Effect of Cortisone Acetate on Experimental Rocky Mountain Spotted Fever in the Guinea Pig. (20223)

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In certain diseases, the administration of cortisone may relieve many of the symptoms and suppress the signs of illness. In spite of this apparent beneficial effect, cortisone therapy may be harmful in diseases such as pneumonia or tuberculosis, since it often accelerates the spread of the infection(1,2). It has been suggested, however, that in the therapy of Rocky Mountain spotted fever and typhoid fever, this hormone may be a valuable adjunct to chloramphenicol(3,4). Because of the intracellular position of rickettsiae as opposed to the extracellular location of most bacteria, one might expect a different response to cortisone in animals or subjects infected with the former microorganisms. The hormone might alter the rate of spread from the site of inoculation, the rate of growth within cells, or the response of the host cells.

The present study was undertaken in an effort to determine the effects of two different dosage schedules of cortisone acetate administered to guinea pigs with experimental spotted fever.

Material and methods. A total of 91 guinea pigs were used. The Bitter Root strain of *R. rickettsii* was passed serially by intraperitoneal injections. Whole bacteriologically sterile, citrated blood obtained by cardiac puncture, or a splenic emulsion in sterile physiologic saline solution was used as passage material. The animals from which passage material was obtained were sacrificed on either the fourth or fifth day of fever. Daily determinations of the body weight and the rectal temperature were made on all animals. Complement fixation tests were performed by a standard method(5). The significance of the difference in the mortality rate between the test and control groups was tested by the chi-square method(6).

Plan of the Experiment. Exp. 1 was designed to determine whether the daily administration of a dose of cortisone acetate comparable to a clinically therapeutic dose in human beings would influence the course of the infection. Five sets of observations were made upon 51 male animals. Each set of animals was given a different preparation of rickettsiae. In each set, the animals were divided into test and control groups of comparable body weight. All animals in the 2 groups were inoculated with the same volume of material containing the rickettsiae. Starting on the day of infection, each animal in the *test group* was given daily subcutaneous injections of 1 mg of cortisone acetate (a dose which is comparable to 150 mg for a 70 Kg man) for 17 days. The *control group* was not given cortisone. All observations were continued for 17 days after infection. *Exp.* 2, performed several months after completion of Exp. 1, was designed to determine whether the administration of a large dose of cortisone acetate for 5 consecutive days at the peak of the clinical illness would alter the mortality rate or the degree of fever. Forty guinea pigs were divided equally into 2 sets, which were studied one month apart. The 20 guinea pigs in each set were observed simultaneously and were given aliquots of a single inoculum of rickettsiae. Ten animals in each set were not given cortisone; the remaining 10 received 5 daily intramuscular injections of

* This work was performed as a Research Fellow of American Heart Assn. sponsored by the 20-30 International Rheumatic Fever Foundation. Work supported in part by U. S. Atomic Energy Commission under contract with Bowman Gray School of Medicine and by grant-in-aid from American Heart Assn. The Bitter Root strain of *R. rickettsii* and the spotted fever antigen were supplied by Dr. Herald R. Cox, Lederle Laboratories, Pearl River, N. Y. Cortisone acetate furnished by Dr. Elmer Alpert, Clinical Research, Merck & Co., Rahway, N. J.

TABLE I. Effect of Cortisone Acetate, 1 mg Daily, on Mortality Rate in Guinea Pigs with Spotted Fever (Exp. 1).

Severity of infection	Total No. animals	Survivals	Deaths
Control group (*R. rickettsii* only)			
Mild, no fatality	3	3	0
Moderate	15	4	11
Severe, fatality rate over 90%	8	1	7
Total	26	8	18
Test group (*R. rickettsii* and cortisone)			
Mild, no fatality	3	3	0
Moderate	14	8	6
Severe, fatality rate over 90%	8	0	8
Total	25	11	14

cortisone acetate, 25 mg, starting on the morning of the fourth day of infection.

