lowing the test dose rises rapidly during the first 4 hours and decreases to basal levels within 24 hours; pyridone is excreted more slowly the first few hours but appears in large amounts later in the day. After graded doses of nicotinamide, the excretion of pyridone increases more rapidly than does that of N^1-Me. In subjects with pellagra, the excretion of these metabolites is lower both on standard diets and following administration of small doses of nicotinamide. These data suggest procedures that may be of use in evaluating niacin nutrition.

The authors wish to thank Janis Gibbens for planning and supervising the diets, and Wilna Ates and Barbara Geer for their technical assistance.

We are indebted to Dr. H. S. Faulkner of General Mills, and Dr. F. N. Peters of Quaker Oats Co., for generously supplying wheat and corn products used in the diets. The nicotinamide used in this study was furnished by Winthrop-Stearns Chemical Co., through the courtesy of Mr. Kenneth Smoot.

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Received July 30, 1953. P.S.E.B.M., 1953, v84.

Observations on Antibiotic Treatment of Bacterial Infections of Cortisone-Treated Mice. (20592)

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Numerous reports on the action of cortisone in promoting progression of various types of infection in man and in experimental animals have appeared in recent years, and in addition, observations on alteration of cellular response to various inflammatory stimuli have been described(1). In previous studies(2) in which *Mycobacterium tuberculosis* infection was induced in mice by the intraperitoneal route, it was observed that mice treated with large doses of isoniazid and cortisone showed much better therapeutic response than did mice treated with isoniazid alone Mice treated with large doses of cortisone and streptomycin did not give such a good response. The work was extended to gain further information on the relationship between invading bacteria and the defense mechanisms of the host; studies have been made in which experimental infections induced in mice by other bacteria were treated with appropriate antibiotics and with or without cortisone.

Methods. CF_1 male mice (18-22 g) were used in all experiments. Mice were infected by the intraperitoneal injection of cultures which had been incubated at 37°C for 24 hours. The strain of streptococcus used in most experiments (*S. zooepidemicus*—Group C) was originally isolated from a casaeus lymphnode of a guinea pig and was maintained on blood agar slants. The culture of *Streptococcus haemolyticus* (Group A) used was obtained through the courtesy of Dr. Fred B. Traub. Cultures for infecting mice were prepared in

TABLE I. Effect of Cortisone on Antibiotic Treatment of Mice Infected with Group C Streptococcus.

Antibiotic	Dosage and administration	Antibiotic + cortisone*				Antibiotic only*			
		Dose of cortisone, mg/day	Mortality† Dead	Total	Survival, %†	Mortality† Dead	Total	Survival, %†
Penicillin (procaine)	0.1 mg/ml drinking water‡	2.5§	5	38	87.	32	38	15.7
	1 " " "	.8	0	8	100.	0	8	100.
	0.25 mg/aqueous subcut. 4× daily	1.	10	10	0	10	10	0
	1 " " " 2× daily	1.	10	10	0	10	10	0
	2 " " " "	1.	10	10	0	10	10	0
	1 mg/oil " "	1.	0	10	100.	0	10	100.
	1 " " " "	.2	0	10	100.	0	10	100.
Aureomycin	1 mg/aqueous " "	2.5§	14	18	12.	0	18	100.
	2 " " "	.8	10	10	0	0	10	100.
	2 " " "	.2	0	10	100.	0	10	100.
Streptomycin	1 " " "	2.5§	25	28	10.7	8	28	71.4
	2 " " "	.8	32	40	20.	5	40	87.5
	2 " " "	.6	5	10	50.	1	10	90.
	2 " " "	.4	4	10	60.	2	10	80.
	2 " " "	.2	2	20	90.	4	20	80.
Terramycin	1 " " "	2.5§	10	10	0	3	10	70.
	2 " " "	.8	10	10	0	1	10	90.
	2 " " "	.2	0	10	100.	0	10	100.

* Mice treated on day 1, 2, and 3. † On the 5th day after infection. ‡ Taken *ad lib.*
§ Cortisone inj. 2.5 mg/kg on day 1, 1 mg/kg on day 2 and 3.

tryptose phosphate broth (Difco) containing 10% human serum. Laboratory stock cultures of *K. pneumoniae, P. multicida,* and *S. pullorum* were grown in tryptose phosphate broth. *Br. abortus* was grown on trypticase soy agar. Cortisone as the acetate was used in all experiments. It was injected subcutaneously as an aqueous suspension and dosage was given once a day beginning on the day of infection, and for the next two days, except in experiments with *Br. abortus* in which the mice were treated with cortisone for 5 days. The doses used in experiments, other than those with *Br. abortus,* was such that 80% to 100% of untreated mice died within 5 days of the time of infection. All of 292 infected, untreated mice, and 292 infected mice treated with cortisone only, used in experiments shown in Table I and II, died within the 5 day period of observation. Other details are described in connection with the various experiments.

