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

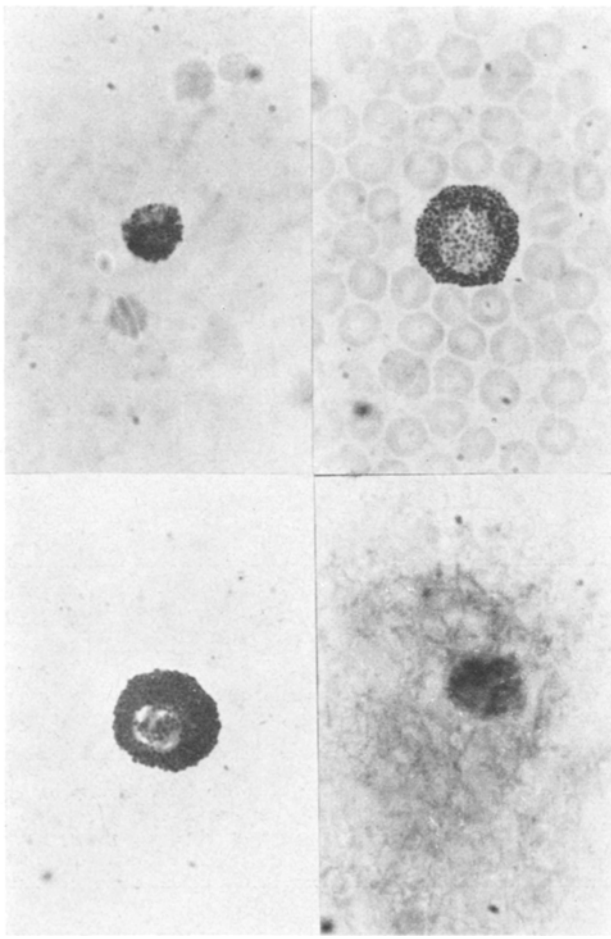

Fig. 2 Fig. 4

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.

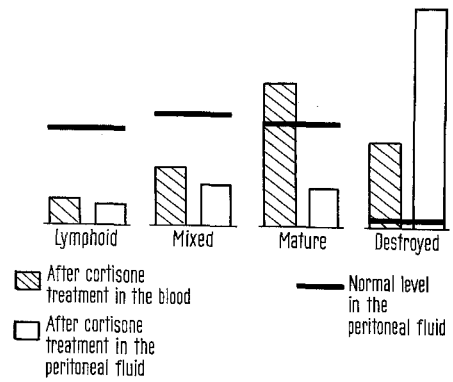

Fig. 5. Effect of cortisone on mast cell proportion. Columns show the proportion of the blood and peritoneal fluid. Dark lines mark values in the peritoneal fluid of untreated animals. Normal blood values are not shown as, due to low values, the proportion could not be established.

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).

similar cells in lymphoid tissues were relatively unaffected.

Methods. Systemic infection was produced in female albino *P* strain mice, 16–18 g, by i.v. inoculation of a suspension of viable *M. lepraemurium* (Douglas strain). Death resulted 5–6 months later. At various intervals after inoculation mice were killed, both lobes of the thymus and the parathymic lymph nodes en bloc[2], as well as liver, spleen and inguinal lymph nodes were removed for histochemical examination.

These tissues were fixed for 3 h in formol-acetic-alcohol and 70% ethanol, and paraffin-wax embedded. In order to demonstrate lipid material, tissue sections (4 μ thickness) were stained by immersing in 0.1% methasol fast blue in 95% ethanol (6–18 h at 60 °C). Slides were rinsed in 70% ethanol and transferred for 10 min to 0.5% lithium carbonate solution. After washing the slides in water, bacilli were stained 3 min with 1% new fuchsin plus 5% phenol in a solvent of 10% ethanol. Then the sections were immersed 1 min in saturated lithium carbonate solution and differentiated 2–5 min in 2 changes of 5% glacial acetic acid in absolute ethanol, dehydrated, cleared and mounted. Bacilli stained bright red, macrophage cytoplasm light blue, lipid aggregates blue to blue-black. Evidence provided by PEARSE[3], and by SALTHOUSE[4,5] strongly suggests that these aggregates are phospholipid.