Results. Exp. 1. Sixty-nine per cent of the control animals infected with spotted fever and 56% of the test animals also given cortisone acetate died; the difference in the overall fatality rate was not significant. However, when the 5 sets of animals were arbitrarily divided into 3 groups, depending upon the severity of the infection, there was a suggestion that the administration of cortisone acetate did have a beneficial effect (Table I). All of the six animals with "mild" infections survived. None developed fever (rectal temperature of 104°F or greater). Complement-fixing antibody to spotted fever antigen was found in all animals on the twenty-second day of infection. This set of animals was given the original lyophilized material, which subsequently increased in virulence upon serial passage in guinea pigs. All but one of the 16 animals with infections classified as "severe" succumbed. There was no significant difference between the test and control groups as to the duration or height of fever or the day of death. In the 29 animals classified as having "moderately severe" infections, 43% of those given cortisone acetate and 73% of the control guinea pigs succumbed. The difference in the mortality rates in the two groups was statistically significant ($P = 0.01$ to 0.02). The mean duration of fever in the test group was 5.5 days, as compared with a mean of 4.1 days in the control group. In the test group, the average day of death was 11.5 days after inoculation; in the control group, 10.4 days. In general, the changes in body weight in the two groups paralleled each other.

Exp. 2. Eighty per cent of the animals in each group died during the period of observation. Only 6 of the 16 deaths in the test group occurred while the animals were receiving cortisone acetate, whereas 14 deaths occurred during the same period in the control group (Table II). Thus, most of the fatalities in the test group occurred after cortisone was discontinued. The mortality rate in the test group during the first 8 days of infection was significantly lower than that in the control ($P =$ less than 0.01). The mean day of death in the infected animals not given cortisone acetate was 8.1 days; that in the test group which received the hormone was 9.9 days. A striking difference was noted clinically in the physical appearance of the test and control animals within 48 hours after the institution of cortisone therapy in the test group. Those given the drug were much livelier and showed less ruffling of the coat than those not receiving cortisone.

The mean daily decrease in body weight in the 2 groups was practically the same during the first 8 days. An abrupt fall in the mean daily rectal temperature occurred in the control group between the sixth and seventh days of infection, and all except 2 of the animals were dead by the ninth day. In the group given cortisone acetate, the highest mean temperature of 104.7°F was found on the fourth day of infection; it subsequently decreased gradually until the thirteenth day of infection.

Comment. Although the daily administration of 1 mg of cortisone acetate did not significantly alter the overall mortality rate in guinea pigs infected with *R. rickettsiae,* analysis of the results according to the severity of the disease suggested that the hormone may be of value if the infection is of moderate severity. The justification for such an arbitrary classification lies in the fact that the efficacy of a therapeutic agent which does not directly inhibit the growth of an infecting organism may not be apparent, either in the presence of a minimal infection without systemic reactions or in the presence of an over-

TABLE II. Effect of Cortisone Acetate, 25 mg Daily, on Period of Survival in Guinea Pigs Infected with *R. rickettsii* (Experiment 2). 20 animals in each group.

	Total deaths	Period of survival (days after infection)										
		6	7	8	9	10	11	12	13	14	15	16	17
Test (Given *R. rickettsii* and cortisone)	16	1	2	3	2	3	1	2		1			1
Control (Given *R. rickettsii*)	16		7	7	1						1		

whelming infection. In the presence of a moderately severe infection, when there is sufficient time for the body's defense mechanisms to be mobilized, any beneficial effect which the drug possesses may become evident. It has been observed that in guinea pigs with fatal infections of *R. rickettsii*, the temperature drops rather precipitously to subnormal values about 12 hours prior to death. The longer mean duration of fever in the group given cortisone acetate and classified as having moderately severe infections has therefore been attributed to a reduction in toxemia resulting from the administration of cortisone. The longer period of survival in the cortisone-treated animals is also compatible with this interpretation.

The observations in Exp. 2 appear to confirm the impression that cortisone acetate has a beneficial effect on the course of moderately severe cases of Rocky Mountain spotted fever. No deaths occurred in either group prior to the fourth day of infection, at which time the administration of large doses of cortisone acetate was begun in the test group. During the period of drug therapy, the number of deaths in the treated group was significantly smaller than that in the control group. In addition, the clinical appearance and behavior of the treated animals was less in keeping with a severe illness. Following discontinuation of cortisone, the number of deaths in the test group was greater than that in the control group, so that the overall mortality rate was identical. It appears, therefore, that the beneficial effects of cortisone acetate were transitory.

