We have now been able to initiate *in vitro* proliferation in cultures of mouse peritoneal macrophages by the addition of inflammatory exudates. Such cultures exhibited extensive nuclear incorporation of tritiated thymidine, a high incidence of mitotic figures and a considerable increase in total cell numbers as demonstrated by direct counting (see Fig. 1) compared with control cultures not exposed to exudate.

Macrophage proliferation was seldom demonstrable earlier than four days after addition of exudate to the culture. Phagocytosis of human red blood cells and heat-killed *M. tuberculosis*, and histochemically evident acid phosphates, seemed higher in cultures treated with exudate than in the controls. Electron microscopy revealed features typical of macrophages, and the cells treated with exudate seemed to have more cytoplasmic organelles and fimbriae than control cells. Preliminary characterisation of the mitogenic factor present in the exudate has so far indicated only that it is relatively thermostable, being unaffected by freezing and thawing, storage for 6 months at −25° C or by incubation for 1 h at 65° C. It is not yet possible to correct it with the macrophage-derived factors which affect mitosis in other cell types[8].

KATHERINE M. WYNNE
W. G. SPECTOR
D. A. WILLOUGHBY

Pathology Department,
St Bartholomew's Hospital Medical College,
London EC1, UK

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Induction of DNA synthesis in rat macrophages *in vitro* by inflammatory exudate

THE proliferation of macrophages in inflammatory exudates[1-3] contrasts with the failure of these cells to divide *in vitro* except in the presence of highly specialised conditioned media[4-7]. Macrophage division *in vivo*, however, could be explained if inflammation produced a local mitogenic factor.

Inflammatory exudates were produced in DFA-specified pathogen-free rats by intrapleural injection of 1 ml 6% Dextran (40,000 daltons) and collected 4 h later. The exudate was centrifuged and the supernatant passed through a Millipore filter (pore size 0.22 μm). The filtered exudate was diluted with medium 199 plus 20% newborn calf serum to make a final concentration of exudate of 30 or 50%.

Macrophages were collected from DFA rats by washing the peritoneum with medium 199. Macrophages were also collected 4 d after intraperitoneal injection of nutrient broth (activated macrophages). The macrophages were then cultured in Leighton tubes[8] and 3 d later the medium was replaced with medium containing exudate. Three to six days after the addition of exudate DNA synthesis was assessed by autoradiography after incorporation of a single 30 min pulse of tritiated thymidine. The results of a typical series of experiments (Fig. 1) show that both normal and activated macrophages responded after 5 d of exposure to exudate *in vitro*. The labelling index of the treated cells rose to 20–30% compared with virtually zero in the control macrophages. Samples taken earlier than 5 d showed much less labelling. The macrophages synthesising DNA showed avid phagocytosis of starch particles added to the medium, which seemed to be greater than in the controls. Preliminary experiments have shown that exudates from Lewis rats are active on peritoneal macrophages from inbred Lewis rats and also on mouse peritoneal macrophages.

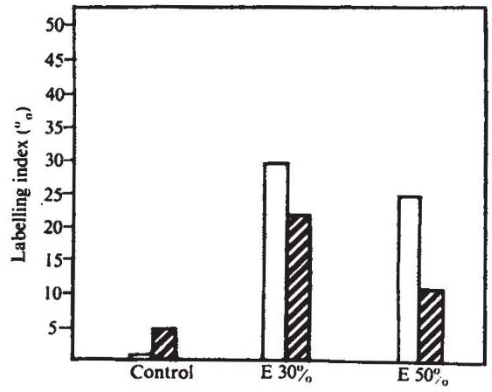

Fig. 1 Uptake of tritiated thymidine by 'normal' and 'activated' macrophages 5 d after culturing with or without exudate added to the culture medium. The open blocks represent normal and the hatched blocks activated macrophages.

The chemical nature of this stimulatory factor is unknown and the possibility of it being a virus cannot be excluded. It does not seem, however, to result from treatment with dextran alone, as dextran added to culture medium and passed through Millipore filters failed to induce DNA synthesis in macrophages. Its relationship to the factors released from peritoneal macrophages which stimulate or inhibit other cell lines remains to be determined[9].

M. ADOLPHE
J. FONTAGNE
M. PELLETIER
J. P. GIROUD

Institute of Pharmacology,
21 rue de l'Ecole de Medecine,
Paris 75006, France

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Effect of antibody emission rates on plaque morphology

CUNNINGHAM and Fordham[1] have used a modified version of the haemolytic plaque technique to show that antibody diversity can be generated after B cells have been stimulated to proliferate. They tested antibody plaque forming cells (PFC) against a mixture of sheep red blood cells (SRBC) from two different sheep and obtained three morphological classes of direct plaques: clear (the plaque radii for both types of SRBC were equal), sombrero (the plaque radii were different for the two types of SRBC), and partial (there was only one plaque radius). For the clear and sombrero plaques both types of SRBC lysed to some extent, while for the partial plaques only one type of SRBC lysed. Cunningham and Fordham used plaque morphology as an antibody specificity marker, that is, if two plaques were different in morphology this was taken to mean that the antibodies produced by the two PFC differed in their specificity for the two types of SRBC. They studied clones produced from single PFC and found in most cases that the plaques produced by the progeny were of the same morphological class, differing only by slight variations in their plaque radii. In 10 of the 93 clones observed, however, the morphology of the plaques differed within the clone. In 7 clones two different morphological classes of plaque were observed, and in the others all three classes. Cunningham and Fordham suggested that within a given clone the antibodies emitted by different cells can have different specificities.

We report here that changes in plaque morphology do not