Effect of Cortisone on Mouse Resistance to Intravenous Toxicity of Influenza Virus.*† (22926)

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While cortisone and ACTH have, in general, been found to depress host resistance to bacterial and viral infections(1) no uniform pattern has been found for effects of these hormones upon host resistance to toxins of bacteria, rickettsiae, and viruses. Although effects on reactions to bacterial toxins have been studied by several investigators in some detail(1), very few studies have been reported involving the so-called toxic reactions produced by rickettsial and viral agents. Jackson and Smadel(2) found that cortisone and ACTH did not alter the reactions of mice to several rickettsial toxins and Kass and coworkers(3) found no effect of ACTH upon intravenous toxicity in mice and rats of influenza A virus or agents of murine and epidemic typhus. There has been one report(4) of increased reactivity of cortisone treated mice to the intracerebral toxicity of influenza B virus.

In the course of studies on tissue damaging effects of influenza virus and factors which alter these effects it was found that appropriate treatment of mice with cortisone increased their susceptibility to the acute lethal effects of large quantities of intravenously injected virus. In view of the paucity of information on the effect of cortisone on reactions to viral toxins, the following studies were carried out.

Materials and methods. The PR8 strain of influenza A, Lee strain of influenza B, and Victorian strain of Newcastle disease virus (NDV) were cultivated in the allantoic sac of embryonated eggs. Allantoic fluid was stored at −70°C until it was shown to be free of bacterial contamination by culture in thioglycollate and nutrient broth. It was then used undiluted or diluted in physiologic saline buffered to pH 7.2 with phosphate. Measurement of the number of agglutinating doses (A.D.) in these preparations was by a pattern technic with a final concentration of 0.5% chicken erythrocytes. Cortisone acetate (Cortone, Merck) was injected subcutaneously in 0.1-0.2 ml volumes. Physiologic saline containing 1.5% benzyl alcohol and buffered to pH 7.2 with phosphate was used in place of cortisone for control injections in most experiments. In some experiments a solution incorporating the suspending agents used in the Merck product was employed. There was no evidence of a difference in the effects of these two control solutions. Allantoic fluid from uninoculated eggs was used as a control substitute for infected allantoic fluid. Immune sera were from immunized rabbits and were heated at 56°C for ½ hour before use. Swiss white mice (female, Webster strain) weighing 10-13 g and 3-4 weeks old were used. Virus suspensions were injected into the tail vein in 1.0 ml volumes. Only deaths occurring within 72 hours after viral injection were considered as due to toxic effects of the virus.

Results. Groups of mice were given 2.5

TABLE I. Effect of Cortisone on Toxicity of IV Injected PR8 and Lee Influenza Viruses and NDV in Mice.

Preliminary treatment	Challenge (i.v.)	No. dead* /No. inj.
Control fluid	PR8 influenza, 500 A.D.	3/20
Cortisone†	*Idem*	19/20
Control fluid	Lee influenza, 2560 A.D.	4/20
Cortisone	*Idem*	19/20
Control fluid	NDV, 2560 A.D.	4/20
Cortisone	*Idem*	17/20
"	Normal allantoic fluid	0/20
"	56°C heated PR8, 2500 A.D.	0/20

* Observation period: 72 hr.
† Mice received 2.5 mg subcut. 24 hr and 3 hr before challenge.

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mg of cortisone 24 hours and again 3 hours before intravenous injection of virus suspensions. Control groups of mice were given an equal volume of 1.5% benzyl alcohol in saline on the same schedule. It was found (Table I) that although the doses of viruses selected for intravenous challenge produced only a few deaths in each control group, many more mice died of the acute effects of intravenous PR8 influenza A, Lee influenza B, and Newcastle disease virus in those groups treated with cortisone. PR8 influenza virus previously heated at 56°C for ½ hour had no apparent toxicity even when given in very large quantities to cortisone treated mice. Comparing the effects in control and cortisone treated groups, P for chi-square was less than 0.001 for each of the active viruses tested indicating that the results were highly significant.

