THE INFLUENCE OF CORTICOTROPHIN AND CERTAIN CORTICOSTEROIDS ON POPULATIONS OF *MYCOBACTERIUM TUBERCULOSIS* IN TISSUES OF MICE

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THE modification of host-parasite relationships by corticotrophin and corticosteroids has been studied extensively in many mammalian species including man with the general conclusion that there is an enhancement of the infective process to the detriment of the host. Most aspects of this field are not clearly understood, however, and although most observations have indicated that the effects of the hormones are deleterious, this may not necessarily always be the case. For example, Lurie, Zappasodi, Dannenberg and Swartz (1951) have demonstrated that experimentally infected rabbits of a highly susceptible strain when treated with cortisone react with certain of the tissue responses characteristic of natively resistant rabbits. At the same time, very large numbers of acid-fast bacilli were observed in the pulmonary lesions of the animals receiving the hormone. In terms of the total well-being of the host these two phenomena represent forces which are acting in opposite directions and the net effect would depend on which predominated. It has also been noted that there is much species variation in action of the hormones both in tuberculosis and in other fields of study. The effects of the different hormones are not uniform. For example, though there is general agreement that increased microbial growth *in vivo* occurs during administration of cortisone, hydrocortisone and their derivatives, there is no unanimity concerning the effect of corticotrophin. In many animals such as the rat and the mouse, the latter produces definite pharmacological effects (Speirs and Meyer, 1949), but causes little or no enhancement of infection, as was shown for mice by Kass, Ingbar, Lungdren and Finland (1951) and for hamsters by Shwartzman (1950).

Knowledge of the action of the hormones in experimental tuberculosis has been obtained mainly from the study of their effect on the survival of infected animals and from the changes in tissue architecture. Much valuable information has been gained with these methods but they have two important disadvantages. The survival time of hormone-treated animals may be diminished by non-tuberculous infections ; and measurement of change in size of the population of tubercle bacilli by microscopy has been made only by approximate estimates and is necessarily inexact. It seemed desirable, therefore, to study this question by the use of more precise techniques for observing the dynamics of the host-parasite relationship. Tubercle bacilli in mouse tissues can be counted with a high degree

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of reproducibility by the method devised by Fenner, Martin and Pierce (1949). This method has been used extensively to measure changes in host resistance (Pierce, Dubos and Schaefer, 1953) and the influence of antimicrobial drugs in experimental infections both with tubercle bacilli (McCune and Tompsett, 1956 ; McCune, Tompsett and McDermott, 1956) and with staphylococci (McCune, Dineen and Batten, 1956). Its use for the study of the effect of corticotrophin and corticosteroids on tuberculosis in mice is reported here. In an accompanying report (Batten and McCune, 1957) observations are presented on the results obtained when antituberculous drugs were administered in various combinations with cortisone or corticotrophin.

MATERIALS AND METHODS

The methods used in this study are similar to those previously reported from this laboratory (McCune and Tompsett, 1956) and will be described only briefly.

Male albino mice (Webster Swiss strain) weighing 18–20 g. were obtained 1 week before infection and were housed in metal cages—not more than 10 animals to a cage. They were fed on standard mouse pellets and water intake was unrestricted.

The mice were inoculated intravenously with 0·2 ml. of a 1/10 dilution of a 7-day Tween-albumin culture of *Mycobacterium tuberculosis hominis* (H37Rv) in 0·1 per cent bovine albumin in saline. In each experiment the size of the inoculum was estimated by surface viable counts ; there were approximately 1–3 $\times$ 10^6 cultivable units per ml. in the inocula used for infecting the animals. The resulting infection was a chronic generalized tuberculosis, predominantly pulmonary, which proved fatal from 5–7 months after infection.

The preparation of hormones and the vehicles used were as follows :

Corticotrophin : (Acthar-Armour) in saline and in hydrolysed gelatin vehicle ; hydrocortisone acetate (Merck) saline suspension ; cortisone acetate (Schering) aqueous crystalline suspension ; hydrolysed gelatine (Armour). All of the preparations were given by intramuscular injection into the thigh, starting on the day of infection and continuing until the end of the experiment.

