In vitro Inhibition of Growth of *M. tuberculosis* by Certain 11-Oxygenated Steroids. (24861)

A. R. HENNES, H. G. MUCHMORE,* H. G. MCCLURE† AND J. F. HAMMARSTEN
Medical Service, V. A. Hospital and University of Oklahoma School of Medicine, Oklahoma City

It has been demonstrated in numerous animal experiments that glucocorticoids decrease the inflammatory response of host to tuberculosis(1). There is ample clinical evidence that administration of these hormones in absence of adequate antibiotic coverage may activate latent tuberculosis in man(2). Despite this large body of evidence about *in vivo* effects of 11-oxygenated steroids, little is known about the effect of these hormones on the tubercle bacillus itself. We found that certain of these hormones can exert a profound suppressant effect on mycobacterial growth *in vitro*.

Procedure. A standard inoculum, 0.1 cc of a 1 x 10^{-3} dilution of stock cultures in logarithmic growth phase at O.D. .509 of H 37 RV and several local isoniazid sensitive and resistant strains of *M. tuberculosis* was used in all studies. Crystalline hydrocortisone or corticosterone was added in several concentrations in distilled water to the following synthetic culture medium: (Aldridge, Muchmore and Felton—in preparation)

Potassium phosphate, monobasic	5.	g
Ammonium chloride	5.	g
Magnesium citrate	1.	g
Potassium sulfate, dibasic	.5	g
Tween 80, certified	.2	ml
Ferric ammonium citrate	.0025	g
Distilled water, to make	980.	ml

Adjust to pH 6.8 with solid KOH; autoclave; add 20 ml of sterile 50% glucose. Bacterial growth was determined by measuring optical density at 525 mμ at various intervals after inoculation. Optical density values represent a mean of 4-5 culture tubes in each instance.

Results. In 5 experiments with 3 isoniazid sensitive strains 100 μg/ml of hydrocortisone produced a striking decrease in bacterial growth and marked increase in apparent lag phase. None of the bacilli grown in this concentration of hydrocortisone entered the usual accelerated growth phase, although some escape from steroid effect was sometimes seen at 22-24 days after inoculation. Ten μg/ml produced slight slowing of growth in some strains. One μg/ml was without apparent effect. Isoniazid resistant organisms isolated from same patients following unsuccessful isoniazid treatment were similarly inhibited by hydrocortisone.

Growth of standard strain H 37 RV obtained from Trudeau Laboratory, was also inhibited by hydrocortisone at concentration of 100 μg/ml. Twenty μg/ml produced slight slowing of growth (Fig. 1). In 1 sensitive and 1 resistant strain 100 μg/ml of corticosterone produced similar depressant effects. In both these strains 1 and 10 μg/ml produced slight slowing of growth. Table I summarizes these data.

No change in acid fastness of bacilli grown in these various concentrations of steroid was noted.

Hydrocortisone inhibition of bacterial growth is apparently limited to certain species. In 8 experiments no effect of hydrocortisone in concentrations of 100 μg/ml could be detected on growth or generation time of *E. coli* isolated from blood of patient who showed

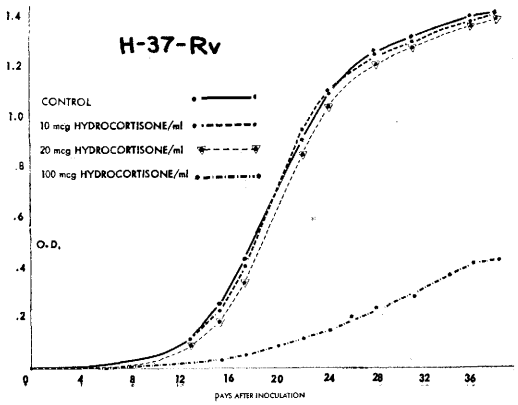

FIG. 1.

* V. A. Clinical Investigator.

† Oklahoma Tuberculosis Assn. Trainee.

TABLE I. Per Cent of Control Optic Density at Approximate Mid-point of Growth Curve.

	INH-S					INH-R			
	L-21-S	L-22-S (1)	L-22-S (2)	L-22-S (3)	H-37-RV	L-21-R	L-22-R (1)	L-22-R (2)	L-22-R (3)
Control	100	100	100	100	100	100	100	100	100
Hydrocortisone									
1 μg/ml	93	93	99			96	102	98	
10	76	89		87	100	75	92	95	98
20				81	87				78
100	23	22	36	29	13	34	21	29	23
Corticosterone									
1 μg/ml		86					82		
10		67					71		
100		10					19		

dramatic improvement after I.V. hydrocortisone semi-succinate.

Discussion. 11-oxygenated adrenal steroids have been previously shown to depress multiplication of cells. Glucocorticoids profoundly suppress mitoses in rat epidermis in culture with glucose, fructose, lactate, or pyruvate as substrate(3). This effect has been confirmed *in vivo*(4).

Perhaps more closely related to the present study is the work of Lester, Stone, and Hechter(5) with *Neurospora crassa*. These authors have recently shown that desoxycorticosterone at concentration of 250 μg/ml of culture medium produces approximately 50% suppression of mycelial growth. Hydrocortisone and cortisone were inactive at comparable concentrations(5). In another study Lester and Hechter found that desoxycorticosterone can inhibit growth of many Gram positive bacteria, but is ineffective against Gram negative bacteria except for *N. catarrhalis*(6). Hirsch, on the other hand, has found that cholesterol stimulates growth of *M. tuberculosis* at a concentration of approximately 100 μg/ml(7). Cortisone had no effect on growth of *M. tuberculosis in vitro*. However, when tubercle bacilli were placed in collodion coated bags into the peritoneal cavity of guinea pigs, parenteral administration of cortisone decreased multiplication of the bacilli(8).

