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Effect of Cortisone and ACTH on Adrenals in Experimental Diphtheria, Shiga, and Meningococcus Intoxication. (19206)

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Involvement of the adrenal glands is a pronounced feature of the intoxication produced in animals by a number of bacterial toxins and in the course of many bacterial infections.

The possible sparing of the adrenals by cortisone was studied in the case of the toxin of *Shigella dysenteriae* (Shiga) and virulent meningococcus infection in mice, and in guinea pigs given a fatal dose of diphtheria toxin. Because of the interrelationship of ascorbic acid and adrenocortical function, experiments were conducted in which cortisone and ascorbic acid were both administered to the same animal even though mice are known to have the ability, not possessed by guinea pigs, to produce their own ascorbic acid. In addition the effect of ACTH was studied in guinea pigs receiving 1 MLD of diphtheria toxin.

Methods. The cortisone used was the dry acetate or free alcohol which was prepared for use by dissolving a weighed amount in the smallest quantity of acetone required to effect complete solution and then quickly bringing up to full volume with physiological saline solution so that the required dose was contained in 0.5 ml. A drop of "Tween 80" per 100 ml helped stabilize the suspension. The ACTH solution was freshly prepared prior to injection by dissolving a weighed amount of the dried powder in sufficient physiological saline solution so that the required dose was contained in 0.5 ml. Weights of ACTH are expressed in terms of Armour Standard No. LA-1A. The animals used in these experiments were guinea pigs weighing 240-280 g and pure line CFW strain white mice weighing 16-20 g. All guinea pigs were weighed daily. All animals were autopsied at death or after sacrifice.

Effect of cortisone and ACTH on guinea pigs given 1 MLD of diphtheria toxin. The hemorrhagic appearance of the adrenals has long been cited as the most conspicuous finding in guinea pigs dying from diphtheria toxin. The course of intoxication following the administration of 1 MLD of diphtheria toxin has been closely observed in this laboratory for a great many years, an experience which provided a background for the detection of any deviation from the expected course which could be attributed to cortisone, cortisone plus ascorbic acid, or ACTH. In 3 experiments all guinea pigs were given 1 MLD of diphtheria toxin subcutaneously. In Exp. 1 and 2 ACTH was given twice daily (0.17 mg) or cortisone daily (0.34 mg) for 15 days, or until death. In Exp. 1 the initial dose was given subcutaneously at the same time as the toxin.

In Exp. 2 the ACTH and cortisone injections were begun on the day prior to toxin administration. The guinea pigs in these first 2 experiments were in groups of 8, a similar number receiving the toxin only, as controls. In the 3rd experiment, which included 56 guinea pigs, ACTH was omitted, the daily dose of cortisone increased from 0.34 to 1.54 mg, and 25 mg ascorbic acid given daily to half of the animals in each group. All animals were examined for subcutaneous edema, local necrosis, ascites, and weakness. At autopsy the presence and degree of subcutaneous edema, subcutaneous hemorrhage, pleural and abdominal fluid, and congestion of the adrenals were noted. The term congestion is used in preference to hemorrhage because the histological appearance of the adrenals of both guinea pigs and mice is that of congestion of the vessels rather than hemorrhage(1). No marked differences were noted between any of the 3 experiments, either before death or at autopsy, although animals receiving cortisone had an average death time of 6 days as against 8.5 days for the controls. The course of action of the diphtheria toxin was unaffected. All animals showed the following signs of diphtheria intoxication: Loss of weight, loss of appetite, local edema and necrosis, increase in pleural and peritoneal fluid, and congested adrenals, with no demonstrable difference in degree between control groups and corresponding treated groups.

Effect of cortisone on Shiga intoxication in mice. In recent studies on the effects of the toxins of *Shigella dysenteriae* (Shiga) in monkeys(2), the most conspicuous finding was that of hemorrhage: petechial hemorrhages in the skin, hemorrhage into lymph nodes in many regions, and especially hemorrhages into adrenals and into heart muscle. It seemed worth while to determine whether or not Shiga toxin produced similar hemorrhages in mice, and if cortisone might have any effect upon their occurrence. A preliminary experiment was done with 120 mice, 1/3 of which were treated with cortisone and 1/3 with cortisone plus ascorbic acid. All received Shiga toxin intravenously.