It is not possible from the present study to determine the exact mechanism of the protective effect apparently afforded by cortisone acetate in guinea pigs infected with *R. rickettsii*. There is no evidence to suggest that it may be due to a suppression of the growth of rickettsiae in the infected animals, and it has been previously observed that cortisone acetate does not affect the rate of growth of *R. rickettsii* in the yolk sac of embryonated chick eggs(7).

Summary. 1. The effect of 2 different dosage schedules of cortisone acetate on guinea pigs infected with Rocky Mountain spotted fever of varying degrees of severity has been investigated. The overall mortality rate in 25 animals given daily intramuscular injections of 1 mg of the drug, starting on the day each received, by intraperitoneal injection, an inoculum of rickettsiae, did not differ significantly from the mortality rate in infected guinea pigs which received no treatment. The severity of the infection induced by different preparations of rickettsiae varied considerably; when this factor was taken into consideration, it appeared that cortisone acetate in doses comparable to those employed in human beings reduced the mortality rate in those animals with moderately severe infections. 2. In another group of 20 animals which received daily intramuscular injections of 25 mg of cortisone acetate for 5 days at the peak of the illness (fourth through the eighth day of infection) the overall mortality rate was identical with that in 20 infected animals not given the hormone. During the period of administration of this relatively huge dose of cortisone acetate, however, the fatality rate was significantly lower than that in the untreated animals.

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Received March 23, 1953. P.S.E.B.M., 1953, v82.

Adipokinetic Activity of Oxycel-Purified Corticotropin.* (20224)

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The administration of crude extracts of anterior pituitary to mice, rats, and guinea pigs was found by Best and Campbell(1,2) to produce a rapid increase in the quantity of lipid in the liver and a decrease in carcass fat. It was subsequently shown that the source of the lipid which accumulated in the liver under these circumstances was the fat storage depots (3,4) and that the increment of hepatic lipid was composed largely of glycerides(5); it was concluded that pituitary extracts promoted the mobilization of depot fat to the liver. Since only a fraction (20-50 per cent) of the fat disappearing from the depots could be accounted for in the liver(2,5), the utilization as well as the migration of fat appeared to have been enhanced. The descriptive term adipokinin has been applied to the pituitary principle responsible for this metabolic effect(6). A number of studies have shown that pituitary extracts rich in one or another of the well-defined anterior pituitary principles (gonadotropins, corticotropin, thyrotropin, growth hormone) may produce various effects on fat metabolism of experimental animals, and it has sometimes been implied that one of these might be the pituitary factor causing these effects. Fatty liver and lipemia have been induced by thyrotropic preparations(7-9), and pronounced adipokinetic activity has been reported in preparations rich in growth hormone(10,11) and prolactin(12). Payne(13), studying a variety of crude and partially purified pituitary extracts, found no correlation between adipokinetic activity and that of any one of the well-characterized hormones, and concluded that the fat-metabolizing factor was a distinct principle.

In the present study estimates were made of the adipokinetic activity of some of the extracts obtained in the course of fractionation of hog anterior pituitary according to the procedures devised in this laboratory for the extraction and purification of corticotropin and growth hormone(14-18). The results indicate that a fraction potent in adipokinin is obtained by these methods and lend support to the idea that the fat-mobilizing principle is a factor distinct from other pituitary hormones.

Experimental. Assays were performed by a modification of the method of Campbell(19). Female mice of the Swiss Webster strain (obtained from Taconic Farms and local dealers) varying in weight from 18 to 24 g were used; in most experiments, mice of nearly uniform weight were selected. The animals were deprived of access to food one hour before pituitary extracts were to be administered, and after injection they were kept in cages containing water but no food. In the earlier experiments the test materials were injected subcutaneously in 0.5 cc volume at pH 4 and the mice were killed seven hours later; in later experiments, particularly when potent extracts

* This study was supported in part by a grant from the Worcester District Chapter of the Mass. Heart Assn. The author is indebted to Dr. E. B. Astwood for many helpful suggestions. The hog anterior pituitary powder and crude corticotropin were supplied through the courtesy of Dr. David Klein of the Wilson Laboratories, Chicago, and the Armour Growth Hormone was generously supplied by Dr. Sanford L. Steelman of the Armour Laboratories, Chicago.