sulated *S. boydii* 2 cultures is reported. An encapsulated culture of *Shigella boydii* 1 also is mentioned. The capsular antigens of *S. boydii* 1 and *S. boydii* 2 are not related. 3. The antigenic relationship of the capsular antigen of *S. boydii* 2 and the capsular antigens of *Klebsiella* types 11 and 21 is reported. This relationship was demonstrated by both agglutination and quellung reactions. No relationship was found between the K antigen of *S. boydii* 2 and any of the 59 K antigens of the *Escherichia* coli group.

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Effect of Cortisone, Related Hormones, and Adrenalectomy on Susceptibility of Mice to Virus Infections.* (18989)

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Cortisone treatment may affect the course of virus infection to the disadvantage of the host. This has been demonstrated for influenza A, influenza B, and mumps viruses in chick embryos(1); for influenza A virus in mice(2); for strain MEF 1 poliomyelitis virus in mice and hamsters(3); for a Coxsackie virus in adult mice(4), and for vaccinia intradermally in the guinea pig(5).

The present work demonstrates that cortisone increases the susceptibility of mice to certain neurotropic viruses, and further investigates the nature of this effect by parallel studies with other steroid hormones, adrenocorticotropic hormone (ACTH), and adrenalectomy.

Methods. Mice. Banks (Swiss) mice weighing 18 to 24 g (6 to 10 weeks old) were used, except for experiment III-12 in which Rockefeller Institute mice of similar age were used. Female mice were used throughout except that males were used for the testosterone treated groups in Exp. IV-27. Adrenalectomies were done about a week before virus inoculation, using ether anesthesia and a dorsal approach. Adrenalectomized mice were given isotonic saline instead of water to drink, but received no other treatment. Autopsies on 38 of the adrenalectomized mice revealed small fragments of adrenal tissue in 11, indicating that about 70% of the mice had been completely adrenalectomized. *Viruses.* All viruses were obtained from Dr. Koprowski of Lederle Laboratories, and were carried in this laboratory by serial intracerebral passage in mice. In the experimental mice all virus inoculations were intraperitoneal using a volume of 1.0 ml of serial 10-fold dilutions of virus-infected mouse brain suspension. Five mice were inoculated with each dilution. Routine virus titrations were performed by intracranial inoculation of Banks mice with 0.03 ml of 10-fold serial dilutions of the material being tested, usually using 3 mice per dilution. Titers, either intraperitoneal or intracranial, are expressed as the LD_{50} dilution using the above volumes. In calculating titers the Reed-Muench formula(6) was used. In Exp. VI-5 the 10^{-1} virus dilution killed only one of the 5 control mice, so in order to calculate an

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TABLE I. Effect of Cortisone on Susceptibility of Mice to Virus Infections.

Virus	Exp. No.	Cortisone dosage: mg per dose	No. doses	Duration (days)	No. doses before virus	Virus titers: LD_{50} dilutions: Controls	Treated	Log difference
West Nile	XII-11	1	5	5	3	$10^{-1.8}$	$10^{-2.5}$	.7
	VI-5	1	5	5	2	$10^{-0.6}$	$10^{-0.8}$	.2
	II-27	2.5	7	10	7	$10^{-1.7}$	$10^{-4.7}$	3
	III-12	2.5	5	5	3	$10^{-2.9}$	$10^{-5.7}$	2.8
	IV-27	2.5	6	9	5	$10^{-1.7}$	$10^{-2.8}$	1.1
	VI-5	2.5	5	5	2	$10^{-0.6}$	$10^{-2.5}$	1.9
	VI-5	10	1	1	1	$10^{-0.6}$	$10^{-1.8}$	1.2
Illheus	I-10	2	9	12	6	$10^{-2.8}$	$10^{-5.2}$	2.4
	II-27	2.5	7	10	7	$10^{-3.3}$	$>10^{-6.0}$	>2.7
	II-27	5	1	1	1	$10^{-3.3}$	$10^{-5.8}$	2.5
Bunyamwera	II-27	2.5	7	10	7	$10^{-2.7}$	$10^{-5.0}$	2.3
	IV-27	2.5	6	9	5	$10^{-4.2}$	$10^{-5.2}$	1
Anopheles B	II-27	2.5	7	10	7	no deaths at 10^{-1}		0

TABLE II. Effect of ACTH on Susceptibility of Mice to West Nile Virus Infection.

