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Susceptibility of Cortisone-treated Mice to Infection with Mouse Hepatitis Virus.* (24263)

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The strain of mouse hepatitis virus (MHV) used has been described by Gledhill(1-3). It is the virus from which the enhancing agent, *Eperythrozoon coccoides,* has been excluded. This virus causes a mild, rarely fatal, infection in weanling Swiss mice and a fatal infection in suckling mice. In the latter case, focal necrotic lesions appear in the liver and evolve into diffuse, cirrhosis-like lesions. It was found that normally resistant weanlings became susceptible to fatal hepatitis infection when subjected to cortisone treatment. In this respect, cortisone has been used extensively to change the native resistance of various animals and embryonated eggs to infection by protozoan, mycotic, bacterial, rickettsial, and viral agents(4).

Materials and methods. Weanling Swiss mice, 8-10 g, and 1-2 day-old suckling mice, were used routinely. Stock virus was made from infected suckling-mouse livers as a 10% emulsion (w/v) in medium 199 or Hank's solution containing 0.2 mg/ml streptomycin and 200 units/ml penicillin. Liver preparations were clarified by centrifugation at 8,000 rpm for 20 min at 4°C, sealed in ampoules, and stored in a dry ice chest. The usual inoculum (0.05 ml) administered *via* the peritoneal route to weanling mice contained $10^{-3.5}$ suckling mice ID_{50}s(5) of MHV. Cortisone acetate (Upjohn Co., aqueous suspension, 25 mg/ml) was injected intramuscularly in single doses of 1.25 mg unless otherwise indicated. Mice were observed daily for 6 to 15 days. Survivors were killed with ether and autopsied. Livers showing lesions of questionable significance were homogenized in saline (10% w/v) and assayed in suckling mice.

Results. Large doses of cortisone alone caused some deaths, ascites, and distinct, pinpoint, whitish liver lesions in a small proportion of mice (Table I). In a series of 2 experiments, a total of 25 mice were given a single injection of cortisone (1.25 mg) and a total of 31 mice were given 4 daily injections of cortisone (total 5.0 mg). Observations were made daily for 12 and 15 days (Table I). Survivors were sacrificed and examined for evidence of liver damage and ascites. Of the mice which received 1.25 mg cortisone, an average of 8% died, none had liver lesions or ascites. Of the mice which received 5.0 mg cortisone, an average of 17% died, 9% had liver lesions, and 5% had ascites. Livers with cortisone-induced lesions were not infective for normal suckling mice. Several forms of unidentified bacteria were cultured in thioglycollate broth from these livers. Similar observations were reported by

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TABLE I. Effect of Cortisone on 8 to 10 g Swiss Mice Showing % Deaths, Liver Lesions, and Ascites.

Cortisone (mg)	No. of mice	Sacrificed (days)	Deaths	Lesions	Ascites
			%		
1.25	11	12	9	0	0
	14	12	7	0	0
5.00	11	12	9	18*	9
	20	15	25	0	0

* Homogenates of livers with cortisone-induced lesions were not infective for suckling mice.

others(6-9) and it is suggested that cortisone may activate a latent infection, transform nonpathogenic residents to pathogenic forms, and/or decrease host resistance to a point where normally nonpathogenic flora assume pathogenic roles.

The effect of single graded doses of cortisone on survival of weanling mice infected with MHV is shown in Fig. 1. One day after virus injection, cortisone was administered to 3 groups of 14 mice each as single graded doses of 0.3, 0.6, and 1.25 mg. A 4th group received only virus and a 5th group received only 1.25 mg cortisone on the 2nd day. There were no deaths in the virus control group after 12 days. In the cortisone control group, 7% of the mice died. There were 22, 57, and 85% deaths in the virus-infected groups which received 0.3, 0.6, and 1.25 mg cortisone, respectively. Cortisone reduced the innate resistance of mice to infection with MHV. Rate of mortality and number of deaths increased to a degree proportional to dosage of the steroid.

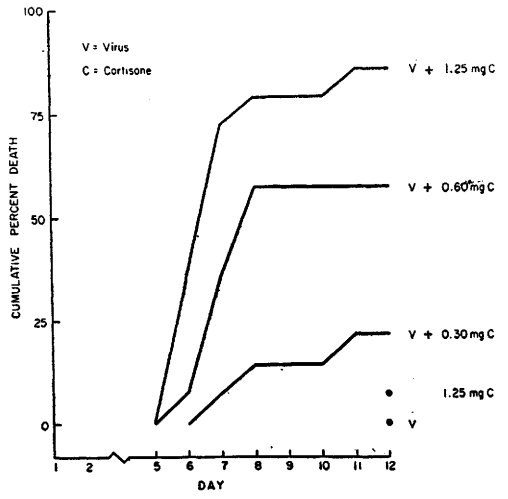

FIG. 1. Effect of single graded doses of cortisone on survival of mice infected with mouse hepatitis virus.

