EFFECT OF CORTISONE ON THE MACROPHAGES OF DIFFERENT SPECIES OF ANIMAL

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Long (1957) has attributed many of the conflicting reports of the effect on infection of treatment with cortisone to variations in the susceptibility of different species of animal. The guinea pig, monkey, and man maintained body weight, γ-globulin synthesis, and antibody production under prolonged treatment with cortisone acetate, whereas rabbits, rats, and mice showed opposite effects, together with an increased susceptibility to infection. The susceptible species often succumb to infection from relatively avirulent organisms such as Actinomyces muris (Long, 1957) or even from saprophytes (Berlin et al., 1952).

Mouse macrophages in cell culture differentiate between virulent and avirulent strains of Salmonella typhimurium, a natural pathogen for the mouse. Avirulent organisms do not multiply after phagocytosis. In contrast, the macrophages kill many of the virulent organisms but the survivors multiply and eventually lyse the cells. The ability of an organism to multiply within mononuclear cells is therefore associated with virulence (Furness, 1955a, b).

Cortisone may increase the susceptibility of rats and mice to infection, either by inhibiting the production of phagocytic cells, or by abolishing (or reducing) the bactericidal power of the mature cells, or by a combination of both effects. The cell culture technique of Furness (1955b) has been used to study the effect of cortisone, administered at a level which divides laboratory animals into cortisone-resistant and susceptible species, on the phagocytic and bactericidal power of macrophages. The results are given in this paper.

MATERIALS AND METHODS

 Cultures. Two strains of bacteria were used as test organisms: S. typhimurium strain M206, which is avirulent for mice, rats, and guinea pigs, and having an MLD > 106 organisms (Furness and Rowley, 1956); the organism is sensitive to 4 μg neomycin per ml in a defined medium (Furness, 1955b). Escherichia coli strain 2242, a streptomycin-resistant mutant of E. coli strain K12, which also is avirulent (Rowley, 1954), has a similar mean generation time to S. typhimurium strain M206 and is sensitive to 6 μg neomycin per ml in a defined medium.

Animals. The following albino strains of laboratory animal were employed: male mice of the CF 1 strain (Carworth Farms, New City, New York) weighing 20 to 25 g, female guinea pigs weighing 400 to 450 g (National Laboratory Animal Company, Mars, Pennsylvania), and male rats of the Wistar strain weighing 175 to 200 g (Holtzmann Farms, Madison, Wisconsin).

Cortisone. Hydrocortisone sodium succinate in aqueous solution (Solu cortef made by Upjohn Company) and cortisone acetate in saline suspension (Cortone prepared by Sharp & Dohme) were used. All animals treated with cortisone acetate received a daily intraperitoneal dose of approximately 36 mg per kg.

Cell culture media. Tyrode’s solution (Rivers, 1948) containing 100 μg streptomycin per ml, 10 units heparin per ml, and 10 per cent homologous serum was used for bactericidal tests with rat and guinea pig cells. All sera were inactivated by heating at 56 C for 30 min. Serum from cortisone-treated animals was added when the cells were derived from cortisone-treated animals. In all other tests, a chemically defined medium containing 10 per cent inactivated calf serum was employed (Lieberman, 1957).

 Cultures of macrophages and bactericidal tests. The technique for cell culture of mouse macrophages has been described (Furness, 1955b). In the case of rats and guinea pigs, the yield of leucocytes from the peritoneum was increased by injecting intraperitoneally 5 and 10 ml, respectively, of a solution containing 0.1 mg glycogen per ml 4 days before the cells were required.
(Chambers and Grand, 1936). The animals were killed with chloroform and the skin over the abdomen reflected. Various amounts (2.5, 15, and 25 ml) of chilled culture medium were injected intraperitoneally into mice, rats, and guinea pigs, respectively. The abdomen was massaged and the cell suspension recovered aseptically through an incision in the abdominal wall. The suspensions were kept on ice until they had been counted in a Petroff-Hauser bacteria counter; then they were pooled and dispensed, in 2-ml amounts, in screw-capped roller tubes. The cell culture medium used for obtaining suspensions of leucocytes included 100 mg streptomycin per ml to prevent the growth of chance contaminants. In experiments with mouse macrophages, the cell cultures were incubated for 40 to 44 hr at 37°C prior to infection with a streptomycin-sensitive test organism, the medium being replaced by streptomycin-free medium after 24 hr incubation of the cell culture. The effect of cortisone treatment on the viability of the cells was unknown. The cultures of cells from cortisone-treated animals were therefore incubated in a slanting position at 37°C for 16 to 18 hr only before infecting with bacteria.

To obviate changing the medium, a streptomycin-resistant test organism was required. Streptomycin-resistant strains have been reported as being of decreased virulence because of slower growth (Seligmann and Wassermann, 1947). A streptomycin-resistant clone of S. typhimurium strain M206 was obtained by transduction (Furness and Rowley, 1956), using phage propagated on a streptomycin-resistant strain having a normal mean generation time. The streptomycin-resistant recombinant, however, had a slower growth rate than the parent. Strains of E. coli were considered to be suitable test organisms as coliforms were reported as being responsible for many of the spontaneous infections in cortisone-treated animals (Berlin et al., 1952). A streptomycin-resistant mutant of E. coli strain K12 known to be avirulent for laboratory animals (Rowley, 1954) and having a similar mean generation time to M206 was therefore chosen.