Exp. No.	ACTH dosage: mg	Doses	Schedule	Vehicle	No. doses before virus	Virus titers: LD_{50} dilutions: Controls	Treated	Log difference
III-12	.5	20	q6h	saline	10	$10^{-2.9}$	$10^{-3.5}$	.6
III-12	2.5	4	q6h	"	2	$10^{-2.9}$	$10^{-2.9}$	0
VI-5	4	10	q12h	gelatin	6	$10^{-0.6}$	$10^{-5.0}$	4.4

TABLE III. Effects of Adrenalectomy, and Steroids Other Than Cortisone on the Course of Virus Infections.

Treatment	Exp. No.	mg per dose	No. doses	Duration (days)	No. doses before virus	Log difference (treated minus controls): West Nile	Bunyamwera
Desoxycorticosterone	IV-27	10	5	9	3	.6	−1.2
Testosterone	IV-27	25	4	9	3	.5	− .6
Estradiol	IV-27	2.5	5	9	3	.5	− .2
Proluton	IV-27	25	5	9	3	1.3	− .8
Adrenalectomy	VI-5	—	—	—	—	.4	—

LD_{50} titer it was assumed that a theoretical 10^{-9} dilution would have been an LD_{100}. Mice dying less than 24 hours after virus inoculation were discarded from the group. All other mice dying within the 14 days following virus inoculation were considered as dying because of virus infection. Confirmation of virus infection as cause of death was obtained in most instances by observation of paralysis for 1 to 3 days before death, or by examination of brains of dead mice for presence of virus (by intracerebral inoculation of serial dilutions into other mice with resulting typical virus death pattern). *Drugs.* All drugs were clinical type preparations. Dosage schedules are indicated in the tables. Cortisone was given intraperitoneally daily for 5 or more days. When more than 5 doses were used, no treatment was given on weekends. Virus was given at least 36 hours after the start of drug treatments. In 2 experiments a single intraperitoneal dose of cortisone was used, followed by virus 4 hours later. Desoxycorticosterone acetate, testosterone propionate, progesterone, and estradiol dipropionate were administered subcutaneously daily.‡ The doses used (see tables) were chosen as the highest practicable dosage that did not cause death

‡ The authors are greatly indebted to the Schering Corporation for a gift of all steroids except cortisone used in these studies, to the Research Division of Armour and Co. for a gift of ACTH and the gelatin vehicle in which it was administered, and to Merck and Co. for cortisone.

of the mice. ACTH‡ was given subcutaneously. When administered in saline it was given every 6 hours, and when administered in 30% gelatin (to prolong absorption) it was given every 12 hours. Saline injections were given to the untreated control mice in Exp. II-27, using the same volume, route, and frequency of injections as in the cortisone treated mice. In all other experiments the controls received no injections.

In order to determine whether the dosage of cortisone used in most of these experiments (2.5 mg i.p. daily) was pharmacologically effective, spleen and adrenal weights were determined on 9 mice 24 hours after the 3rd, 4th, or 7th doses. Spleens represented 0.25% of total body weights in treated mice and 0.47% in 9 control mice. Adrenal weights showed no consistent difference between treated and untreated groups. All 3 dosage schedules of ACTH (Table II) also caused an average shrinkage of spleens to 50% of normal weight. The 5-day ACTH treatment schedules caused adrenal hypertrophy (adrenals of the 6 mice studied represented 0.058% of body weight in contrast to 0.036% in the 9 controls). The one-day ACTH treatment caused no adrenal hypertrophy when measured 6 hours after the last injection.

Results. Cortisone. Treatment with 2.0 or 2.5 mg of cortisone daily for 5 or more days rendered mice consistently more susceptible to lethal infection by the intraperitoneal route by West Nile, Ilheus, and Bunyamwera viruses. The increase in susceptibility varied from 10-fold to 1000-fold as measured by the LD_{50} dilution of virus. The data from all experiments with cortisone are recorded in Table I. In addition to the decrease in minimum amount of virus causing death, the effect of cortisone usually was also indicated by earlier deaths in the cortisone treated mice as compared with control mice receiving the same dose of virus.

