THE INHIBITION BY CORTISONE OF THE CYTOTOXIC ACTIVITY OF PPD ON TUBERCULIN-HYPERSENSITIVE CELLS IN TISSUE CULTURE

By ROBERT H. LEAHY and HERBERT R. MORGAN, M.D.

(From the Department of Bacteriology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

PLATE 25

(Received for publication, July 23, 1952)

Several investigators have shown that cortisone will suppress the local reaction of hypersensitive animals and man to the intracutaneous injection of tuberculin (1–3). In this type of hypersensitivity the importance of the mononuclear cell has been demonstrated by both histological examination (4) and passive transfer experiments (5). Furthermore, Rich and Lewis (6) demonstrated *in vitro* a cytotoxic action of tuberculin upon macrophages from splenic tissue of tuberculin-hypersensitive animals. In the present experiments, it was decided to investigate the effect of cortisone on the reaction of such hypersensitive cells to PPD in tissue culture, which provides a system in which the cells can be studied in the absence of blood-borne substances, vascular effects, and non-specific tissue responses.

Materials and Methods

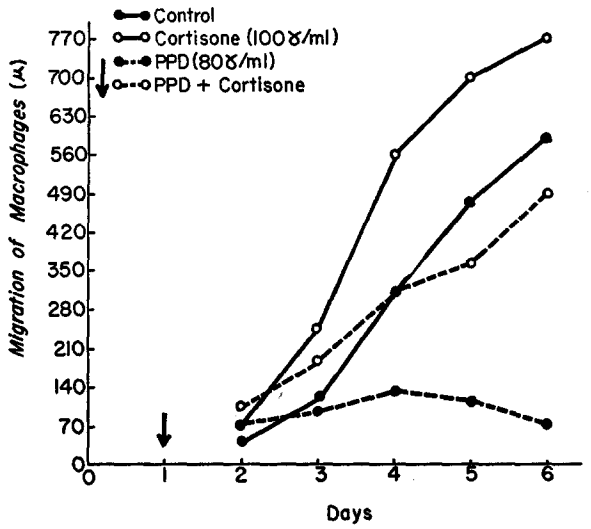
Animals and Sensitization.—Albino guinea pigs weighing approximately 250 gm. were injected intraperitoneally with 1.0 ml. (20 mg.) of *Mycobacterium tuberculosis* of the bovine strain (BCG) and subcutaneously in the inguinal region with 0.5 ml. (10 mg.). After a minimum period of 5 weeks, the animals were tested for hypersensitivity by the intracutaneous injection of 0.1 ml. of a 1:10 dilution of old tuberculin (supplied by Parke, Davis and Co.). These areas were examined at 48 hours and generally exhibited an area of erythema and induration measuring approximately 2 by 3 cm., with central necrosis.

Tissue Cultures.—The roller tube method of tissue culture used here was similar to that described previously (7). The spleens were removed from the animals, minced with scissors, and fragments approximately 1.0 mm. in size were selected. These were washed twice in Hanks's (8) balanced salt solution without sodium bicarbonate. Eight fragments were planted in the plasma lining each tube. 2 ml. of nutrient medium containing an appropriate test substance was added to each tube.

The nutrient medium used consisted of 18 ml. of Hanks's balanced salt solution, 7.0 ml. of ox serum ultrafiltrate (Microbiological Associates, Inc.), and 2.0 ml. of 50 per cent beef embryo extract, to which were added penicillin and streptomycin for a final concentration of 50 units of each per ml. Either of the following materials was then added to the nutrient solution: cortisone acetate suspension (supplied by Merck and Co., Inc.) to give a concentration of 100 gamma per ml.; PPD, the purified protein derivative of tuberculin (furnished by

Dr. Florence B. Seibert), to give a concentration of 80 gamma per ml.; or both in the same concentrations.