In another series of experiments, an attempt was made to determine the time interval between virus and cortisone injection which was most effective in decreasing host resistance. Five groups of mice were inoculated with virus on the 1st day. Following intervals of 1, 2, 3, and 5 days, 4 consecutive daily doses of cortisone (total 5.0 mg) were administered to each group of mice (Fig. 2).

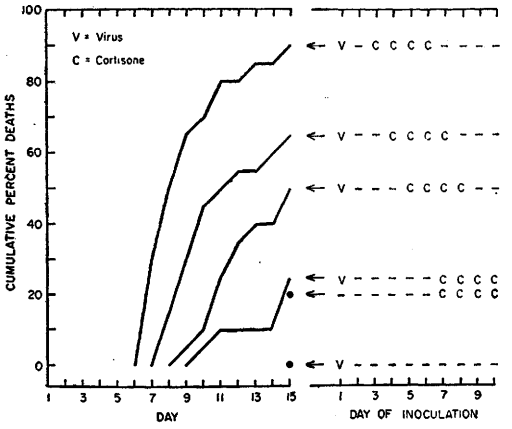

FIG. 2. Effect of cortisone on susceptibility of mice to hepatitis infection.

Deaths were not noted in the virus control group and cortisone alone accounted for 20% deaths. There was a definite decrease in rate of mortality as the time interval between virus and cortisone administration was increased from 1-5 days.

In other tests, cortisone and virus combinations were administered to 5 groups (I to V) of 18 mice each over a period of 5 days as shown in Table II. Deaths and liver lesions were noted on animals dying within 6 days at which time survivors were sacrificed. Table II shows the percentage of deaths incurred with and without liver lesions and the percentage of the survivors with liver lesions. Cortisone alone accounted for 6 and 22% deaths in cortisone-control groups IV (1.25 mg cortisone) and V (5.0 mg cortisone), respectively. However, 22% of the survivors of group IV had liver lesions. Comparison

TABLE II. Effect of Cortisone (C) and Virus (V) on 8-10 g Swiss Mice Showing % Deaths and % Survivors with Liver Lesions on the 6th Day.

Group	Day of inoc. 1	2	3	4	5	Deaths, no lesions	Deaths, with lesions	Survivors, with lesions
I	C	V	–	–	–	0	28	77
II	C	V	C	C	C	11	50	100
III	–	V	–	–	–	0	0	39
IV	C	–	–	–	–	6	0	22*
V	C	–	C	C	C	22	0	0

* Homogenates of livers with cortisone-induced lesions were not infective for suckling mice.

with the data in Table I shows that all of these survivors in group IV would have lived for at least 12 days. There were no deaths in virus control group III and 39% of the mice had liver lesions. As shown in Fig. 1 and 2, all of the mice in the virus control group III would have survived. Comparison of the percent deaths and percent liver lesions observed in groups I and II with their corresponding cortisone control groups IV and V again shows the effectiveness of cortisone in decreasing host resistance to infection with MHV (Table II).

Summary. A small proportion of mice inoculated with cortisone alone died or developed ascites and/or liver lesions. Bacteria were isolated from some of the livers showing cortisone-induced lesions. Homogenates of such livers were not infective for normal suckling Swiss mice. Cortisone acetate reduced susceptibility of normally resistant weanling Swiss mice to infection with the Gledhill strain of mouse hepatitis virus. The effectiveness of cortisone in decreasing host resistance was enhanced as dosage of the steroid was increased. There was a decrease in this cortisone effect as the time interval between virus and cortisone administration was increased from 1 to 5 days.

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Response of Germ-Free and Conventionally Reared Turkey Poults to Dietary Supplementation with Penicillin and Oleandomycin.* (24264)

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The mechanism of the growth-promoting effect of antibiotics in poultry rations has not been clearly established. The prevailing opinion seems to be that antibiotics act mainly by modifying the intestinal microflora(1-4). A basic question is whether antibiotics exert a growth-promoting effect in the absence of an intestinal microflora. Luckey(5) reported that no stimulation of growth was seen when germ-free chickens and turkeys were fed antibiotics at a level of 50 mg/kg diet. In subsequent studies Luckey, *et al.*(6) reported that

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