To determine whether cortisone treated and control mice differed to this degree in their susceptibility to a range of doses of virus, control and cortisone treated mice in groups of 20 were injected intravenously with stepwise dilutions of a pool of PR8 infected allantoic fluid. Dilutions were made in 0.15 $\log_{10}$ steps. The results are presented in Fig. 1 and it can be seen that for both cortisone

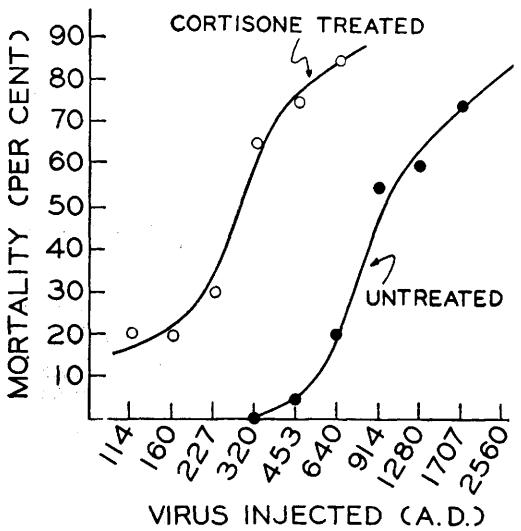

FIG. 1. Effect of cortisone in reducing resistance of mice to a range of doses of intravenous PR8 influenza A virus. Mortality is that occurring within 72 hr after virus injection into groups of 20 mice.

TABLE II. Effect of Immune Serum on IV Toxicity of PR8 Influenza Virus in Cortisone Treated Mice.

1st inj.	Time interval	2nd inj.	No. dead /No. inj.
PR8 I.S.* + PR8 virus†	—	None	0/20
Normal serum	1 hr	PR8 virus	19/20
PR8 immune serum	"	*Idem*	1/20
Lee " "	"	"	18/20
PR8 virus	15 min.	Saline	18/20
Idem	"	PR8 immune serum	7/20
"	"	Normal serum	16/20
"	3 hr	PR8 immune serum	19/20
"	"	Normal serum	17/20

* Rabbit PR8-immune serum. † 640 A.D.

treated and control animals the points, representing the percentage of deaths in each experimental group, fall into curves roughly sigmoid in shape and that these are approximately parallel throughout their courses.

It was important to determine whether the lethal effect of intravenous influenza virus for cortisone treated mice could be prevented by addition of specific immune serum to the virus inoculum or by passive immunization of mice before or after virus injection. An experiment designed to test this is outlined in Table II. A mixture of 640 A. D. of PR8 virus and PR8 antiserum in 1:20 final dilution was without lethal effect when injected into cortisone treated mice. PR8 antiserum (0.2 ml, undiluted) given by intravenous injection one hour before PR8 virus had a marked protective effect as compared to normal serum or Lee antiserum. When specific antiserum was injected intravenously only 15 minutes after an injection of virus the number of resulting deaths was reduced somewhat but when antiserum administration was delayed for 3 hours it had no significant effect on the outcome.

Stained smears and cultures in thioglycollate broth and on blood agar of blood and tissues from cortisone treated mice gave no evidence that bacterial infections activated by cortisone treatment played any part in these results.

To determine the influence of quantity of

TABLE III. Effect of Dose Size and Time of Cortisone Treatment on Resistance of Mice to IV Toxicity of PR8 Influenza Virus.

Cortisone treatment, mg (s.c.) Hr before challenge*				
72	48	24	3	No. dead/No. inj.
		C.f.†	C.f.	1/20
		.5	.5	7/20
		1.0	1.0	10/20
		2.0	2.0	16/20
		2.5	2.5	18/20
.5	.5	.5	.5	17/20
5.0				9/20
	5.0			17/20
		5.0		16/20
			5.0	3/20

* 500 A.D. of PR8 influenza i.v.
† Control fluid (0.2 cc).

cortisone and time of cortisone treatment relative to virus injection, groups of mice were treated with cortisone by several schedules and then injected with 500 A.D. of PR8 influenza virus. The most important of these groups are presented in Table III. Control mice for each of the cortisone schedules were treated with cortisone but injected with normal allantoic fluid. No deaths occurred in cortisone treated controls and details of these controls are, therefore, not included in the table. It was found that when cortisone was given in two injections, one 24 hours and one 3 hours before virus injection, susceptibility was increased as cortisone dosage was increased from 0.5 mg to 2.5 mg per injection. Larger doses had little further effect on susceptibility to viral toxicity and resulted in some deaths apparently due to the cortisone itself. A slight increase in susceptibility to viral toxicity was demonstrable when a third dose of 2.5 mg of cortisone was given 24 hours after virus injection. Although a total of 2.0 mg of cortisone given in two 1.0 mg doses beginning 24 hours before virus injection resulted in only 10 deaths in a group of 20 mice, the number of mice succumbing was increased to 17 when 2.0 mg was given in 0.5 mg doses beginning 72 hours before challenge. The importance of a sufficient interval between the initiation of cortisone treatment and injection of the virus challenge was further indicated by the finding that as much as 5 mg of cortisone given 3 hours before challenge had little effect on susceptibility while 5 mg given 24 or 48 hours before challenge markedly increased susceptibility. The influence of a single 5 mg injection of cortisone had apparently begun to wane when challenge was deferred for 72 hours.

