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Effect of Methyl Cellulose on the Phagocytosis of *Mycobacterium lepraemurium**

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Does the reported beneficial effect of Lampren (G 30 320 or B 663) in lepromatous leprosy depend on the fact that it is taken up selectively by the cells of the reticulo-endothelial system and so may there modify the reaction to phagocytosed leprosy bacilli? This study shows that in rats and mice experimentally infected with mycobacteria, the injection of methyl cellulose did not modify the infection, some macrophages ingesting the polymer and some the bacilli but few ingesting both. It is suggested that Lampren and similar drugs may have to be given either in high dosage or over a long period in order to be effective.

INTRODUCTION

The macrophage cell plays a central role in leprosy. The intracellular habitat it provides for the bacilli may condition the immunological response, since it protects them against drugs that cannot penetrate the cell membrane. The rimino-phenazine compound B 663 (Lampren), however, is taken up selectively by the cells of the reticulo-endothelial system and can be identified in them in particulate form (Conalty and Jackson, 1962), and this may account in part for its therapeutic effect in lepromatous leprosy (Browne and Hogerzeil, 1962; Pettit and Rees, 1966; Warren, 1968). The present experiments were undertaken to find out whether the ingestion of inert particles is sufficient to modify the reaction to phagocytosed leprosy bacilli.

Methyl cellulose is a chemically inert polymer of dextrose which persists for long periods in the tissues after parenteral administration. Repeated intravenous administration causes splenomegaly in rabbits and dogs (Hueper, 1942) and polymer is lodged in macrophages. The lesions described in rats by Teoh (1961) include aggregates of foam cells which bear a superficial resemblance to lepromatous lesions. Although the polymer is chemically inert, its presence in some way induces erythrophagocytosis by reticulo-endothelial cells and this is associated with an anaemia that appears soon after it is given (Teoh, 1961).

We have attempted to modify experimental infections with *Mycobacterium lepraemurium* by giving methyl cellulose and have studied the behaviour of tissue macrophages when they are engaged at the same time in the phagocytosis of methyl cellulose. Additional observations on phagocytosis by macrophages of peritoneal exudates were carried out with another mycobacterium.

MATERIALS AND METHODS

Inoculation of mice

Twenty adult female albino mice (Strong A strain) were injected intravenously with 0.2 ml. of *Myco. lepraemurium* suspension containing 1.007×10^6 organisms per ml. Ten of the mice were given at the same time a single subcutaneous dose of 0.2 ml. of a 2% solution of methyl cellulose (B.D.H.: approximately 450 centipoises; average mol. wt 92,000). From each group 2 mice were killed on the day of infection and further pairs at 2-monthly intervals thereafter. The whole liver and spleen of each mouse were homogenized together in 1.5 ml. of 1% albumin-formalin-saline for 15 min. and the bacilli in a known volume were counted by the method of Hart and Rees (1960).

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Inoculation of rats

Twenty albino rats weighting 125 to 200 g were used. Each rat received an intraperitoneal inoculum of 0.5 ml. of the *Myco. lepraemurium* suspension used for the mice. Ten of the rats received in addition 2 ml. of a 2% methyl cellulose solution intraperitoneally twice a week for the whole duration of the experiment. Blood was taken from the tail vein at weekly intervals and the haemoglobin value estimated. After 6 months the surviving animals were killed and the livers and spleens were weighed and divided into parts for bacillary counts, by the technique used for the mice, and for histological examination. The fixative was 10% formal-saline. Paraffin sections were stained with haematoxylin and eosin and by Fite's method for leprosy bacilli. The Congo-red method of Horváth *et al.* (1956) was used to stain the methyl cellulose.

Phagocytosis of Myco. tuberculosis

A large loopful of *Myco. tuberculosis bovis* (B.C.G. strain) was emulsified in 1 ml. of 2% methyl cellulose solution and given by intra-peritoneal injection to 6 rats; 48 hr later the rats were killed and peritoneal washings collected. The washings were concentrated by centrifugation, smeared on slides, fixed in acetic alcohol (1 : 3 v/v), stained for bacilli with the auramine-O modification of the Ziehl-Neelsen technique, and examined by phase-contrast microscopy.

