch eine lokale Umwandlung lymphoider Elemente in Mastzellen wird experimentell wahrscheinlich gemacht.

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Increased Lipid in Thymic Macrophages Parasitized by *Mycobacterium lepraemurium*

Macrophages engulf mycobacteria in vivo and in vitro, and lysosomal enzymes attempt, too often unsuccessfully, to kill and degrade these bacilli[1] (non-specific cell resistance). In mice, the macrophage is the sole site of proliferation of the 'rat leprosy' bacillus (*M. lepraemurium*) which therefore is an *obligate intracellular parasite*, and has a generation time of approximately 7 days. Experimental disease is chronic and the macrophages in almost all body tissues, including the reticulo-endothelial system, become heavily involved. Here, in a preliminary investigation we found lipid material abundant within parasitized macrophages in the mouse thymus, whereas

[1] G. B. Mackaness, Am. Rev. resp. Dis. *97*, 37 (1968).