For the first 24 hours of growth, tissues were maintained with plain nutrient medium or nutrient containing cortisone acetate. These media then were replaced respectively by nutrient containing PPD or nutrient containing both PPD and cortisone. Throughout the experiments, controls were maintained with plain nutrient or nutrient containing cortisone. Splenic tissue from normal guinea pigs was treated in a similar manner in order to observe any effects of cortisone or PPD on normal tissue. All tissues were observed microscopically each day and the migration of the macrophages away from the fragments was measured with an ocular micrometer. Four cultures were prepared for each experimental condition and the experiments were repeated several times, using additional experimental animals.



TEXT-FIG. 1. Extent of migration of macrophages from representative fragments of tuberculous tissues subjected to the various experimental conditions in a typical experiment. The PPD was placed on the proper cultures on the 1st day after the cultures were prepared. No appreciable migration of cells was visible until the second day.

Other Compounds Tested.—Compounds tested in the same manner as cortisone included desoxycorticosterone glucoside (supplied by Ciba Pharmaceutical Products, Inc.), compound F (provided by The Upjohn Company), and the free alcohol of cortisone (provided by Merck and Co., Inc.).

RESULTS

The differences in the rates and the extent of migration of the macrophages from representative fragments of tuberculous tissues subjected to the various experimental conditions in a typical experiment are summarized in Text-fig. 1. Control tuberculous tissues maintained in plain nutrient showed migration of a moderate number of cells which moved away from the fragments at a fairly uniform rate (Text-fig. 1). The cells were ameboid in contour and contained a

relatively refractile cytoplasm (Fig. 1 *a*). Cells exposed to 80 gamma per ml. of PPD exhibited little migration after the 2nd day of growth (Text-fig. 1) and the macrophages present lost their typical appearance and became rounded, small and granular (Fig. 1 *b*). Concentrations of PPD less than 80 gamma per ml. were found to be insufficient to cause this toxic picture regularly with tuberculin-hypersensitive cells, whereas concentrations greater than 100 gamma per ml. were found to be slightly toxic to normal cells. Cells treated with cortisone alone showed a consistently greater rate of migration than the untreated macrophages (Text-fig. 1, Fig. 1 *c*). Fragments that were exposed to cortisone for 24 hours and then received a fresh medium containing both cortisone and PPD (Fig. 1 *d*) produced cells that appeared no different from cells of control tissues. Though these macrophages edged out somewhat more slowly and did not disperse freely from one another, their final migration approximated that of control cells (Fig. 1). The results obtained with other tissue fragments in this and additional experiments were similar.

During the first 24 hour exposure to cortisone, the microscopically visible crystals of cortisone acetate in the nutrient disappeared, indicating absorption by the cells or a rapid conversion to a more soluble form or derivative.

Studies of the migration of macrophages from normal tissues showed that no stimulating or toxic effects were produced by cortisone or PPD in the concentrations used.

In earlier experiments, attempts were made to obtain protection by the simultaneous addition of cortisone and PPD to the cultures at the time of preparation. It was not possible to demonstrate any protection by cortisone against the toxic action of PPD on hypersensitive cells under these conditions, indicating that the pretreatment of the cells with cortisone was necessary.

The purified cortisone alcohol preparations (100 gamma per ml.) gave results similar to those obtained with the cortisone acetate. Compound F (100 gamma per ml.) also afforded protection to the macrophages against PPD. The desoxycorticosterone glucoside (140 gamma per ml.) and estrone (100 gamma per ml.) had no protective action against the cytotoxic effects of PPD.

In the case of the tuberculous tissue, the presence of PPD greatly inhibited the outgrowth of fibroblasts from the tissue explants, indicating that these cells also possess a hypersensitivity to PPD similar to that observed with the macrophages.

DISCUSSION

The tuberculin reaction of the hypersensitive subject is a complex affair involving humoral and vascular and other cellular elements. Previous *in vivo* investigations have shown that cortisone reduces the loss of vascular tone and endothelial damage caused by the introduction of tubercle bacilli into hypersensitive tissues (9). Others have shown that the cellular exudation at the

site of the tuberculin reaction is suppressed by cortisone (4). The tissue culture technic employed in these experiments, by eliminating blood-borne factors, vascular phenomena, and associated tissue reactions, has permitted the investigation of the activity of cortisone in tuberculin hypersensitivity at a purely cellular level.