RESULTS

Infections with Myco. lepraemurium *in mice*

The average log. bacillary counts carried out on the day of infection on the pooled liver and spleen homogenates were 4.000 per g both in the 2 mice given methyl cellulose in addition to infection and in the 2 mice infected only. At 2 months after infection the average log. bacillary count was 7.3685 in the mice treated with methyl cellulose and 7.4554 in the mice infected alone. At 4 months the corresponding counts were 9.3972 and 9.0339: the remaining counts showed equivalent and parallel increases. The observations covered 6 months only, because 2 mice from each group died and were not examined. Bacilli multiplied in both groups and there was no enhancement or reduction of infection statistically significent at the 5% level in the group treated with methyl cellulose when compared with the group infected alone.

Infections with Myco. lepraemurium *in rats*

All the 10 rats that received repeated injections of methyl cellulose showed a reduction in haemoglobin level, from 14.4 g per 100 ml blood in the first week to 11 g between the second and fourth weeks. Five of the rats infected only, and 5 of those that received methyl cellulose in addition, died from intercurrent infections and were not examined. The surviving 5 rats from each group were killed 6 months after infection. In the rats infected and treated with methyl cellulose the mean log. counts were 5.4527 per g of liver homogenate and 5.8422 per g of spleen homogenate. In the control group receiving *Myco. lepraemurium* only, the respective counts were 4.8328 and 5.2051. Statistical analyses show no differences significant at the 5% level either between the liver counts or between the spleen counts of the 2 groups.

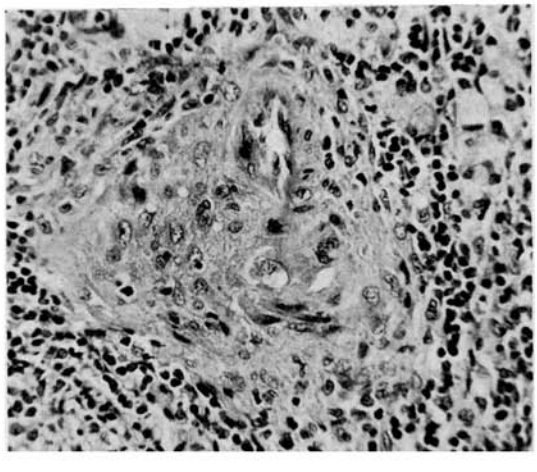

FIG. 1

Discrete nodular lepromatous lesion in spleen of rat 6 months after administration of *Myco. lepraemurium* and methyl cellulose. The lesion includes a giant cell and no methyl cellulose. A few foam cells containing polymer lie outside the lesion (Congo red × 300).

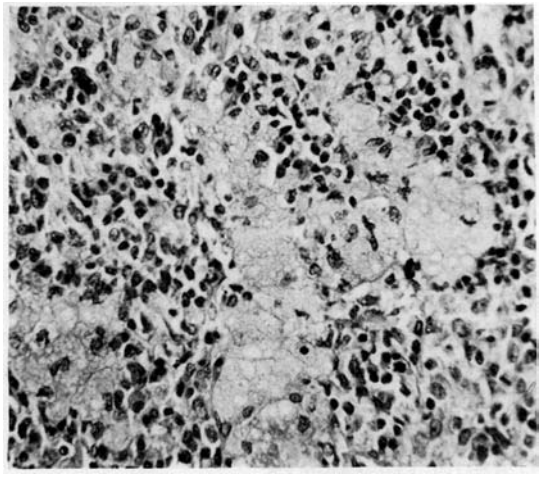

FIG. 2
Foamy macrophages containing methyl cellulose line splenic sinuses (Congo red × 300).

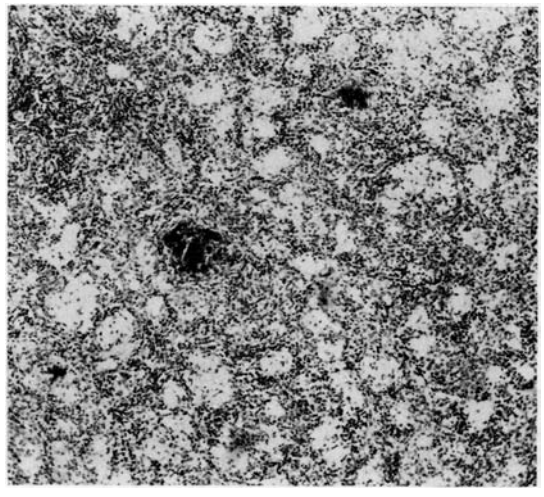

FIG. 3
Discrete clusters of foamy macrophages in spleen of rat which had received methyl cellulose and *Myco. lepraemurium* 6 months previously (H & E × 70).