Results. In comparison with lymphoid tissues entry of bacilli into the thymus was delayed and occurred a few weeks after bacillary multiplication began elsewhere in the body. Therefore it is likely that parasitized macrophages resided in lymphoid masses for this period of time prior to their migration into the thymus. Macrophages

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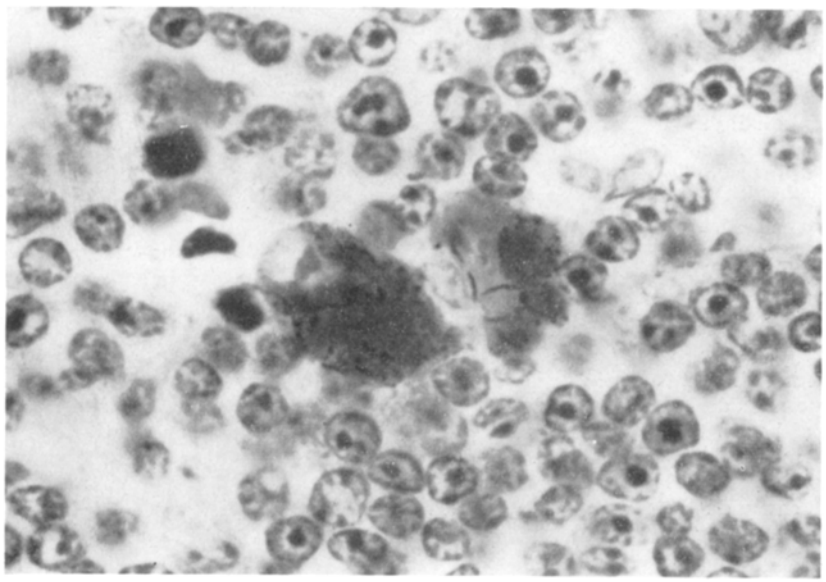

Fig. 1. A small area of the thymic cortex 2 months after inception of infection showing 2 adjacent macrophages in which the bacilli are almost obscured by tightly packed lipid granules (probably phospholipid). Methasol fast blue plus new fuchsin stains. × 1950.

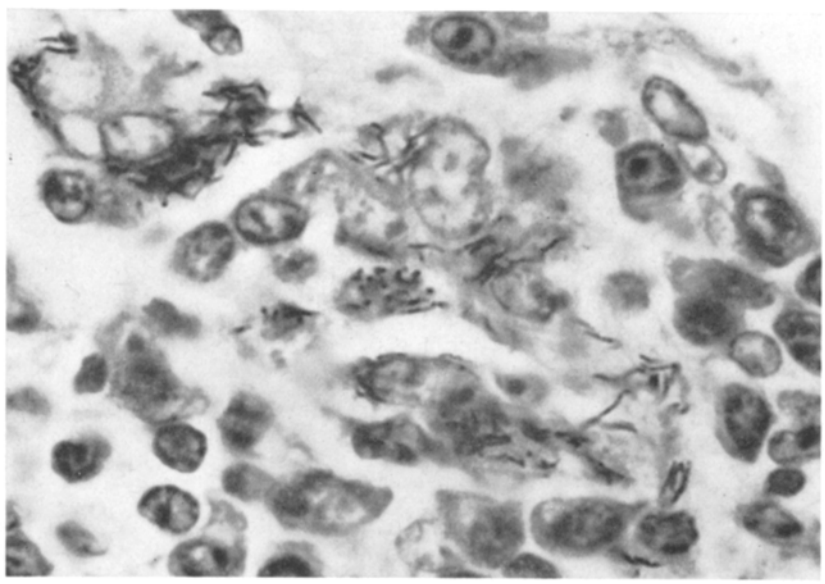

Fig. 2. A cluster of parasitized macrophages within the medulla of the parathymic node (same mouse) and free of lipid material, 2 months after inception of infection. Some of the bacilli are 'beaded' in appearance. Methasol fast blue plus new fuchsin stains. × 1950.