Experimental. Mice infected with Group C streptococcus were treated with oral or subcutaneous penicillin with or without injections of cortisone. Procaine penicillin (1000 units/mg) was given in drinking water, by subcutaneous injection as aqueous solution twice, or 4 times a day in divided doses between 9 a.m. and 5 p.m., or by subcutaneous injection of a suspension in oil with aluminum mono-stearate further diluted in sesame oil to give the desired dose in 0.1 ml. Mice were infected between 9 and 10 a.m. by the intraperitoneal injection of 0.1 ml of serum broth culture. *Ad libitum* or subcutaneous dosage with penicillin was begun 2-3 hours after infection, and cortisone was injected within 1 hour of the time of infection. Treatment was given for 3 days, and survivors were totaled after 5 days. Groups of mice which received no treatment and groups treated with cortisone alone were included in each experiment.

It is seen in Table I that mice infected by intraperitoneal injection of Group C streptococci survive when treated with penicillin or penicillin and cortisone, provided the dosage of antibiotic is adequate. Thus, the infected mice which drank daily 1000 to 10000 units of penicillin in water or which received 1000 units in oil each day for the 3 day treatment period, usually survived, but when aqueous penicillin was injected subcutaneously 2 or 4 times during the day, but not during the night, there were no survivors. It is of interest that a greater number of mice survived among those which were given drinking water containing 100 units of penicillin per ml, and which were injected with large doses of corti-

TABLE II. Effect of Cortisone on Antibiotic Treatment of Mice Infected with Various Bacteria.

Infection	Antibiotic treatment* and dosage		Cortisone,† mg/day	Mortality‡ Dead	Mortality‡ Total	Survival, %	Antibiotic only Mortality‡ Dead	Antibiotic only Mortality‡ Total	Antibiotic only Survival, %
Streptococcus (Group A)	Penicillin 0.1 mg/ml drinking water		1.	6	10	40.	7	10	30.
		mg subcut. 2× daily							
	Neomycin	.5	1.	10	10	0	4	10	60.
P. multicida	Terramycin	1	2.5§	10	10	0	2	10	80.
S. pullorum	Streptomycin	1	2.5§	20	20	0	14	20	30.
Brucella abortus	Streptomycin	.25	.8	18	20	10.	12	20	40.
	Aureomycin	"	.8	20	20	0	5	20	75.
	Terramycin	"	.8	15	20	25.	8	20	60.
Klebsiella pneumoniae	Streptomycin	1	2.5§	16	20	20.	0	20	100.
	"	1	.8	15	20	25.	0	20	100.
	"	2	.8	0	20	100.	0	20	100.
	Aureomycin	2	.8	17	20	15.	0	20	100.
	Polymyxin B	.2	1.	8	10	20.	0	10	100.
	Neomycin	1	1.	5	10	50.	0	10	100.
	Chloramphenicol	1	2.5§	9	10	10.	0	10	100.
	Terramycin	1	.8	9	10	10.	0	10	100.
	"	"	.6	8	10	20.	0	10	100.
	"	"	.4	6	10	40.	0	10	100.
	"	"	.2	2	10	80.	0	10	100.

* Mice treated on day 1, 2, and 3.
† Aqueous suspension of cortisone acetate inj. subcut. once a day, day 1, 2, and 3.
‡ After 5 days with the exception of those infected with *Brucella abortus* which were tabulated after 14 days.
§ Cortisone inj. subcut. 2.5 mg on day 1, 1 mg on day 2 and 3.

sone (2.5, 1, 1 mg per mouse on 3 successive days) than among those given the same amount of antibiotic but no cortisone.

Mice infected with Group C streptococcus were treated with aureomycin, streptomycin or terramycin alone and with cortisone acetate. These antibiotics were given subcutaneously twice a day, the first injection being 2-3 hours after infection. Cortisone was injected 1 hour after infection, as in the experiments with penicillin. Results of these experiments are also shown in Table I. It is seen that, in contrast to the results with penicillin, the great majority of streptococcus-infected mice injected with large doses of cortisone and adequate doses of aureomycin, streptomycin or terramycin failed to survive.