Twenty-four hours after inoculation and at intervals up to 8 weeks, 3–5 animals in each treatment group were killed with chloroform and autopsies were performed aseptically. The lungs and spleen were removed and their volumes measured by displacement of 5 ml. of diluent (2 per cent bovine albumin in saline) in graduated centrifuge tubes. The tissues were then transferred to Pyrex tubes and emulsified with a plastic (Teflon) grinder. Three 0·02 ml. aliquots of appropriate dilutions of the homogenates were cultivated on oleic acid albumin agar. Colony counts were carried out by two independent observers after 2 and 4 weeks of incubation at 37°. Taking into account the various dilution factors the number of " cultivable units " of tubercle bacilli was estimated for each unit (ml.) of tissue. The results were expressed logarithmically and are presented graphically.

With this method it is theoretically possible to detect 70–90 tubercle bacilli present in either lung or spleen. For detection of fewer organisms, the technique may be modified by alteration of dilution factors so that it is theoretically possible to detect 1–3 cultivable units in either of the two organs. As this modification becomes relevant only in the succeeding paper it will be discussed there.

RESULTS

A preliminary experiment was designed to see if corticotrophin exerted an enhancing effect on the microbial populations. Attempts were also made to discover the most satisfactory dose and preparation of the hormone and the most effective frequency of administration.

Three mice were killed 24 hr. after infection for determination of initial population levels. Five mice were killed 20 days later in each of the 10 groups which are specified in the Table. It will be noted that two dosage levels (4·0 and 0·4 mg./day) were used and were administered in two vehicles : 0·2 ml. saline

TABLE.—*The Effect of Altering Time-dose Relationships and the Method of Corticotrophin Administration on Populations of* Myco. tuberculosis (*H37Rv*) *in Lungs and Spleens of Mice. Infecting Inoculum:* $1{\cdot}8 \times 10^6$ *Cultivable Units Tubercle Bacilli*

Daily regimen				Viable units tubercle bacilli (H37Rv)* per ml. tissue. × 10^6	
Dose (mg.)	Number of injections	Vehicles		Lung	Spleen
—	—	—	1 day	0·3	1·9
—	—	—	21 days	36	7
—	2	S		36	13
—	2	G		33	17
0·4	2	S		36	8
0·4	4	S		500	31
0·4	1	G		40	12
0·4	2	G		400	45
4·0	2	S		66	18
4·0	4	S		700	200
4·0	1	G		300	53
4·0	2	G		1500	300

* Average of 5 animals in each group.
† S = saline; G = hydrolysed gelatin.

given twice or four times daily and 0·2 ml. hydrolysed gelatin given once or twice daily.

Animals in two groups received the same volume of one or the other vehicle alone and were included together with infected untreated animals as controls.

The results are presented in the Table. There was a 20-fold increase in the populations of tubercle bacilli in both the lung and the spleen of the animals which received 4·0 mg./day of corticotrophin when 4 doses were given daily in saline or twice daily in gelatin. In contrast, no significant effects on the microbial populations were produced by the 0·4 mg. dose of hormone given once daily in gelatin or twice daily in saline, nor by the vehicles alone. The increase in populations in the groups on intermediate dosage did not achieve significance on statistical analysis because of the small numbers of animals used. Nevertheless, there was a definite trend throughout the groups which indicated that an increase in population size resulting from corticotrophin administration depended not only on total daily dose, but also on frequency of administration. No animals died in the course of this experiment.

In view of these results an effort was made in all subsequent experiments to maintain a continuously high concentration of hormone in the blood. For this purpose a dose of 2 mg. of corticotrophin in gelatin administered twice daily was chosen.