The bactericidal test has been described in detail (Furness, 1958b). Cell cultures were infected with bacteria and incubated for 90 min to allow phagocytosis to occur. The uningested organisms were then killed by the addition of neomycin to the cell culture medium. At intervals, the cell culture medium was decanted, 2 ml distilled water added, and the cells resuspended by means of a rubber policeman. Viable counts were then made of the organisms within the cells.

RESULTS

Number of leucocytes. Leucocytes in the peritoneal washings of each animal were counted. The number obtained from rats averaged $3.4 \times 10^4$ per ml, the lowest count being $2.2 \times 10^4$ per ml, and the highest $4 \times 10^4$ per ml. The average for the guinea pig was $1.15 \times 10^4$, lowest count $8.0 \times 10^3$, and highest $1.9 \times 10^4$. Leucocyte suspensions from normal rats were occasionally diluted, but not below $2 \times 10^4$ ml. Cell suspensions from cortisone-treated animals were always dispensed undiluted.

Although there was no significant reduction in the number of cells obtained from guinea pigs injected intraperitoneally with 36 mg cortisone acetate per kg daily over a period of 6 days, there was a significant reduction in the number obtained from rats (figure 1).

Morphological effect of hydrocortisone on cell cultures. Mouse, rat, and guinea pig cells were incubated for 40 to 44 hr at 37°C in a defined medium containing 10 per cent inactivated calf serum. Then 0.1-ml amounts of varying concentrations of hydrocortisone were added to give a final concentration in the cell culture medium of 1000, 100, 10, and 1 mg per ml. The cells were examined after a further 6 to 8 and 24 hr incubation. After 6 to 8 hr incubation, only those cell cultures containing 1000 mg hydrocortisone per ml were visibly affected, the cells being smaller, more rounded and refractile than the controls. After 24 hr incubation, the guinea pig cells treated with 1000 mg hydrocortisone per ml had left the glass; the morphology of the cells in the cultures treated with lower concentrations of hydrocortisone was, however, unaffected. Rat and mouse cells showed a graded response to hydrocortisone with few cells becoming detached from the glass. In tubes containing 1000 mg hydrocortisone per ml, practically 100 per cent of the cells were rounded and opaque, 100 mg hydrocortisone per ml affected the majority of the cells, whereas 10 mg per ml gave variable results, many cultures being similar to the controls. Thus, guinea pig cells responded to hydrocortisone over a narrower range of concentration than rat and mouse cells.
Figure 1. Effect of intraperitoneal cortisone acetate on leucocytes in the peritoneum.

Physiological effect of hydrocortisone on cell cultures. Mouse macrophages were cultured in a medium containing 100 µg hydrocortisone per ml for up to 16 hr prior to infection. There was no significant reduction in phagocytic or bactericidal power of these treated cells using S. typhimurium as the test organism.

Cells from cortisone-treated animals. Cell cultures of leucocytes in Tyrode's solution containing 10 per cent homologous serum from cortisone-treated animals were tested for their bactericidal power after 16 to 18 hr incubation using E. coli as the test organism. In the majority of the experiments 100 µg hydrocortisone per ml were added to the culture medium so that the cells were exposed to its action in vivo and in vitro. There was no diminution of the bactericidal or phagocytic power of the cells from rats and guinea pigs treated with cortisone. Neither did addition of hydrocortisone to the cell culture medium affect the results with these cells.

DISCUSSION

The dosage of cortisone used has been shown to render the rat and mouse susceptible to infection without altering the resistance of the guinea pig. Results obtained using this level indicate that the division into "cortisone-sensitive" and "cortisone-resistant" species (Long, 1957) on the basis of response of the whole animal to injection of cortisone acetate applies also at the cellular level, i.e., in cell culture. The fact that cortisone decreased the number of leucocytes in the peritoneal exudate of rats is in keeping with earlier observations on rats and rabbits in which mineral oil was used as the stimulant (Cummings et al., 1952). But no such effect was observed in the guinea pig which bears a closer analogy to man in this respect than does either the rat or the rabbit (Long, 1957).

The advent of water-soluble hydrocortisone made it possible to carry out toxicity tests in cell cultures using pharmacological doses of the steroid. Under these conditions, the species difference was confirmed, the dose required to affect guinea pig cells being 10 to 100 times as great as that which caused changes in rat and mouse cells. These observations suggest that there is a basic difference in the metabolic behavior of cells from...
the two species and they provide a convenient technique for investigating the nature of such differences.

The knowledge that macrophages obtained by these techniques do not multiply in cell culture is important because any antianabolic action of cortisone would have little scope for producing an effect. On the other hand, the reduction in the number of leucocytes obtained from the cortisone-treated rat is probably due to an antianabolic effect in the intact animal.

The finding that those cells which were obtained, although few in number, maintained their phagocytic and bactericidal power is in keeping with the suggestion of Udall (1955) that cortisone prevents maturation of the precursors of lymphocytes, and of Benaceraff and his colleagues (Benaceraff et al., 1954) that it impairs the formation of new reticuloendothelial cells. Although cortisone causes changes in leucocytes, the experiments described suggest that their functions are relatively unimpaired and that increased susceptibility to infection in cortisone-sensitive species is due to a reduction in the number of cells.

SUMMARY

Guinea pig macrophages are 10 to 100 times as resistant to hydrocortisone succinate as those of the rat and mouse. The effect of the steroid differs qualitatively as well as quantitatively. Macrophages which survive the in vitro and in vivo action of cortisone do not differ in phagocytic and bactericidal power from normal macrophages.

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