In mice receiving Shiga toxin there is a minimum asymptomatic period of at least 18 hours, often extending to 4 or even 6 days. The signs of intoxication are those of an ascending spastic paralysis(3). The mice in the present experiments were observed for 7 days after injection of the toxin. Neither cortisone, ascorbic acid, nor combination of the 2 appreciably lengthened the life of the mice. Ten mice died within 48 hours and were autopsied. Of these, 2 had received cortisone, 4 were controls that received toxin only, and 4 had received toxin and ascorbic acid. Three of the 10 had intensely red adrenals; 2 of these were controls and one had received toxin and ascorbic acid.

In a second similar experiment, 2 groups of 30 mice each were used. One group was given 0.5 mg cortisone on each day of the study. The other group received no cortisone. On the second day both groups were given varying doses of Shiga toxin intravenously. All mice dying in this experiment were autopsied. All those surviving the 7 day period of observation were killed and examined. The time of death seemed uninfluenced by administration of cortisone, but fewer of the cortisone-treated mice showed congested adrenals than those receiving toxin alone. This difference, while not great, seemed definite and this point was then examined further in 3 more extensive experiments, summarized together in Table I.

Those surviving 7 days were sacrificed. All mice were autopsied and the adrenals examined. Many adrenals were bright red, corresponding in appearance to those of the guinea pigs dying from the effects of diphtheria toxin. The appearance of the adrenals was recorded according to intensity of congestion as *red, pink,* or *pale.* The results indicated that cortisone did appear to exert a sparing effect on the adrenals as judged by the degree of congestion observed. More than twice as many untreated animals showed marked congestion as compared with those receiving cortisone.

Effect of cortisone on meningococcus toxin and infection in mice. Massive hemorrhage into the adrenals is the most conspicuous finding at autopsy in patients dying with the Waterhouse-Friderichsen syndrome. Until fairly recently such overwhelming meningo-

TABLE I. Effects of Cortisone on Mice Receiving Varying Doses of Shiga Toxin.

Inoculum of Shiga toxin	Total No. mice T*	Total No. mice U*	Deaths T	Deaths U	Adrenals: Red T	Red U	Pink T	Pink U	Pale T	Pale U
.5 ml of 1/25	10	10	9	9	0	4	9	6	1	0
1/50	20	17†	16	15	4	11	11	6	5	0
1/100	30	30	20	28	5	12	13	14	12	4
1/200	20	25	9	14	3	7	5	6	12	12
1/300	10	20	3	8	3	3	2	4	5	13
1/400	0	14	—	4	—	4	—	3	—	7
Total	90	116	57	78	15	41	40	39	35	36
% T or U			63	67	17	35	44	34	39	31

* T = treated mice; U = untreated mice.

† Animals consumed by others in the group not counted.

Combined results of 3 exp., 60 mice each, 30 in each exp. given .5 mg cortisone daily subcut. On 2nd day all mice given graded doses of Shiga toxin BS-24 intravenously. Additional groups of normal mice—same age and wt—given higher toxin dilutions to confirm LD_{50}.

coccus infections, with the accompanying intoxication, were considered invariably fatal. Within the last few years, a number of recoveries have been reported(4-12). These have been attributed to combination therapy with sulfonamides, antibiotics, large amounts of fluid given intravenously, and whole cortical extract.* The possibility of producing a fatal intoxication in mice and of studying the effect of cortisone upon such a condition was investigated. Meningococcus "endotoxin" was prepared from a heavy suspension of 18 hour agar growth of a mouse-virulent Group I meningococcus (NIH 1027). Titration by intraperitoneal injection into mice showed the LD_{50} to be 0.5 ml. Mice dying from this amount of toxin showed general hyperemia and varying degrees of injection of the axillary and inguinal blood vessels. The adrenals were bright red. Injection of larger amounts of the toxin was followed by conspicuous hemorrhage into the lymphatic tissue of the intestinal walls.

This meningococcus "endotoxin" was unreliable. Either the intoxication seemed to be overwhelming or else it was not constantly fatal. Production of meningococcus infection in mice promised a more consistent picture. This same strain of meningococcus (NIH 1027) was suspended in 3% mucin and titrated by intraperitoneal injection into mice. The LD_{50} was found to be approximately 20 organisms. The mice that died in this titration all showed bright red adrenals, much hyperemia, injection of blood vessels, and usually hemorrhage into the axillary and inguinal lymph nodes, a picture closely paralleling that produced by the "endotoxin." The appearance of the adrenals was similar to that in mice receiving fatal doses of Shiga toxin. In a histological study of the adrenals of mice infected with meningococci, Ruml and Bohnhoff(13) found depletion of fat, engorgement of blood vessels and inflammatory cell infiltration.