Observations on Microbial Populations Influenced by Cortisone and Corticotrophin over an Eight-week Period

In the next experiments in which the effects of cortisone and corticotrophin on the populations of tubercle bacilli were compared, the same method was extended over an 8-week period. Data were gathered from 4 identical experiments and the results are presented in Fig. 1 and 2. Each symbol represents the

mean of the logarithm values of the populations in the lungs and spleens of 9–18 animals killed at the intervals of time indicated. The dose of corticotrophin was 2·0 mg. administered in gelatin vehicle twice daily by the intramuscular route ;

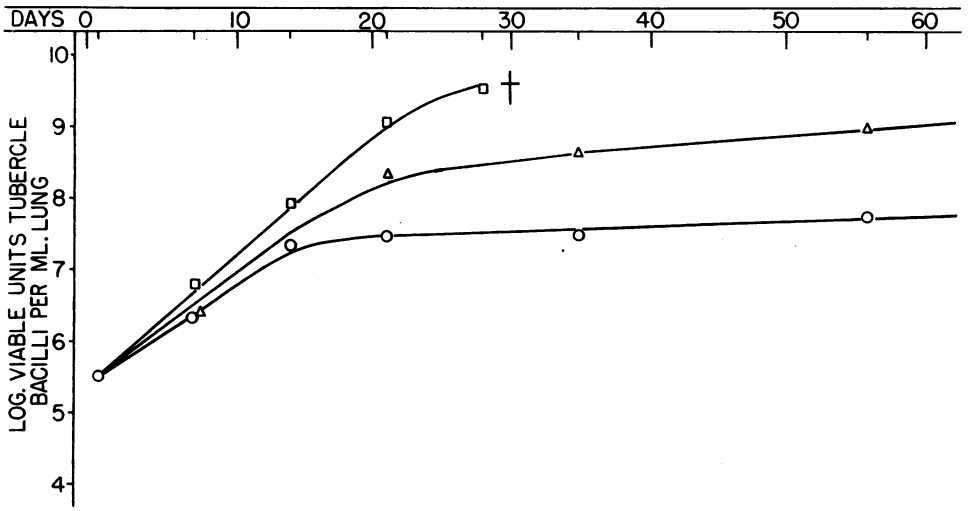

FIG. 1.—Influence of corticotrophin and cortisone on populations of *Myco. tuberculosis* (H37Rv) in mouse lungs. Infecting inoculum : $3 \cdot 4 \times 10^6$ cultivable units tubercle bacilli.

○——○ control.
△——△ cortisone.
□——□ corticotrophin.

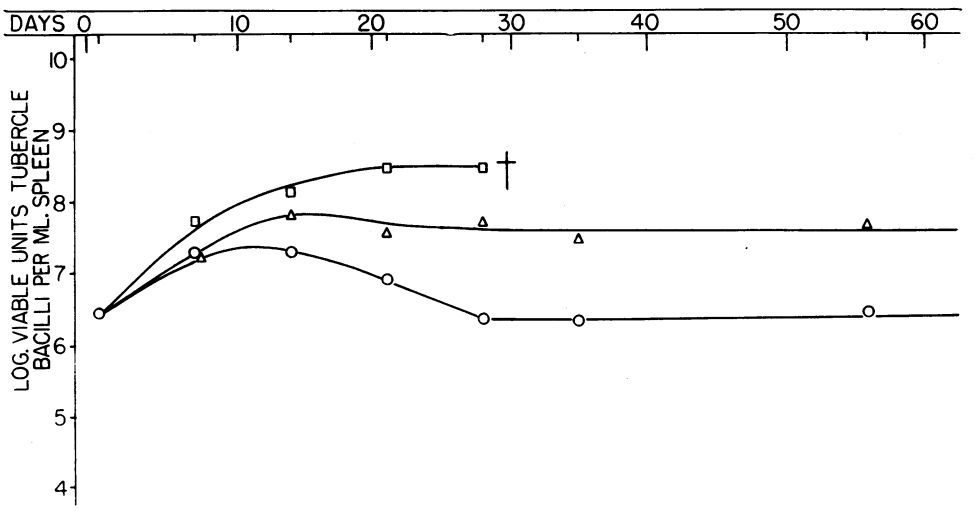

FIG. 2.—Influence of corticotrophin and cortisone on populations of *Myco. tuberculosis* (H37Rv) in spleens of the same animals whose lung populations are shown in Fig. 1.

○——○ control.
△——△ cortisone.
□——□ corticotrophin.

0·1 mg. cortisone acetate was given as a saline suspension once daily by the same route.

Infected untreated mice

The changes which occurred in the size of the populations of tubercle bacilli on both lungs and spleen throughout the experimental period were of the same nature as those previously described from this laboratory (McCune and Tompsett, 1956).