Histological studies on the rat infections: The mean liver weight of the rats treated with methyl cellulose was 5.9 g per 100 g body weight, in comparison with 4.43 g for the rats infected only, and 3.16 g for normal controls. The mean spleen weight of the treated rats was 0.84 g per 100 g body weight in comparison with 0.38 g in the rats infected only and 0.19 g for normal rat controls.

Microscopically, nodular lesions of leprosy were found in the livers and spleens in both groups. They were composed of foci of macrophage cells with a finely granular cytoplasm (Fig. 1) resembling Virchow cells of human leprosy and generally containing acid-fast rods often in large numbers. Giant cells of corresponding type were present occasionally. Methyl cellulose was not demonstrated in this type of cell by Congo-red staining. In the rats treated with methyl cellulose the polymer was recognizable in phagocytic cells in the livers and chiefly in Kupffer cells. In the spleens individual cells in the cords of the red pulp were distended with methyl cellulose which gave the cytoplasm a foamy appearance and was stained with Congo red. Foamy macrophages were also located in the sinuses (Fig. 2) and they appeared as prominent pale bands or clusters (Fig. 3). The foamy macrophages did not form an integral part of the lepromatous nodules. Acid-fast bacilli were not present in any cells containing more than minute quantities of methyl cellulose. It appeared that the cells were engaged in the phagocytosis either of bacilli or of polymer and only to a very limited degree of both together.

Phagocytosis in peritoneal exudates

Smears of peritoneal exudates of 6 rats were examined 48 hr after the intraperitoneal injection of *Myco. tuberculosis bovis* (B.C.G. strain) together with methyl cellulose. The use of phase contrast permitted the polymer to be seen clearly at the same time as the bacilli stained with the fluorochrome auramine-O. Numerous macrophages contained either methyl cellulose or B.C.G., but cells containing one did not contain the other.

DISCUSSION

Anaemia was noted in all the 10 rats given repeated injections of methyl cellulose. This anaemia is associated with erythrophagocytosis (Teoh, 1961). Although methyl cellulose induces

proliferation of reticulo-endothelial cells, the anaemia and erythrophagocytosis may be due to changes in the red cells because there is no evidence from our experiments that the presence of the polymer modifies or enhances phagocytosis by individual cells. There were no significant differences between the bacillary counts in the methyl-cellulose-treated rats and mice and those in the animals infected alone and nothing to suggest that the experimental infection was altered.

The presence of methyl cellulose did however have some effect on the behaviour of individual macrophages that ingested it in both the rat-leprosy experiments and in the study of peritoneal exudates in B.C.G. infection. Individual macrophages ingested the polymer or the bacilli preferentially and not both equally. Apparently the reserve capacity for proliferation of the reticulo-endothelial system sufficed to supply both demands and these were not met by an enhancement of the phagocytic activity of individual cells. Evidently the action of slowly metabolized drugs, such as B 663, which are taken up by macrophages in particulate form cannot be ascribed to an enhancement of phagocytosis alone. Doubtless the nature of the surface and other properties of particles affect their phagocytosis, but if drugs of this nature are dealt with in the same way as the particles of methyl cellulose, then there is a greater probability of such drugs entering a normal macrophage than of their entering a Virchow cell. It follows that in order to reach the bacilli within the cells in effective concentration such drugs must be given in a dosage high enough to exert some extra effect, or over periods sufficiently prolonged to span the life of several macrophages.

SUMMARY

Methyl cellulose, a chemically inert macromolecule which is stored in macrophages in particulate form, was given parenterally to rats and mice concurrently infected with *Myco. lepraemurium*. There was no evidence that this treatment modified the infection.

The lepromatous lesions in the rats included cells like human Virchow cells containing numerous bacilli but little or no methyl cellulose. Further experiments with *Myco. tuberculosis bovis* (B.C.G.) confirmed the impression that individual macrophages ingest preferentially either bacilli or polymer.

It is concluded that drugs like the riminophenazine B 663 (Lampren) which are stored in particulate form in macrophages must be administered either in high dosage or over prolonged periods in order to gain access to bacilli lying within cells.

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