It was of interest to see if lesions produced in cortisone treated mice were different in location and characteristics from those in untreated mice. During the course of several experiments in which graded doses of PR8 influenza were employed mice from cortisone treated and control groups were killed at 6-12 hour intervals and examined for gross and microscopic pathology. Gross and microscopic lesions appeared in cortisone treated mice with lower doses of virus, but their time of appearance (6-12 hours after challenge), location and characteristics were essentially similar to those seen in untreated mice given at least four-fold greater quantities of virus. The lesions produced in mice by intravenous injection of influenza virus have been described previously(5) and details need not be repeated here. In cortisone treated mice, as in untreated controls, the major damage was found in the liver.

Discussion. The experiments described show that cortisone lowers resistance in mice to the intravenous toxicity of influenza viruses and NDV providing cortisone treatment is begun 24 hours or more before challenge. How this is brought about is not yet apparent. Since in these experiments only deaths occurring within 72 hours after challenge were considered as due to viral toxicity and since passive immunization of cortisone treated mice 3 hours after viral injection had no effect on the outcome, it appears unlikely that suppression of antibody production by cortisone was of any importance as a cause of the increased susceptibility. The similarity of location and characteristics of lesions produced in cortisone treated and untreated mice suggest that it was not due to additional tissues becoming susceptible to viral damage. The appearance of histologic evidence of tissue damage with smaller doses of virus in cortisone treated mice as compared to controls suggests that affected cells may be damaged by smaller quantities of virus, or that

under the influence of cortisone the cells may support some degree of multiplication to reach levels of virus which result in cellular destruction. Work is being continued to explore these possibilities.

Summary. 1. Treatment of mice with cortisone significantly reduced their resistance to the intravenous toxicity of PR8 influenza A, Lee influenza B, and Newcastle disease viruses. 2. Deaths of cortisone treated mice were prevented by mixing specific immune serum with the viral inoculum or by passive immunization of mice prior to challenge. Immune serum injected intravenously 15 minutes after challenge gave some protection but was without effect when injected 3 hours after challenge. 3. For full effect, doses of 2.5 mg of cortisone had to be used when treatment was begun 24 hours before challenge. Smaller doses were effective if begun earlier. Doses as large as 5.0 mg had no significant effect if given only 3 hours before challenge.

1. Kass, E. H., and Finland, M., *Ann. Rev. Microbiol.*, 1953, v7, 361.

2. Jackson, E. B., and Smadel, J. E., *J. Immunol.*, 1951, v66, 621.

3. Kass, E. H., Neva, F. A., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 650.

4. Bozzo, A., Jacono, A., and Ricciardi, S., *Riv. Ist. Sieroter, Italiano,* 1954, v29, 324.

5. Henle, G., and Henle, W. J., *J. Exp. Med.,* 1946, v84, 639.

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Progestational Activity of 19-norprogesterone and 19-norethisterone in the Rhesus Monkey. (22927)

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The synthesis of 19-norprogesterone and 19-norethisterone has been previously reported(1,2). These 19-nor steroids have been shown to possess high progestational potency(3,4). 19-norprogesterone was found to be 4 to 8 times as active as progesterone when administered parenterally(3). The degree of endometrial proliferation in the rabbit uterus was used as the criterion of progestational activity. Oral administration of norethisterone (17α-ethinyl-19-nortestosterone) gave a five-fold increase in activity over that of orally administered ethisterone (17α-ethinyltestosterone) in the rabbit assay(4).

This report concerns further observations on these compounds with respect to their relative ability to produce progestational effects in the ovariectomized rhesus monkey.

Materials and methods. Bilaterally ovariectomized rhesus monkeys (*Macacus rhesus*) weighing 3-4 kg were given daily subcutaneous injections of 10 μg estradiol-17β in 0.25 cc sesame oil for 20 days. Beginning on the twenty-first day, daily saline vaginal lavage was used to determine the onset of withdrawal bleeding. This procedure was continued until withdrawal bleeding was completed. The interval between cessation of estrogen administration to the beginning of estrogen-withdrawal bleeding was checked at least twice in each animal before determining the effectiveness of the test compounds in preventing estrogen-withdrawal bleeding. After pretreatment with estrogen as previously indicated, daily administration of test compounds was begun on the twenty-first day. Ethisterone and norethisterone were given orally in tablet form (except as noted in Table I). Solutions of 19-norprogesterone and progesterone were administered subcutaneously in sesame oil. The onset and completion of withdrawal bleeding were determined as noted above.

Results. In Table I, 19-norprogesterone and progesterone have been compared with respect to the characteristic inhibition of estrogen-withdrawal bleeding by progesterone. There was a delay in withdrawal bleeding of