In the spleen, there was an initial increase of the populations of tubercle bacilli with a fall to the original census within a period of 3 weeks after institution of infection. The census stabilized at this point (approximately 10^6 tubercle bacilli per ml. of spleen) and remained constant throughout the remainder of the experiment. In the lung there was a steady increase in the population to about 10^8 organisms per ml. 8 weeks after infection. No animals died in this group.

Infected mice which received corticotrophin

In both the spleen and lung of the mice which received corticotrophin there was a considerable rise in the populations of tubercle bacilli when compared with those of the untreated animals (Fig. 1 and 2). In the spleen there were approximately 5×10^8 organisms after 3 weeks. Despite this great increase in population the numbers stabilized as in control animals, although at a high level. In the lungs of the same animals there was a rapid rise to a very high level (5×10^9) after the same period of time. As may be seen in Fig. 3 no animals survived beyond the end of the fourth week of infection.

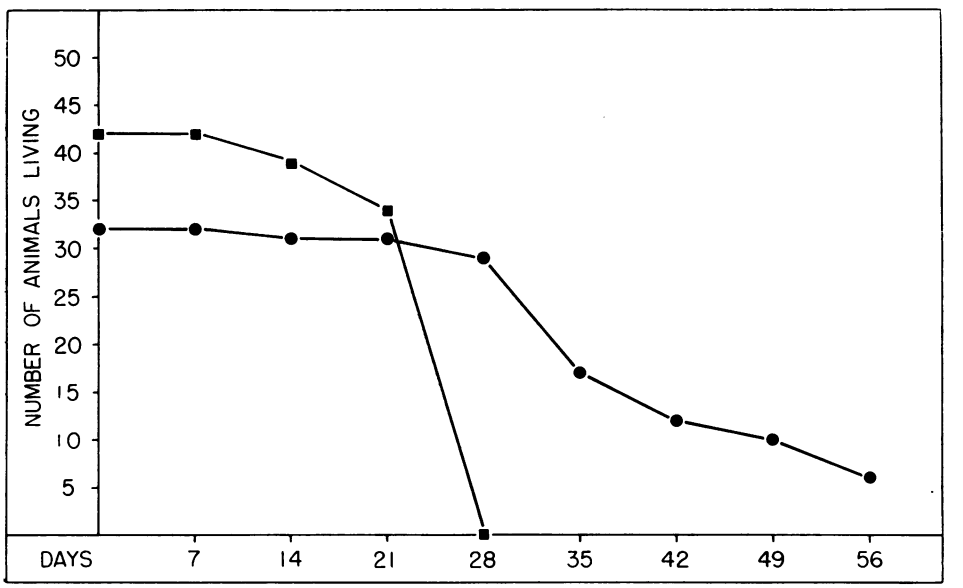

FIG. 3.—Influence of corticotrophin and cortisone on death rate of mice infected with *Myco. tuberculosis* (H37Rv).

■———■ animals given corticotrophin.
●———● ,, ,, cortisone.

Infected mice which received cortisone

The behaviour of the populations of tubercle bacilli in the lungs and spleens of the group of mice which received cortisone is also presented in Fig. 1 and 2. In both organs, there was a greater than 10-fold increase in population size above that which occurred in the control animals. In the spleen there was no fall in population after an initial increase to about 5×10^7 organisms. At this level stabilization occurred, and persisted throughout the latter part of the experiment. In the lung there was a marked initial multiplication which diminished in degree as the experiment progressed. The populations of tubercle bacilli reached a level of about 10^9 organisms at the end of 8 weeks.

There was a gradual increase in death rate beginning at the end of the fourth week. Seventy per cent of the animals in this group which received cortisone died within the 8-week period (Fig. 3).

Comparison of Cortisone and Hydrocortisone

The comparative effects of cortisone and hydrocortisone on the populations of tubercle bacilli are presented in Fig. 4 and 5. Both hormones were administered intramuscularly in a dose of 0·1 mg. daily. Each symbol in the Figures represents the population of tubercle bacilli in the lung or spleen of a single animal.

The population trends in the animals which received cortisone and the animals which received no treatment are similar to those observed in the 8-week experiment previously described. The present experiment, however, was continued for 12 weeks. No cortisone-treated animals lived longer than 8 weeks.