Concentration of corticosteroid which markedly inhibits *in vitro* mycobacterial growth is well above that obtainable in blood after rapid I.V. injection of 100 mg(9). However, corticosteroids may be concentrated in tissues (10). Further, under certain circumstances such as intrathecal administration of hydrocortisone for tuberculosis(11) concentrations in spinal fluid may equal or exceed those required for marked inhibition *in vitro*.

It is possible that the total effect of administration of certain corticosteroids may be a balance between an effect on decreasing multiplication of *M. tuberculosis* and an effect on decreasing host response to infection, the latter predominating under usual circumstances. There is, however, no necessary correlation between depression of host response and suppression of bacillary growth. Corticosterone, though possessing only ⅓ - ⅕ the anti-inflammatory potency of hydrocortisone in man, is as effective as hydrocortisone in inhibiting mycobacterial growth.

A bacterial system of this type may perhaps be of use both for determining nutritional requirements of the tubercle bacillus and for studying *in vitro* effects of certain mammalian steroid hormones.

Summary. Hydrocortisone and corticosterone in a concentration of 100 μg/ml markedly inhibit growth of isoniazid sensitive and resistant strains of tubercle bacilli grown in a simple synthetic culture medium. Ten and 20 μg/ml produce slight slowing of growth in some strains.

1. Spain, D. M., Molomut, N., *Am. Rev. Tuberc.*, 1950, v62, 337.

2. Muchmore, H. G., Welsh, J. D., Harvey, C. M., *J. Okla. State Med. Assn.*, 1958, v51, 593.

3. Bullough, W. S., *Vitamins and Hormones*, 1955, v13, 261.

4. Ghadially, F. N., Green, H. W., *Brit. J. Exp. Path.*, 1957, v38, 100.

5. Lester, G., Stone, D., Hechter, O., *Arch. Biochem. Biophys.*, 1958, v75, 196.

6. Lester, G., Hechter, O., *J. Bact.*, 1958, v76, 365.

7. Hirsch, J. G., *Am. Rev. Tuberc.*, 1954, v70, 977.

8. Lurie, M. B., Zapposodi, P, Dannenberg, A. M., Cardona-Lynch, E., *Ann. N. Y. Acad. Sci.*, 1953, v56, 779.

9. Scheuer, J., Bondy, P. K., *J. Clin. Invest.* 1957, v36, 67.

10. Dougherty, T. F., Schneebeli, G. L., *Ann. N. Y. Acad. Sci.*, 1955, v61, 328.

11. Wasz-Hockert, O., *Ann. Pediatria. Fenn.*, 1957, v3, 90.

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Effect of Certain Androgenic Steroids and Cortisone on Gastric Ulcerogenesis in Fasting Rats. (24862)

K. KOWALEWSKI

McEachern Cancer Research Lab. and Dept. of Surgery, University of Alberta, Edmonton

When intensive corticoid treatment is associated with 4 days fasting in rats, steroid gastric ulcers develop in about 90% of animals. These ulcers are always found in glandular portion of stomach and closely resemble human peptic ulcers. Fasting alone, on the other hand, results in forestomach ulcers, similar to typical lesions of the Shay rat. Robert and Nezamis(1) propose this technic of ulcer production as assay for ulcerogenic property of steroids. Several factors may be responsible for steroid gastric lesions. Most frequently quoted hypotheses concern post-steroid changes in gastric secretion, antiphlogistic role of corticoids and possible decrease of gastric mucus synthesis(1,2). We showed that sulphated mucosubstances are reduced in both gastric tissue and gastric juices of rats treated with cortisone(3). The mechanism of this steroid action is, however, not clear. That corticoids exert a general anti-anabolic effect will be taken into consideration when interpreting any local effect of these hormones. It is known, for example, that the effect of cortisone on tissue healing and on synthesis of collagen is anti-anabolic and that the antiphlogistic role of corticoids is partly explained by this effect(4,5). Some anti-anabolic effects of cortisone may be offset however by certain anabolic androgens(5,6,7), and it seemed interesting to investigate this problem using above mentioned method of producing cortisone ulcers in rats. The present report concerns investigation of possible protective action of some synthetic androgens on development of gastric ulcers in fasting cortisone treated rats.

Materials and methods. Male rats of Wistar strain, of body weight 195 to 220 g were placed in individual cages and fasted 4 days. Some animals received hormonal treatment for 10 days preceding fasting period. 17-ethyl-19-nortestosterone (E.N.T.) (Nilevar, Searle & Co.) was given 5 mg/os daily, crushed tablet mixed with food(5). Testosterone enanthate (T.E.) (Delatestryl, Squibb & Sons) was injected intramuscularly, one 100 mg dose 10 days before beginning of fasting. Cortisone acetate (Merck & Co.) was injected subcutaneously in 10 mg dose once daily, only during fasting period(1). The following 6 groups of rats were used: 1. Pretreated with E.N.T. before fasting; 2. Pretreated with T.E. before fasting; 3. Fasted only; 4. Pretreated with E.N.T. and given cortisone during fasting; 5. Pretreated with T.E. and given cortisone during fasting; 6. Given cortisone during fasting. Isotope was administered 24 hours before killing of rats. Radiosulphur (S^{35} in H_2SO_4, Atomic Energy, Canada) was injected subcutaneously, one mc/kg body weight. The dose was dissolved in 5 ml distilled water together with 40 mg of sodium sulphate(6). Animals were killed with ether, the stomachs dissected, opened along great curvature and the mucosa examined under dissecting microscope (X 10). Stomachs were then studied for S^{35} radioactive mucopolysaccharides following modification(3) of method described previously(8,9).