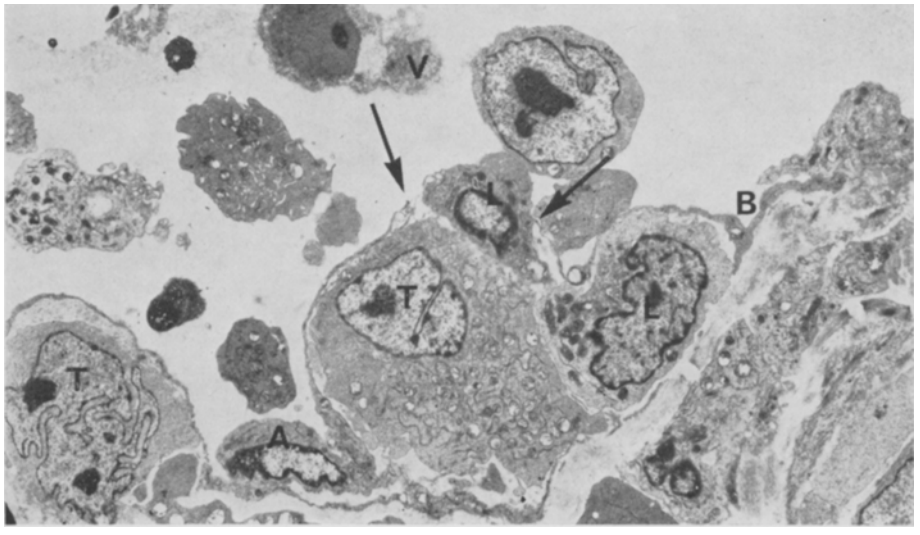


Electron micrograph of the wall of a lymphatic vessel at the edge of the tumour mass present in a rat footpad 7 days after injection of 20 million Rd/3 cells into the footpad. Tumour cells (T) and lymphoid cells (L) are migrating through the lymphatic wall, into the lumen of the lymphatic vessel (V). The endothelium is invaginated between points (A) and (B) and there is an open gap between endothelial cells at the points arrowed. ×3200.

footpad and metastasize to the ipsilateral popliteal lymph node; direct intralymphatic injection has been excluded. The model is a fair, though accelerated model of natural tumour metastasis. In this model continued recruitment of tumour cells from the primary site to the lymph node occurs. The present report describes the ultrastructure of the lymphatic vessels at the edge of the footpad tumour 7 days after injection of 20 million tumour cells into the footpad using glutaraldehyde-osmium fixation, araldite embedding and transmission electron microscopy of thin sections.

The lymphatic endothelial junctions are normally closed; beneath the lymphatic endothelium, there may be seen sometimes a basement membrane and usually an interlacing network of collagen fibres through which neoplastic cells have to penetrate to gain access to the lymphatic. Rd/3 tumour cells in suspension or in non-metastasizing situations are usually of circular or ovoid profile. Around lymphatic vessels on the other hand they are elongated with organelle-free pseudopodia at both ends.

Tumour cell processes and tumour cells lie in patent inter-endothelial gaps with endothelial cytoplasm applied closely to tumour cell cytoplasm (Figure). Collagen fibres very close to the tumour cells show no significant changes; on morphological grounds it seems unlikely that the tumour cells secrete a collagenase which acts to destroy the collagen barrier. However short-range collagenolytic activity cannot be excluded. The direction of movement of the neoplastic cells is virtually certainly from without inwards because experiments have shown consistent metastasis in this direction. Macrophages and lymphocytes migrate between endothelial cells in a similar way either alone, or with tumour cells, so that the lymphatics come to contain as many lymphoreticular cells as tumour cells. It seems likely that tumour cells and lymphoreticular cells gain access to the vessels independently and in the same way. Blood vessels are not similarly invaded by tumour cells in this model, possibly because their walls are thicker or not so well held open by collagen bands.

These findings support the view that in this model of lymphatic metastasis, tumour cells gain access to lymphatic vessels, in significant numbers, by a process of reverse diapedesis.

Résumé. Dans un modèle animal de métastasie lymphatique, on a pu démontrer que les cellules tumorales migraient entre les cellules endothéliales pour entrer dans les vaisseaux lymphatiques (diapédèse inversée). Les cellules endothéliales des vaisseaux sanguins ne laissent pas passer les cellules tumorales.

I. Carr, P. Norris and F. McGinty[3]

Department of Pathology, Sheffield University and Weston Park Hospital, Sheffield S10 2SJ (England), 14 January 1975.

[1] I. Carr and F. McGinty, J. Path. *133*, 85 (1974).
[2] I. Carr, F. McGinty, C. Potter and S. Westby, Experientia *30*, 185 (1974).
[3] This work was supported by the Cancer Research Campaign (Yorkshire Branch).