Infected mice which received hydrocortisone

In the animals which received hydrocortisone, the populations of tubercle bacilli in the spleen showed an increase which closely followed the trend observed in the mice which received cortisone. In the lung, however, there was very rapid multiplication of the tubercle bacilli to a level of 5×10^9 4 weeks after infection, no animals surviving after this time.

Tissue Changes in Infected Mice which Received Corticosteroids or Corticotrophin

Gross examination

At autopsy all animals which received any one of the three hormones were smaller in size than the control animals. In the lungs widely scattered lesions greyish-yellow in colour were seen. Some of the lesions were discrete, measuring 2 or 3 mm. in diameter, others were lobar in extent. The intervening lung tissue was bright red in colour and the lungs were firm and tended to sink on immersion in water. The spleens were extremely small and were one-quarter or less of the volume of the spleens in the control animals. It was also noted that the thymus was small in all animals and there was a considerable reduction of retroperitoneal fat. All of these changes were most marked in the animals which received corticotrophin. The adrenal glands in these latter were hypertrophied and presented a noticeable contrast to the atrophied spleens. In the animals which received hydrocortisone or cortisone, the adrenal glands appeared normal or reduced in size.

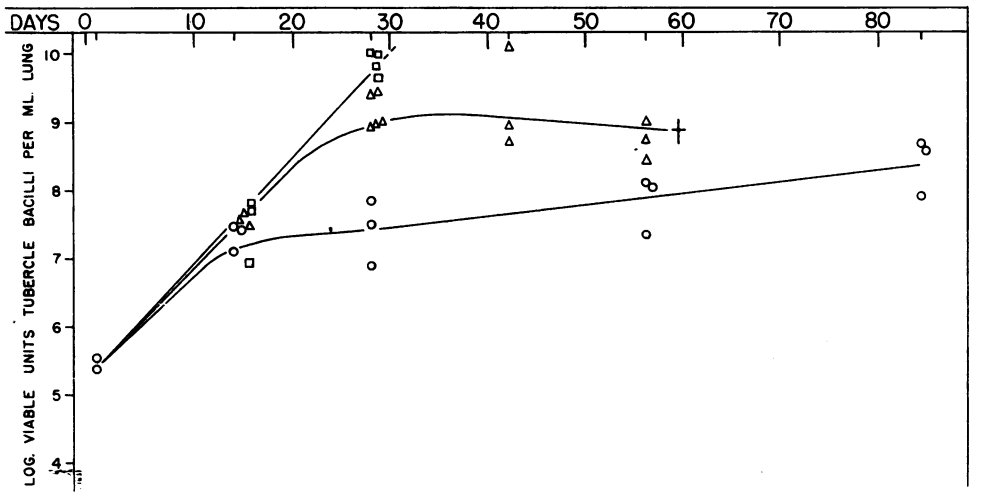

FIG. 4.—Influence of hydrocortisone and cortisone on populations of tubercle bacilli (H37Rv) in mouse lungs. Infecting inoculum: $3 \cdot 2 \times 10^6$ cultivable units tubercle bacilli. No animals survived after treatment for 28 days with hydrocortisone or 56 days with cortisone.

○———○ control.
□———□ hydrocortisone.
△———△ cortisone.