Cortisone at a dose of 1 mg daily failed to show any effect on susceptibility to West Nile virus in 2 experiments. Two experiments were done with a single large dose of cortisone. Mice treated with a single 10 mg dose of cortisone 4 hours before West Nile virus showed a slight increase in sensitivity, and a single dose of 5 mg of cortisone caused a significant increase in sensitivity to Ilheus virus.

A single experiment was performed with Anopheles B virus, which is infective for normal mice only if administered intracerebrally, to see if cortisone-treated mice would be susceptible to intraperitoneally-administered virus. No increase in susceptibility to this virus was observed, although simultaneous studies showed that cortisone-treated mice were much more susceptible to infection by each of the 3 above mentioned viruses.

In order to see if cortisone had any effect on a virus *in vitro,* a West Nile virus preparation was mixed with cortisone and isotonic saline so as to give a final virus dilution of 10^{-1} and cortisone concentrations of 10, 1, and 0.1 mg/ml. These mixtures and a control virus preparation containing no cortisone were incubated for 2 hours at 37°C and then titered intraperitoneally and intracerebrally in mice. The results showed no difference in virus titer after exposure to 1 or 0.1 mg/ml of cortisone, as compared to the control. There was a decrease of approximately 100-fold (2 logs) in virus infectivity in the tube containing 10 mg of cortisone per ml. This concentration is greatly in excess of what would be expected within the body of a cortisone-treated mouse. It is not known whether this anti-viral effect was due to cortisone itself or to the suspending and preserving agents in the cortisone suspension (Merck). It is clear that there was no stimulating effect on virus growth resulting from the *in vitro* exposure to cortisone

ACTH. Adrenocorticotropic hormone at a dosage of 0.5 mg every 6 hours for 5 days failed to influence either favorably or unfavorably the course of infection with West Nile or Ilheus virus inoculated intraperitoneally after the 10th dose of the hormone. Similar results have been reported with other viruses(2,3,7,8). However, when the dose was quadrupled, to 4 mg every 12 hours, and administered in

7. Loosli, C. G., Hull, R. B., Berlin, B. S., and Alexander, E. R., *J. Lab. and Clin. Med.*, 1951, v37, 464.

8. Hull, R. B., and Loosli, C. G., *J. Lab. and Clin. Med.*, 1951, v37, 603.

gelatin to prolong absorption, a cortisone-like effect on virus susceptibility was observed (Table II). Four treatment controls (no virus inoculation) survived more than 2 weeks after this ACTH treatment without apparent ill effect.

Adrenalectomy. The LD_{50} titer for West Nile virus in adrenalectomized mice (approximately 30% were incompletely adrenalectomized) was $10^{-1.0}$ as compared to $10^{-0.6}$ in parallel normal controls. This difference is insignificant, indicating that adrenalectomy had no effect, either adverse or therapeutic, on this virus infection.

Steroids other than cortisone. Testosterone, estradiol, progesterone, and desoxycorticosterone in maximum tolerated dosage appear to have no significant effect on the course of the virus infections (Table III). As judged by the LD_{50} titers, desoxycorticosterone had an apparent slight therapeutic effect (indicated in the table as a negative difference in the logs of the LD_{50} titers) on Bunyamwera virus, but not on West Nile virus. Conversely, progesterone appeared to have an adverse effect on mice infected with West Nile virus, but a very slight therapeutic effect on mice inoculated with Bunyamwera virus. It is felt that these slight and inconsistent differences are not significant.

Clinical studies. Four patients with advanced inoperable cancers were inoculated with West Nile virus while receiving large doses of cortisone. This study was part of a clinical evaluation of the anti-neoplastic effects of several viruses in man(9). West Nile virus produces no subjective evidence of disease in man. Infection is evidenced only by occurrence of viremia for a few days after virus inoculation. Cortisone dosage was 400 mg every 12 hours intramuscularly in 3 patients and 200 mg every 6 hours by mouth in one patient. The drug was given for 2 to 4 days prior to virus inoculation (2.5 ml of 20% infected mouse brain suspension) and was continued for a total of 10 to 14 days. These doses caused rapid fluid retention but no other clinical evidence of toxicity. By clinical standards this is massive cortisone dosage. It probably closely approaches the maximum that can safely be administered to man. Virus infection was established in only one patient and caused no signs or symptoms. Infection with West Nile virus was established 4 times in 18 similar patients without cortisone treatment. Thus, in this limited clinical trial cortisone treatment did not appear to influence West Nile virus infection in man as judged by susceptibility to infection (occurrence of viremia) or clinical course. Obviously, these criteria are in no way comparable to the criteria used in the mouse experiments.