Macrophage Activation in Mice Lacking Thymus-Derived (T) Cells

T-cell-mediated immunity (CMI) plays a major role in protection from *Listeria monocytogenes* infection in mice. Sensitized T lymphocytes trigger protection by activating macrophages[1–5], which then kill the bacteria. Spleen cells from mice which were adult-thymectomized, lethally irradiated and bone marrow reconstituted (ATx-BM) before immunization do not transfer protection[4], but surprisingly, ATx-BM mice survive primary infection. Evidence is presented here that ATx-BM mice possess activated macrophages before infection, thus explaining this paradox. Macrophages of athymic (nude) mice also show some evidence of activation.

[1] G. B. Mackaness, J. exp. Med. *129*, 973 (1969).
[2] R. M. Zinkernagel, R. V. Blanden and R. E. Langman, J. Immun. *112*, 496 (1974).
[3] F. C. Lane and E. R. Unanue, J. exp. Med. *135*, 1104 (1972).
[4] R. V. Blanden and R. E. Langman, Scand. J. Immun. *1*, 379 (1972).
[5] R. J. North, Cell. Immun. *7*, 166 (1973).

Materials and methods. CBA/H and nude mice (nu/nu) were bred locally and used as young adults. The strain of *Listeria monocytogenes*, bacteriological techniques, production of ATx-BM mice and evaluation of peritoneal macrophage activity have been described elsewhere[4–7].

Results. ATx-BM mice differed from normal in several ways (Table I). First, i.v. injected *Listeria* were cleared more efficiently from blood. Second, less of these bacteria localized in spleen. Third, *Listeria* was less successful at parasitizing livers and spleens. The fall in bacterial numbers in liver over the first 4 h of infection was greater in ATx-BM mice ($p < 0.01$), and the increase between 4 h and 24 h was less ($p < 0.01$). In spleens the increase in bacterial numbers over the first 24 h of infection was again less than normal ($p < 0.01$). Since these effects were detected within minutes or hours after the injection of *Listeria*, it seemed likely that the macrophages of ATx-BM mice which were responsible for blood clearance and in which ingested organisms first resided, possessed improved phagocytic and antibacterial powers before infection.

Beyond the first 24 h after infection data varied considerably and clear interpretation of trends was difficult; statistical significance was obtained only with the spleen. However, in 2 experiments, the elimination of bacteria from the tissues from the 3rd to 6th day of infection was impaired in ATx-BM mice.

Congenitally athymic nude mice (nu/nu) also seem to possess nonspecifically activated macrophages as shown by their ability to prevent multiplication of *Listeria* in the liver and spleen[8]. Therefore peritoneal macrophages of nude mice were investigated. Macrophages of nude mice exhibited accelerated spreading[9] (Figure). *Listeria* were phagocytized more efficiently in nude mice as indicated by lower numbers of free bacteria in the peritoneal washing (χ^2 test gave $p < 0.001$, Table II).

Although less extracellular *Listeria* were recovered from the peritoneal cavities of nude mice the bactericidal capacity of the macrophages obtained was significantly worse than that of their litter mates under the experimental conditions employed (χ^2 test gave $p < 0.001$, Table II).

Discussion. The reasons for the existence of activated macrophages in ATx-BM, nude[8], or neonatally thymectomized mice[10], and probably also mice which are lethally irradiated and bone marrow reconstituted[4] are unknown. However, the presence of activated macrophages in mice lacking T cells for such a variety of reasons suggests first a causal relationship between T cell (or thymic) absence and macrophage activation, and second, that T cells (and CMI) are not always essential for the process of macrophage activation. Two possible explanations are as follows. First, evidence is accumulating that B cells are able to produce factors influencing macrophages, eg. macrophage migration inhibitory factor (MIF)[11]. This B cell function may be suppressed normally by unsensitized T cells[12]. Second, bacteria phospholipid extracts are apparently able to activate macrophages[13]. Thus, substances shed from the gut flora may activate macrophages, especially in the absende of a controlling influence of T cells.

The present investigation of nude mouse macrophages gave paradoxical findings. First, as a population, the resident peritoneal macrophages of nude mice appeared to be more efficient at phagocytosis. Second, fewer cell-associated bacteria were recovered from nude mice, suggesting that many of the phagocytes which ingested bacteria were not removed by the washing technique employed. Finally, despite increased spreading in vitro (Figure), a finding compatible with increased phagocytic ability[9], the nude mouse macrophages which were recovered in the peritoneal washings were significantly inferior to normal controls in bactericidal activity.

