

bial degradation of this insecticide. This opinion is based on the observations that the bacterium (a) caused a marked loss of lindane from anaerobically incubated reaction mixtures consisting of bacterial cells, phosphate buffer and lindane, and (b) released the covalently linked chlorine of lindane as chloride ion.

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New Evidence for the Existence of Long Lived Macrophages

PROLONGED observation of labelled macrophages within inflammatory reactions in the ear chambers of rabbits has led to the suggestion that some of these cells may be very long lived^{1,2}. Objective evidence in favour of the existence of long lived macrophages has, however, been lacking. In addition, study of granulomata induced by complete Freund adjuvant indicates a high rate of turnover of participating macrophages, with no evidence of persistence beyond 1-2 weeks of the mononuclear cells that originally emigrated into the lesion from the circulation³. We have found a similar high rate of turnover among the macrophages in chronic inflammatory reactions provoked by *B. pertussis* vaccine or paraffin oil.

Carrageenin, a crude extract of seaweed, has long been known to induce a granulomatous inflammation characterized by massive macrophage infiltration⁴. We injected 0.05 ml. of a 1 per cent solution (Seakem 402A.P. Marine Colloids) intradermally into the dorsum of rats' feet. The macrophage precursor cells had been previously labelled *in vivo* with tritiated thymidine (3HT)⁵, and 60-80 per cent of the macrophages in 2-3 day carrageenin lesions were found to contain the marker when autoradiographs of the tissue sections were prepared⁵. Studies in radiation chimerae showed that these recently divided circulating precursor cells were of marrow origin, as in other inflammatory states⁵⁻⁸.

In the granulomata hitherto studied, the percentage of macrophages labelled with 3HT and their average count of nuclear grains fall steeply after the first few days as a result of proliferation and fresh emigration, and reach zero after about 4 weeks³. In carrageenin granulomata, however, both the percentage and the average count of nuclear grains of labelled cells decline only slightly over the same period. This suggests that a high proportion of the macrophages originally arriving from the circulation persisted for months intact within the lesion without dividing.

To measure the level of DNA synthesis and thus of mitosis in the macrophages, a single pulse of 3HT was given 30 min before death³. In carrageenin reactions lasting 2-12 weeks, only 0.1-0.4 per cent of the macrophages in-

corporated the isotope into nuclear DNA as compared with 3-4 per cent in corresponding lesions provoked by complete or incomplete Freund adjuvant or *B. pertussis* vaccine^{3,9}. Finally, the rate of migration of circulating mononuclears into established carrageenin granulomata was measured by exchange transfusion of the tritiated cells¹⁰. The number was found to be negligible (less than 10,000/24 h) compared with that in Freund adjuvant lesions (200,000/24 h)¹⁰. Because the size of carrageenin lesions stays constant over a long period, this is further evidence of the stability of the macrophage population. The possibility remained that most macrophages in the carrageenin reactions were dead or moribund, although their appearance suggested the contrary. To test this, 30 min before death the lesion was perfused for 5 min with tritiated uridine through the common iliac artery. Many of the macrophages in question were found to incorporate the isotopic uridine when autoradiographs were prepared. The uptake of uridine was taken as evidence of RNA synthesis and of a healthily functioning metabolism.

The results indicate therefore that macrophages derived from the rapidly proliferating marrow cell, now thought to be their usual precursor^{8,11}, may exist in at least one type of granuloma for up to 3 months, without dividing, dying or being replaced. Because other persistent irritants induce similar cells to proliferate at a high rate, it would seem that the natural history of the macrophage may be as variable as that of the lymphocyte and greatly influenced by the nature of the substances with which it is called upon to deal.

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Mycoplasmacidal Action of Normal Tissue Extracts

WHILE studying experimental infection with various mycoplasmas, we found that concentrated homogenates of infected animal tissues sometimes yielded negative cultures, while higher dilutions of the same homogenates were positive. Further examination revealed the presence of a mycoplasmacidal factor in extracts of normal tissues, which appears during incubation at 37°C and kills the organisms by lysis.

Tissues were homogenized in sterile physiological saline, or in PPLO (pleuropneumonia-like organisms) broth, to make 25 per cent suspensions by wet weight. They were centrifuged for 30 min at 12,000g, and the sediment was discarded. Two-fold dilutions were made in PPLO broth¹ containing 1,000 u/ml. of penicillin, 20 per cent fresh horse serum, 10 per cent yeast extract and 0.5 per cent glucose and phenol red as indicator. *M. neurolyticum*² was used for all studies of the tissue factor. Cultures of this organism in PPLO broth yielded approximately 10⁹ organisms/ml. after incubation for 24 h at