Discussion. In causing this adverse effect on virus infection, cortisone might act directly or indirectly. By direct action it might so affect the virus as to make it more virulent, or it might so affect the host as to make it more susceptible. Such an effect on the host might result from changes in susceptible cells such as to favor virus invasion or propagation, or from interference with cellular and humoral defense mechanisms. Cortisone might act indirectly through its effect on the pituitary and adrenal cortex, thereby causing changes in other hormones which in turn might be the agents directly responsible for the effects observed. From the present data, it appears unlikely that the cortisone effect is dependent on suppression of pituitary or adrenal function, because the other steroid hormones tested did not affect susceptibility to the viruses, and because adrenalectomy did not significantly alter virus susceptibility. Furthermore, if the cortisone effect were due to reduced output of a protective adrenal substance, as has been hypothesized by Schwartzman(3), one might expect that stimulation of the adrenal by ACTH would increase the output of this substance in proportion to the cortisone output and therefore would have a therapeutic effect or no effect at all on virus infection. Since the opposite was observed when very large doses of ACTH were used, it appears that the endogenous cortisone or cortisone-like steroids produced in response to ACTH were unopposed by any hypothetical protective material. Studies by other workers(2,3,7,8) on the effect of ACTH on virus

9. Southam, C. M., and Moore, A. E., *Am. J. Trop. Med.*, 1951, in press.

infections have shown either no effect or suggestive indications of a cortisone-like effect. The ACTH dosage used by these workers has never equalled, and has usually been much smaller than that used in the present studies. The necessity for large doses of porcine ACTH for adrenal stimulation in the mouse has been emphasized by Kass *et al.*(10). Kalter and coworkers(11) have reported that small doses of ACTH or cortisone decrease, and small doses of testosterone increase, the rate of influenza virus propagation in the mouse for 48 hours after inoculation, but apparently not thereafter. This phenomenon is probably unrelated to that studied in the present experiments. The fact that virus virulence was unaffected by *in vitro* incubation with cortisone, makes improbable the hypothesis that cortisone acts directly on the virus rather than through the host. In summary then, the evidence at hand suggests that the adverse effect of cortisone upon virus infections is a direct result of the action of cortisone itself upon the host. The observation that cortisone inhibits the early stages of the inflammatory reaction(12) suggests a possible mechanism for this effect. It is not yet known if other compounds of the cortisone type (11-oxy steroids) have this same effect on virus infections.

It is of interest that while cortisone treatment rendered mice more susceptible to some viruses, it did not make mice susceptible to Anopheles B virus by the intraperitoneal route. Kilbourne and Horsfall(4) have demonstrated that a Coxsackie virus will infect adult cortisone-treated mice whereas it will not infect normal adult mice. These observations are readily reconcilable if one reasons that cortisone causes merely a quantitative diminution in host resistance. Thus, it may be that cortisone has a marked effect on West Nile, Ilheus, Bunyamwera, and Coxsackie virus infection because with these viruses there is a delicate adjustment between virus invasiveness and host resistance, while Anopheles B virus has insufficient invasive ability to cause death even with the "help" of cortisone. If this hypothesis is correct, one might expect that if a highly virulent virus were studied, cortisone might have little effect on the course of infection because such a virus would be so efficiently invasive that it can readily overwhelm host resistance even without the "help" of cortisone.

Summary. Large doses of cortisone greatly increased the susceptibility of mice to lethal infection by West Nile, Ilheus, and Bunyamwera viruses. ACTH in massive dosage produced the same effect with West Nile virus. Desoxycorticosterone, testosterone, progesterone, and estradiol in massive doses had no significant effect on these virus infections in mice. Anopheles B virus was not infective by intraperitoneal route even in cortisone treated mice. Adrenalectomy did not influence susceptibility to virus infection. The possible mechanisms of the cortisone effect are discussed and it is concluded that the effect of cortisone upon virus infection is probably due to a direct effect of cortisone upon the host.

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