Table I. Distribution and behaviour of i.v. injected *Listeria monocytogenes*[a] in ATx-BM mice

Time after *Listeria* injection	Mice	Viable counts[b] of *Listeria* in		
		Blood	Liver	Spleen
6 min	ATx-BM	3.48 ± 0.15[c]	—	—
	Normal	4.11 ± 0.04	—	—
8 min	ATx-BM	—	4.83 ± 0.01	3.08 ± 0.04[c]
	Normal	—	4.71 ± 0.02	3.63 ± 0.06
4 h	ATx-BM	—	4.06 ± 0.12[d]	3.27 ± 0.12[c]
	Normal	—	4.37 ± 0.07	3.94 ± 0.06
1 day	ATx-BM	—	6.13 ± 0.16[c]	5.38 ± 0.13[c]
	Normal	—	7.25 ± 0.13	6.44 ± 0.04
3 days	ATx-BM	—	—	5.38 ± 0.20
	Normal	—	—	5.52 ± 0.13
6 days	ATx-BM	—	—	4.24 ± 0.16[c]
	Normal	—	—	2.84 ± 0.11

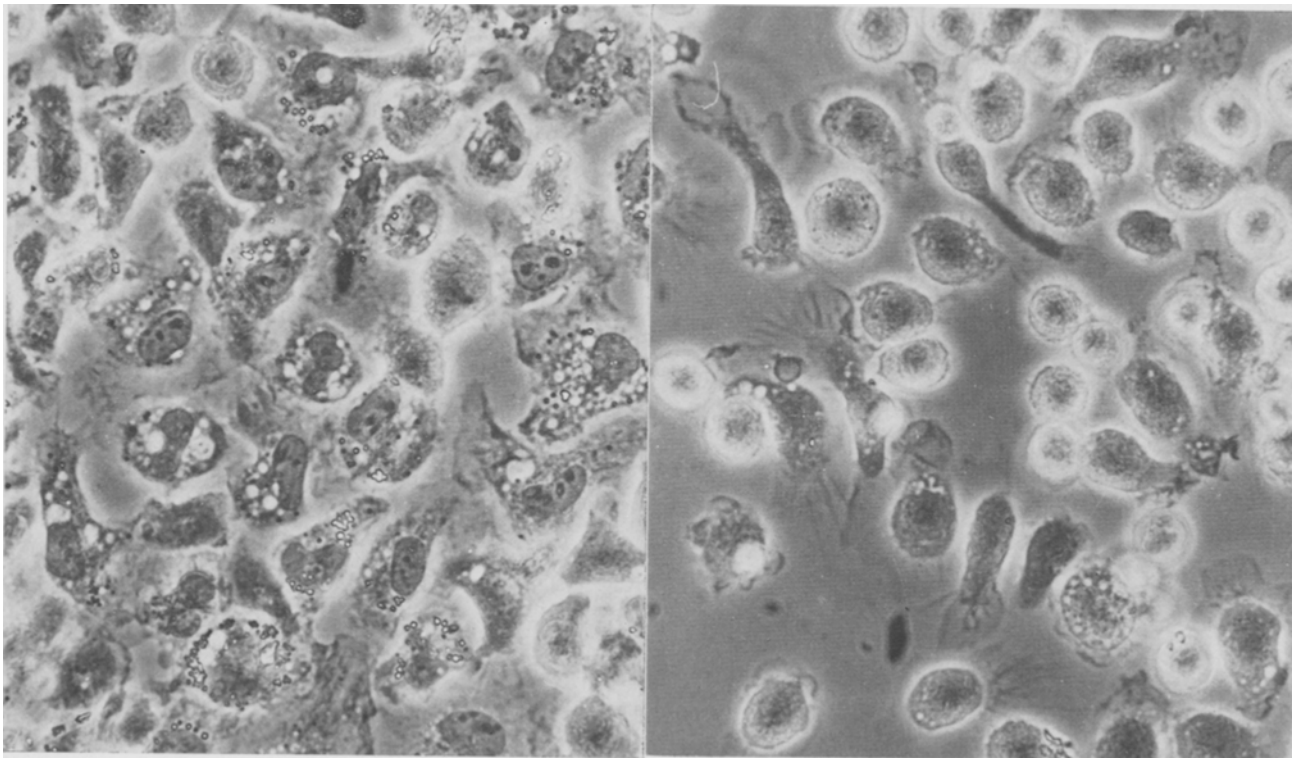
[a] 8.8×10^4 viable *Listeria* per mouse for time points from 6 min to 1 day. 5×10^8 viable *Listeria* for 3 day and 6 day data. [b] Mean $\log_{10}$ viable bacteria per organ $\pm$ standard error of the mean in groups of 4–5 mice. [c] Significantly less than normal controls $p < 0.01$. [d] Significantly less than normal controls $p < 0.05$. [e] Significantly greater than normal controls $p < 0.001$.