The *in vitro* observations presented here suggest that the inhibition of the tuberculin reaction by cortisone *in vivo* may be caused in part by a direct protective action on hypersensitive macrophages.

It is interesting to speculate about possible mechanisms of this specific protective capacity of cortisone exhibited in tissue cultures. It might be due to: (*a*) an increase in the number of migrating macrophages, which would render the quantity of PPD present insufficient to demonstrate its cytotoxic action; (*b*) loss of activity of the PPD secondary to a direct reaction with the cortisone; or (*c*) some alteration of the cells which renders them no longer specifically susceptible to the cytotoxic action of PPD. The volume of fluid and the concentration of PPD employed here appear to be more than adequate to eliminate the first possibility, as judged from previous experiments (6). The second mechanism does not seem a likely one, since protection was not observed when both cortisone and PPD were added to tissue not previously conditioned by cortisone. Furthermore, the mixtures of cortisone and PPD used were often prepared 24 hours before being placed on the cultures, thus allowing ample time for any direct reactions between the PPD and cortisone to occur.

The third possibility appears the most likely, *i.e.*, that the hypersensitive macrophages are definitely altered in some way by cortisone to render them no longer specifically reactive to PPD. The disappearance of the crystals of the insoluble cortisone acetate from the nutrient solution soon after coming in contact with the tissues indicates a rapid reaction with the cells, leading to absorption of the cortisone or a transformation to a more soluble form. It is possible that in this process the metabolism of the cell is altered by cortisone in a specific manner that eliminates the mechanism by which PPD affects the cell.

The specificity of the activity of cortisone is emphasized by the failure of other steroids, such as desoxycorticosterone and estrone, to have any effect upon the hypersensitivity of the tuberculous macrophages to PPD.

SUMMARY

Using the roller tube method of tissue culture, it has been shown that cortisone and compound F inhibit the cytotoxic action of PPD upon macrophages from the splenic tissue of tuberculous guinea pigs. Pretreatment of the cells for 24 hours with hormones before exposure to PPD was found to be essential. Estrone and desoxycorticosterone glucoside had no protective activity.

Certain advantages of the tissue culture method in studies of tuberculin hypersensitivity and possible mechanisms and implications of this protective action of cortisone are discussed.

BIBLIOGRAPHY

1. Long, J. B., and Favour, C. B., *Bull. Johns Hopkins Hosp.*, 1950, **87,** 186.
2. Harris, S., and Harris, T. N., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 186.
3. Long, D. A., and Miles, A. A., *Lancet*, 1950, **1,** 492.
4. Gell, P. G. H., and Hinde, I. T., *Brit. J. Exp. Path.*, 1951, **32,** 516.
5. Chase, M. W., *Proc. Soc. Exp. Biol. and Med.*, 1945, **59,** 134.
6. Rich, A. R., and Lewis, M. R., *Bull. Johns Hopkins Hosp.*, 1932, **50,** 115.
7. Morgan, H. R., and Wiseman, R., *J. Infect. Dis.*, 1946, **79,** 131.
8. Hanks, J. H., and Wallace, R. E., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71,** 196.
9. Ebert, R. H., *Am. Rev. Tuberc.*, 1952, **65,** 64.