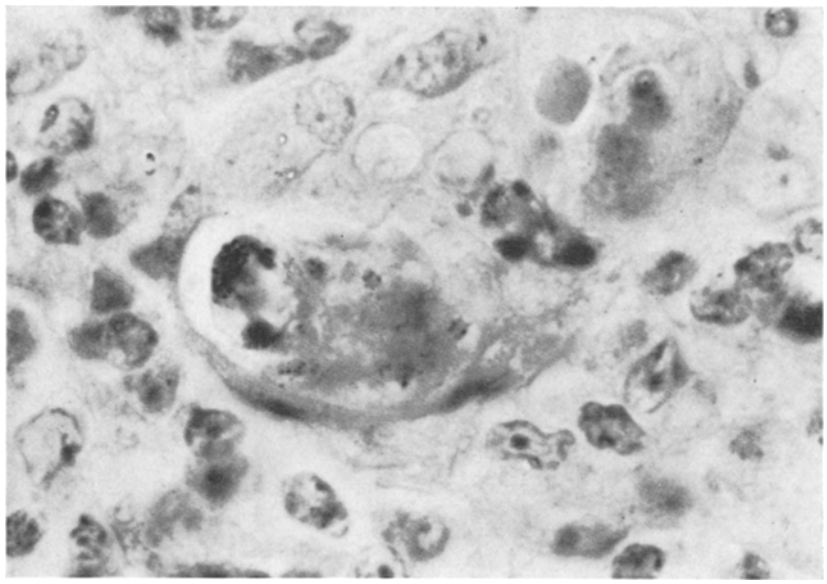

Fig. 3. Hassall's corpuscle containing lipid granules in the thymic medulla about 2 months after inception of infection. Methasol fast blue plus new fuchsin stains. × 1950.

carrying bacilli appeared to infiltrate into the thymic cortex by passing through the walls of blood vessels located in the intra- and inter-lobular septa. About 2 months after inception of the infection tightly packed lipid granules (or methasol fast blue positive material) appeared in parasitized thymic macrophages as shown in Figure 1, and in giant cells. Similar cells clustered together in the medulla of parathymic and other lymph nodes, as well as all other tissues examined, were without demonstrable lipid (Figure 2). Also at this stage it appeared that macrophages occasionally passed from the thymic cortex and into the medulla, retaining their content of lipid. However, in the cortex of a lymph node only a few small parasitized cells were found, although these contained lipid material the amount was markedly less than that in the thymus. The lipid material is probably phospholipid containing choline[6], and was easily demonstrated only before the entire cytoplasm of the host cell was replaced by bacilli, which occurred by about 3 months.

By 4–6 months later in the infection, macrophages preponderated and were slightly more heavily parasitized in the thymus than those in lymphoid tissues. Hence lipid material may somehow have enhanced bacillary multiplication.

It is of interest that only those parasitized macrophages in contact with either lymph node lymphocytes, or more especially cortical thymocytes, produced lipid material. It is extremely doubtful whether the intracellular bacilli themselves are capable of producing this amount of lipid material and depositing it outside of their own bodies and phagocytic vacuoles. Hassall's corpuscles (or 'cysts') in the mouse thymus are invariably small and scanty. When present by about 2 months in infected glands they contained fairly small deposits of lipid granules (Figure 3).

Discussion. There is no doubt that development of the thymus gland precedes and influences that of lymph nodes and is essential for the maturation of immune competence in both animals and man[7]. Hence it is of considerable interest to the study of mycobacterial immunity that, for example, after i.v. inoculation of *M. tuberculosis* a small proportion enter the mouse thymus[8]. Because the host soon dies of pulmonary complications these particular bacilli do not have much opportunity for proliferation and local dissemination throughout the gland itself. Thus *M. lepraemurium*, like the tubercle bacillus, ultimately penetrates the so-called 'thymic barrier' to antigens[9]. In addition, KÖLSCH[10] has recently reported that macrophages carrying antigen migrate into the mouse thymus.

Finally, the preceding findings give some support to the recent discovery by BLAU[11–14] in which an intimate though transient relationship was demonstrated between migrant macrophages, containing either particulate matter or foreign protein, and Hassall's corpuscles in guinea-pigs during local and temporary irradiation induced involution of the thymus. Such involution is simulated by sickness, cortisone, sex steroids, or starvation.

If the lipid material which is produced in macrophages is indeed important for a defence mechanism reaction against bacteria its role is, so far, unknown.

Zusammenfassung. Mycobacterium lepraemurium wird in vivo nur in Makrophagen der Maus gefunden. Diese parasitierten Makrophagen finden sich einige Wochen nach der Infektion im Thymus, wobei diese Zellen bald dicht bepackt mit Lipidgranula sind.

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National Institute for Medical Research, Mill Hill, London N.W.7 (England), 30 January 1969.

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