Table II. The bactericidal activity of peritoneal macrophages of nude mice nu/nu and of their litter mates nu/+ and +/+ against *Listeria monocytogenes*

Mice	Numbers of extracellular bacteria[a]		Numbers of cell-associated viable bacteria[a]	
	0 min	35 min	0 min	35 min
nu/nu	1.60×10^4	1.35×10^4	2.16×10^5	1.08×10^5
nu+/++	2.28×10^4	2.14×10^4	4.02×10^5	1.20×10^5

[a] Actual numbers obtained as described in Materials and methods.

[6] G. B. MACKANESS, J. exp. Med. *116*, 381 (1972).
[7] R. V. BLANDEN and C. A. MIMS, Aust. J. exp. Biol. med. Sci. *51*, 393 (1973).
[8] C. CHEERS, personal communication 1974.
[9] R. J. NORTH, J. exp. Med. *130*, 299 (1969).
[10] K. TAKEYA, R. MORI and N. IMAIZUMI, Nature, Lond. *218*, 1174 (1968).
[11] S.F. SCHLOSSMAN, in *Proc. 2nd International Congress of Immunology* (Eds. L. BRENT and J. HOLBOROW; North Holland, Amsterdam 1974), in press.
[12] P. A. CAMPBELL, B. L. MARTENS, H. R. COOPER and J. K. MCCLATCHY, J. Immun. *112*, 1407 (1974).
[13] R. M. FAUVE and B. HERIN, Proc. natn. Acad. Sci., USA *71*, 573 (1974).



Peritoneal macrophages from nude mice nu/nu (a) and their litter mates nu/+ and +/+ (b) 30 min after plating into plastic dishes at 37 °C. The macrophages of nude mice show extensive spreading and some phase-dense and phase-lucent vesicles. ×400.

This is superficially incompatible with findings of CHEERS[8] which indicate suppression of the growth of i.v. inoculated *Listeria* in the liver and spleen. But the disagreement can be reconciled if it is assumed that macrophages of nude mice are heterogenous with respect to bactericidal activity, and that fixed macrophages in the liver and spleen and perhaps those remaining in the peritoneum under our experimental conditions are much superior to those obtained in peritoneal washings.

Zusammenfassung. Ein gewisser Grad von Makrophagenaktivierung, gemessen an der Fähigkeit der Listerien-Elimination, wird auch in adult thymektomierten, mit Knochenmark rekonstituierten Mäusen sowie in nude/nude-Mäusen beobachtet und daraus der Schluss gezogen, dass die Makrophagenaktivierung nicht unter allen Umständen mit der Anwesenheit von T-Lymphozyten verknüpft sein muss.

R. M. ZINKERNAGEL and R. V. BLANDEN

The John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra (A.C.T., Australia), 28 November 1974.

Flashing Phenomenon in Blood Platelets Stained with Fluorescent Basic Drugs

Fluorescent basic drugs such as mepacrine, acridine orange and daunomycin (an antitumor agent) have been shown to selectively accumulate in the 5-hydroxytryptamine (5HT) storage organelles of blood platelets[1,2]. The present work deals with the fluorescence properties of isolated platelets loaded in vitro or in vivo with these compounds.

Materials and methods. Rabbits, guinea-pigs and rats were exsanguinated using disodiumethylene diaminetetracetate (EDTA, 1/10 vol. 5%) as anticoagulant[3]. Platelet-rich plasma obtained by centrifugation of the blood for 20 min at 300 × *g* was incubated (37 °C, 30 min) with various concentrations (10^{-5}, 5×10^{-5}, *M*) of mepacrine (K & K Labs, USA), acridine orange (DIFCO Labs, U.K.) or daunomycin (Farmitalia, Italy). Platelet-rich plasma was also obtained from rabbits 60 min after i.v. injection of 10 mg/kg of mepacrine, acridine orange or daunomycin. The platelets were then sedimented and washed twice with modified Tyrode buffer[4], and the whole platelets or their 5HT organelles, isolated as previously described[3,4], submitted to fluorescence microscopy.

For qualitative fluorescence microscopy a mercury super pressure lamp HBO 200 W/4 was used. Epi-illumination was performed with a Leitz fluorescence vertical illuminator[5] equipped with an interference dividing plate

[1] M. DA PRADA and A. PLETSCHER, Eur. J. Pharmac., in press.
[2] G. B. PICOTTI and I. GALATULAS, Archo it. Pat. Chim. Tumori *16*, 101 (1973).
[3] M. DA PRADA, K. VON BERLEPSCH and A. PLETSCHER, Naunyn-Schmiedeberg's Arch. Pharmac. *275*, 315 (1972).
[4] M. DA PRADA and A. PLETSCHER, Br. J. Pharmac. *34*, 591 (1968).
[5] J. S. PLOEM, Leitz-Mitt. Wiss. Tech. *4*, 225 (1969).