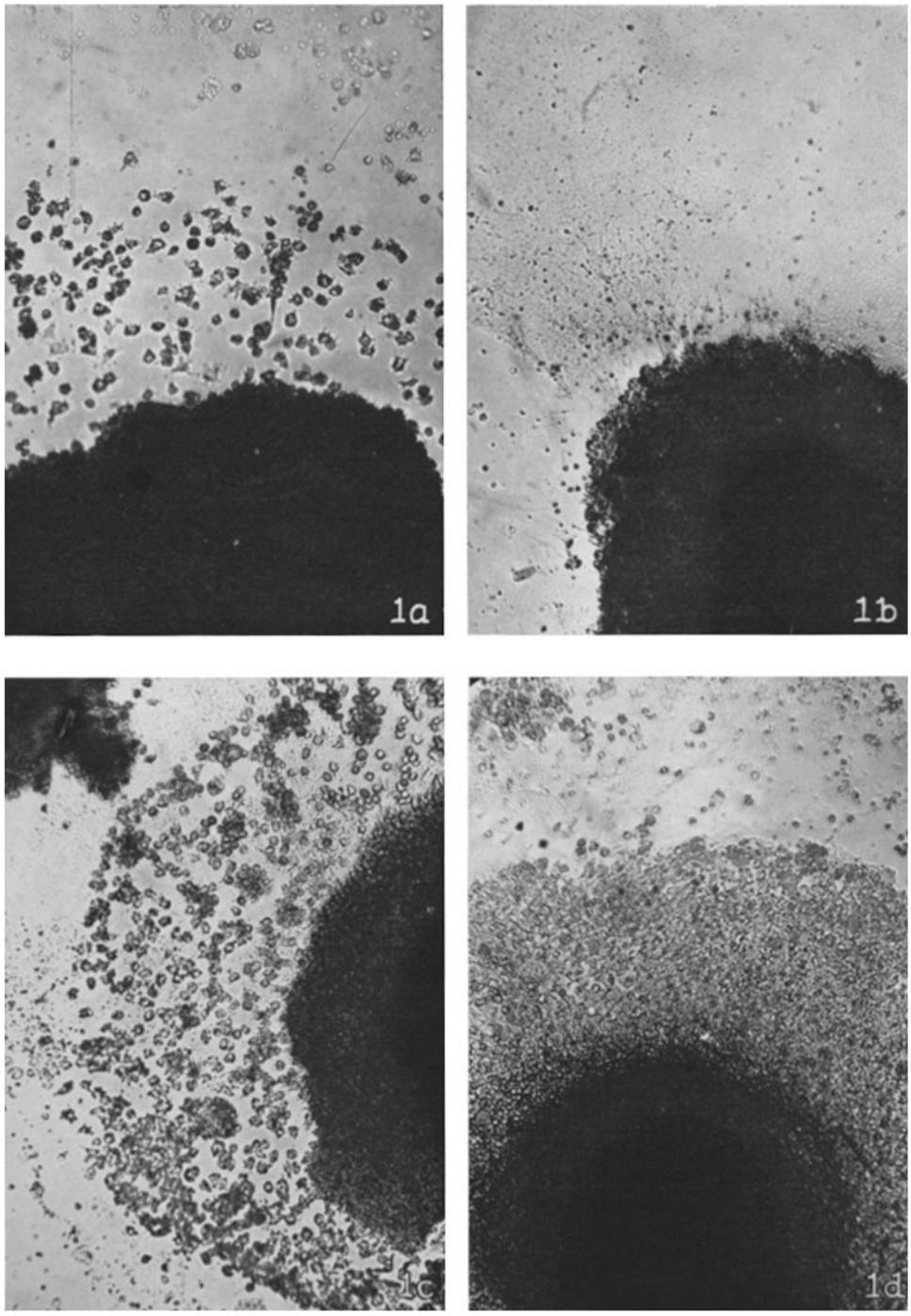
EXPLANATION OF PLATE 25

FIG. 1 *a*. Spleen fragment from guinea pig infected with *Mycobacterium tuberculosis* (BCG) in tissue culture, showing migration of macrophages on the 5th day of cultivation. × 330.

FIG. 1 *b*. Spleen fragment showing suppression of migration of macrophages by PPD. 5th day. × 330.

FIG. 1 *c*. Spleen fragment exhibiting increased migration of macrophages in the presence of cortisone acetate. 5th day. × 330.

FIG. 1 *d*. Spleen fragment with many migrating macrophages after exposure to cortisone acetate and PPD. 5th day. × 330.



(Leahy and Morgan: Effect of cortisone on tuberculin cytoxicity)