Effect of Cortisone on Mice Infected with Chromogens and with Isoniazid Resistant *Tubercle bacilli.* (25906)

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D'Arcy Hart and Rees were the first to show that the severity of a tuberculous infection (H37Rv) in mice could be increased by concurrent administration of massive doses of cortisone(1). We were interested in investigating the effect of cortisone on experimental infections with chromogenic acid-fast and with isonicotinic acid hydrazide resistant (INHR) organisms.

Methods. Groups of 5 or 10 mice of the CF_1 strain, 16-18 g in weight, were infected by intravenous route with 0.25 ml of the appropriate culture. They were fed Purina chow *ad libitum* and weighed at weekly intervals. The strains of organisms used were: the human, virulent H37Rv; the H37Rv strain rendered resistant to 54 γ/ml of INH by serial passage in increasing quantities of drug, and 20 chromogenic acid-fast organisms of human origin obtained from Dr. Ernest Runyon. Cultures were grown in Dubos' albumin medium in Erlenmeyer flasks for 5 to 8 days depending on the strain, diluted with the same medium to give a reading of 65-70 in the Klett-Summerson colorimeter, then further diluted 1:4. Cortisone acetate suspended in 0.5% tragacanth, was administered by subcutaneous route for 7 days in a volume of 0.1 ml beginning the day of infection. Chromogen-infected animals that did not succumb were sacrificed between 6th and 8th week after injection. Mice infected with the INHR strain of H37Rv were observed for over 100 days and cultures made of spleens and lungs in Dubos' medium from sacrificed and from spontaneously dead mice. Survival time greater than or less than that of controls was not considered significant unless it exceeded 2 days. Autopsies were performed on some mice from all groups.

Results. As a base line for work with the other strains, we determined for an H37Rv infection minimal cortisone dose which when given for several days beginning the day of infection would result in significant decreased survival. Seven doses of 0.5 mg/day were required to produce this result (Table I). A dose of 0.25 mg for 7 days, though resulting in loss of weight, had no effect on survival, but did hasten death if given for 9 days. On the other hand, treatment with cortisone for a week *before* infection resulted in no increase in susceptibility. The 1 mg dose administered by gavage was probably insufficient to decrease survival markedly. Actual numbers of tubercle bacilli in mice treated from day of infection were found to be greatly increased even when the cortisone treatment was not lethal (Table II). Heavier growth occurred from spleens of treated than from those of infected, untreated mice sacrificed on 12th or 13th day after infection. This has been demonstrated more quantitatively by Batten and McCune(2).

Strains of tubercle bacilli highly resistant to INH produce chronic, slowly progressing disease in mice. Under our conditions, mortality was low and greatly delayed. Examples of the effect of cortisone on this infection are shown in Table III. Mortality is greater when the hormone is administered. That deaths were not due to other than acid-fast organisms was clearly shown in cultures of lungs

TABLE I. Effect of Varying Dosage of Cortisone on Infection with H37Rv.

Dose/day, mg	No. of doses†	Days' avg treated	Survival time, controls
.5	9	11.9	19.6
.25	9	12.6	21.0
.125	9	18.8	"
.025	9	21.0	"
.5	7	12.2	20.2
.25	7	18.6	"
1.0 *	9	17.6	19.6
.5	7‡	19.5	21.2
.2	7	22.0	"
.05	7	24.0	"

* By gavage.
† Administered by subcut. route beginning day of infection.
‡ Treatment started 7 days before infection.

TABLE II. Growth of H37Rv from Spleens of Cortisone-Treated and Control Mice.*

	Dilution of minced spleen									
	10^{-2}		10^{-3}		10^{-4}		10^{-5}		10^{-6}	
	Days of incubation									
	7	14	7	14	7	14	7	14	7	14
Cortisone, 7 doses, .5 mg	3†	>4	1	>4	0	>4	0	3	0	2
Control	2	4	0	3	0	1	0	0	0	0
Cortisone, 7 doses, .25 mg	3	>4	±	>4	0	4	0	2	0	1
Control	2	>4	0	3	0	1	0	0	0	0

* Dubos' albumin medium used. Growth recorded at 7 and 14 days.
† 0 = no growth; 4 = heavy growth, etc.

and spleens made to test the sensitivity of recovered tubercle bacilli. Contaminants were rarely present. Whether from sacrificed or spontaneously dead mice, these tissues yielded heavy cultures of acid-fast bacilli still completely resistant to 54 γ/ml of INH.