Similar experiments were made in which mice infected by i.p. injection of other bacteria were treated with antibiotics known to be effective against them. Dosages of cultures used to infect mice were: *S. pullorum* 1.0 ml, streptococcus (Group A) 0.1 ml; *K. pneumoniae* 0.1 ml of culture diluted 1:30; *P. multicida* 0.1 ml of culture diluted 1:50. Cortisone was first injected 1 hour, and the antibiotics 2-3 hours after infection and treatment was continued for 3 days. In the experiments with *Br. abortus* mice were infected with 0.1 ml of a suspension obtained by washing the growth from a 24 hour culture with 3 ml of saline. The mice in the various groups were treated with cortisone for 5 days and with antibiotics for 7 days starting 24 hours after infection. Survivors were totaled after 2 weeks. None of the untreated controls, or those treated with cortisone, survived this period of observation. Results of treatment of mice infected with the various bacteria and treated with appropriate antibiotics are shown in Table II.

Penicillin given in drinking water protected the majority of mice infected with the Group A streptococcus regardless of whether they received cortisone. However, the therapeutic effect of polymyxin B, streptomycin, neomycin, chloramphenicol or terramycin obtained in mice infected with *K. pneumoniae* was reversed by concomitant treatment with cortisone and *P. multicida, S. pullorum* and *Br. abortus* infections were not controlled when cortisone was given together with the doses of

aureomycin, streptomycin, or terramycin which when used alone insured survival of mice.

Impairment of the effect of antibiotics appears to depend upon the administration of massive doses of cortisone. This is shown by results of treatment of streptococcus infected mice treated with streptomycin, terramycin, and aureomycin (Table I) and by *K. pneumoniae* infected mice treated with terramycin (Table II). If the amount of cortisone given during the 3 day treatment period is decreased below a critical level, reversal of successful treatment by these antibiotics failed to occur.

Discussion. Under the conditions of these experiments, penicillin protects infected mice treated with large doses of cortisone as well as those treated with penicillin alone, and when mice were treated by giving 100 units per ml of drinking water, those which received large doses of cortisone in addition were better protected than those which received no cortisone. Experiments by Mogabgab and Thomas(3) have shown that penicillin in large doses was effective in the treatment of cortisone induced streptococcus infection in rabbits; and Kaplan *et al.*(4) have shown that penicillin prevents deaths due to experimental streptococcal infection in mice exhibiting profound leukopenia as a result of irradiation. The latter authors considered that penicillin, because of its bactericidal action, was effective in the absence of the host's defense mechanisms, and that the lesser effectiveness of aureomycin under these conditions was probably a function of the low dosages employed. In the present experiments, dosages of aureomycin, terramycin, and streptomycin greatly in excess of the amount needed to protect streptococcus-infected mice which received no cortisone failed to protect mice injected with large doses of cortisone. Similarly, these antibiotics and neomycin, polymyxin B and chloramphenicol, when given in doses which protected non-cortisone treated controls failed to protect mice treated with large doses of cortisone against infections caused by various gram negative bacteria. One exception was noted, however, in an experiment in which mice, infected with *K. pneumoniae,* were protected by being treated with 2 mg of streptomycin twice a day although they received cortisone. Most of the cortisone-treated mice which received 1 mg of streptomycin twice a day died, although this amount of streptomycin protected controls. *K. pneumoniae* is notably susceptible to streptomycin and it is probable that at the 2 mg 2 x day level, the infecting organisms are quickly destroyed. In this connection, it should be noted that Miller *et al.*(5) found streptomycin to be the most effective of a number of antibiotics studied in controlling post-irradiation infection.

On the basis of the present experiments, it appears that with the exception of penicillin, the antibiotics studied are unable to complete their chemotherapeutic effect in mice in which normal defense mechanisms have been impaired by large doses of cortisone. Jawetz(6) has briefly described experiments in mice of similar importance in which cortisone interfered more with the protective effect of aureomycin than with that of penicillin.

Summary. The influence of cortisone on the chemotherapeutic effect of antibiotics in experimental bacterial infections in mice has been studied. Cortisone has little or no effect on the protective action of penicillin against streptococcus infections in mice. When administered in doses which regularly protect non-cortisone treated mice, streptomycin, aureomycin, terramycin, chloramphenicol, neomycin and polymyxin B, failed to protect mice treated with cortisone. This reversal of successful antibiotic treatment occurred in mice treated with large doses (0.8 mg) but did not occur in mice treated with small doses (0.2 mg) of cortisone.

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Received July 31, 1953. P.S.E.B.M., 1953, v84.