In the experiment summarized in the upper portion of Table II, there was an apparent tendency for the adrenals to be less involved, as judged by the degree of congestion, in those animals to which cortisone was given. This experiment was repeated, using another strain of a different serological group of meningococcus (NIH 2103) Group II. Titration in 5% mucin indicated an LD_{50} of 2-10 bacteria. Results are shown in the lower portion of Table II. Strain NIH 2103 affected the adrenals less than did strain NIH 1027, but here again some sparing action seems to have taken place in the mice receiving the cortisone. The excess of ascorbic acid seems to have been without effect in either case.

With both of these strains excessive doses of culture were used. Possibly a more obvious sparing action may have been evident had smaller inocula been given. A further

* Since this paper went to press, two clinical reports of the successful treatment of Waterhouse-Friderichsen syndrome with cortisone have appeared; Nelson, J. and Goldstein, N., *J.A.M.A.*, 1951, v146, 1193; Newman, L. R., *Ibid.*, v146, 1229.

TABLE II. Effect of Cortisone and Ascorbic Acid on Mice Receiving Varying Doses of Meningococci in Mucin.*

Inoculum (LD_{50})	No. mice	Deaths CA†	Deaths C†	Deaths O†	Appearance of adrenals: Red CA	Red C	Red O	Pink CA	Pink C	Pink O	Pale CA	Pale C	Pale O
Exp. 1													
(NIH 1027) 100000	30	10	10	9	1	0	3	8	6	5	1	4	2
10000	30	10	9	10	0	0	8	2	6	1	8	4	1
Total	60	20	19	19	1	0	11	10	12	6	9	8	3
%		100	95	95	5	0	55	50	60	30	45	40	15
Exp. 1													
(NIH 2103) 10000	30	10	10	9	1	3	8	2	0	0	7	7	2
1000	30	10	10	8	3	2	6	0	2	1	7	6	3
100	29	10	10	8	2	4	8	2	3	1	6	3	0
Total	89	30	30	25	6	9	22	4	5	2	20	16	5
%		100	100	86	20	30	76	13	16	7	67	53	17

* Exp. 1—3 groups 20 mice each. 1 group received 1 mg ascorbic acid and .5 mg cortisone daily. 1 group received .5 mg cortisone daily. 1 group received neither cortisone nor ascorbic acid. On 3rd day all mice given from 1000 to 100000 LD_{50} of 5 hr culture of meningococcus NIH 1027 (I) in 1 ml of 3% mucin intraper. Exp. 2—3 groups of 30 mice each. Cortisone and ascorbic acid given as in Exp. 1. On 3rd day all mice given from 100 to 10000 LD_{50} of 5 hr culture of meningococcus NIH 2103 (II) in 1 cc of 5% mucin intraper. All animals autopsied.

† C, cortisone; A, ascorbic acid; O, mice receiving neither ascorbic acid nor cortisone.

series of experiments designed to demonstrate more fully the tendencies indicated above was next carried out using 2 Group I strains, NIH 1027 and NIH 1966. These were arranged in the same manner except that ascorbic acid was not administered. The infecting meningococci were suspended in 3% mucin and the doses varied from 5 LD_{50} to 10000 LD_{50}. Of 60 treated animals receiving strain NIH 1966 the numbers showing red, pink, and pale adrenals were 20, 31, and 9, while for 60 receiving no treatment the figures were 38, 18, and 4. In the case of 30 treated and 30 untreated animals receiving strain NIH 1027 the corresponding figures were 12, 9, 9, and 20, 6, 4. Thus fewer animals receiving cortisone showed congestion of the adrenals as compared with corresponding animals receiving the same dose of organisms but no cortisone. Cortisone did not affect the mortality.

Conclusions. (1) Neither ACTH, cortisone, nor cortisone in conjunction with ascorbic acid appeared to have any effect on the outward course of intoxication of guinea pigs given lethal doses of diphtheria toxin. (2) Neither cortisone nor cortisone in conjunction with ascorbic acid affected the mortality of mice receiving fatal doses of either Shiga toxin or meningococci suspended in mucin. (3) Cortisone appeared to exert a protective action on the adrenals of mice receiving fatal doses of Shiga toxin and meningococci suspended in mucin, as judged by the degree of congestion.

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