The atypical acid-fast organisms used in this study were not highly pathogenic for mice. A few deaths occurred in our animals infected with photochromogens No. 4 and 8 and not treated with cortisone (Table IV). The effect of cortisone administration is apparent. Virulence of strains No. 4 and 8 was enhanced as well as that of 4 additional cultures (No. 5, 6, 16, 26) as evidenced by increased mortality. If deaths occurred earlier than 16 days after infection, no lesions visible to the naked eye were observed in lungs. However, large numbers of acid-fast organisms were present in stained smears. When surviving mice were sacrificed 6 to 8 weeks after infection, lung lesions were found in those infected with cultures No. 1, 4, 8, 16, 18, 21 and 26, though not in every mouse of every group, whether they had received cortisone or not. In the case of culture No. 24, lung lesions were present only in cortisone treated animals. Kidney lesions were noted in mice infected with No. 4, 8 and 16, treated or not treated with cortisone. No. 21 and 26, on the other hand, did not provoke kidney lesions unless cortisone was administered. (More deaths might have resulted had heavier inocula been used. We preferred to use H37Rv as a reference point and to employ inocula of the same density as used in our routine tests with this strain.)

TABLE III. Effect of Cortisone on Infection with INH-Resistant H37Rv.*

Treatment	No. of doses	No. of mice dead	ST_{50}†	ST_{100}
Cortisone, .5 mg	7	7/10	47	>103
Controls	0	1/10	>108	
Cortisone, .5 mg	9	8/10	41	> 71
Controls	0	2/10	>110	

* Strain resistant to 54 μg/ml of INH.
† ST_{50} and ST_{100} = survival time in days of 50 and 100% of mice.

Discussion. The dose of cortisone used in these experiments to elicit an enhancing ef-

TABLE IV. Effect of Cortisone on Infections with Atypical Acid-Fasts.

Pkge. No.*	Group*	Deaths
1 & 1c†	photochromogen	0/5
4		2/10
4c		5/10
8		1/10
8c		9/10
16		0/10
16c		3/10
18 & 18c		0/5
21 & 21c		0/10
22 & 22c		0/5
24 & 24c		"
26		0/10
26c		2/10
5	scotochromogen	0/10
5c		3/10
6		0/10
6c		2/10
15 & 15c		0/5
19 & 19c		"
2 & 2c	Battey	"
3 & 3c		"
7 & 7c		0/10
17 & 17c		0/5
20 & 20c		"
23 & 23c		"
25 & 25c		"
9 & 9c	H37Rv	10/10
11 & 11c	*M. phlei*	0/10

* No. and group designations of Dr. Runyon's Sunmount collection.
† c denotes cortisone-treated mice, 0.5 mg/mouse /day for 7 doses.

fect on murine tuberculosis was greatly in excess of the equivalent dose customarily employed in management of human disease. On a weight basis, five-tenths mg/day of cortisone for a 20 g mouse for example, is 17 times the 100 mg/day maintenance dose frequently used for man.

The position of INHR organisms in human disease is still a matter of controversy although they have been isolated on primary culture from sputum and spinal fluid(3,4). The increase in virulence of INHR tubercle bacilli and of some chromogens when mice were treated with cortisone might have its parallel in human infections under conditions of prolonged stress or in anxiety states. In this connection, Steenken and co-workers have shown(5) that INHR organisms can cause progressive disease in silicotic guinea pigs.

Summary. 1) The virulence for mice of INHR laboratory strains of H37Rv was increased when cortisone was administered. 2) Cortisone also increased the pathogenicity for mice to some extent of 2 of 4 scotochromogens and 3 of 9 photochromogens tested. Seven Battey strains were unaffected.

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Stability of Isoniazid in Aqueous Solutions and Plasma. (25907)

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In our work on isoniazid (INH) blood level determinations in men and animals, the impression was gained that lower levels were found if the chemical test could not be completed immediately after blood sample was obtained. This raised the question of stability of the drug in solution. The following factors were considered: (a) Influence of temperature and pH in aqueous solution. (b) The possibility that the drug after becoming absorbed through the intestinal tract might be in a state in serum different from that in a simple solution; *e.g.,* INH could be bound by a protein fraction which might influence its stability.

Methods. The method used has been published(1). We have since modified this method as follows: Before medication was given patient's plasma was collected and used for blank and standard determinations to avoid the influence of the coloration by bile constituents which vary from one person to another.* Analytical error of the method is $\pm$ 0.3 μg. Two patients with polycythemia and healthy volunteers were available for blood donations. In each case 250 cc of blood was taken before breakfast and before medication for blank determinations. Ninety minutes after oral administration of 4 or 8 mg INH/kg another 250 cc of blood was obtained and first determination was made without delay. A pool of rabbit plasma was obtained from the carotid artery by exsanguinating male rabbits kept on a standard diet. Four rabbits received 20 mg INH/kg intravenously, their total blood was taken after 10 to 15 minutes and the plasma pooled. Another batch of pooled, untreated rabbit plasma was divided into 2 equal portions for the blank and for *in vitro* tests in which INH was added. Plasma specimens were kept at tem-

* This same modification has been introduced, independently, by Dr. John W. Jenne, Veterans Adm. Hosp., Minneapolis, Minn. Personal communication.