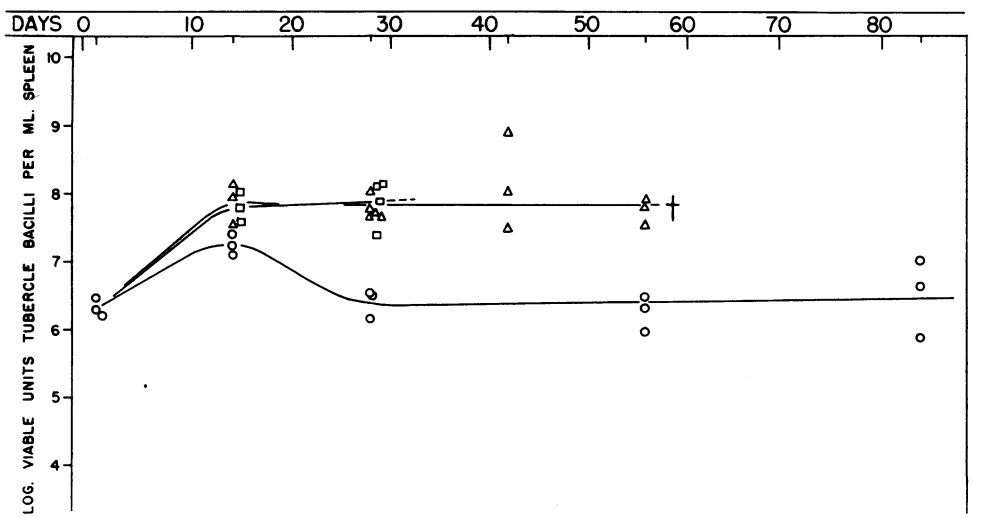

FIG. 5.—Influence of hydrocortisone and cortisone on populations of tubercle bacilli (H37Rv) in spleens of the same animals whose lung populations are shown in Fig. 4.

○———○ control.
□———□ hydrocortisone.
△———△ cortisone.

Microscopic examination

Three weeks after infection and the start of corticotrophin and hydrocortisone administration there were marked histological changes in the lungs. There was mononuclear cell infiltration of the alveolar walls and many focal pneumonic lesions were present. The lesions varied in size and consisted chiefly of mononuclear and epithelioid type cells without giant cell formation. There was necrosis in some of the focal areas, but no fibrosis was observed. On staining by the Ziehl-Neelsen method very great numbers of acid-fast bacilli were visible.

At a similar time, the lungs of the animals which had received cortisone showed changes like those described above, but much less in degree and no necrosis was seen. Moderate numbers of acid-fast forms were found. At 8 weeks after the start of infection and cortisone administration, however, the histological changes were pronounced and the pneumonic areas were widespread and confluent. Necrosis was observed and great numbers of acid-fast bacilli were visible.

In the animals which had received no hormone, the histological changes in the lungs at 3 weeks closely resembled those observed at this time in the animals treated with cortisone. There was a greater degree of generalized mononuclear cell infiltration in the infected control group but only scanty acid-fast bacilli were seen.

DISCUSSION

The results of these experiments permit the conclusion that in albino mice infected with the virulent human strain of *Myco. tuberculosis* (H37Rv), corticotrophin, cortisone and hydrocortisone in appropriate doses gave rise to a significant increase in the microbial populations in lungs and spleens. Furthermore, there did not appear to be any qualitative difference between the effects of corticotrophin on the one hand and cortisone and hydrocortisone on the other.

In the present studies the determination of the numbers of tubercle bacilli within the organs appeared to be a much more sensitive index of the effects of the hormones than the studies of the character of the tuberculous lesions. This is in agreement with previous studies in this laboratory (McCune and Tompsett, 1956) in which histological examination was correlated with microbial enumeration in animals treated with various antituberculous drugs.

Although the present results are at variance with many studies concerning the effect of corticotrophin on experimental infections, it is believed that these differences can be explained by the species variation in adrenal response to corticotrophin and the differences of the time-dose relationships employed in the various studies. In the present experiments it was observed that by varying the time-dose relationships, widely different results were obtained when the same total daily dosage of corticotrophin was administered.

There are studies concerned with the depression of resistance to several bacterial and viral infections in mice in which corticotrophin was observed to be inactive (Smith, Murphy and Mirick, 1951 ; Kass, Hechter, Mou and Lurie, 1955). In one experiment (Southam and Babcock, 1951), however, corticotrophin was given in a dose of 4 mg. in gelatin vehicle at 12-hour intervals and was considered to depress resistance to the West Nile virus.

In a review (Johnson and Davey, 1954) of the influence of steroids on tuberculosis in other animal species only one instance could be found where cortico-

trophin had enhanced the experimental infection : namely in ocular tuberculosis in rabbits. In this instance Bunn and Drobeck (1952) observed that there was more extensive progression of the disease, greater destruction of tissue, and greater frequency of distant spread in animals receiving corticotrophin. Contrariwise, in two studies (Le Maistre and Tompsett, 1951 ; Weimer *et al.*, 1953) in which relatively high dosages of corticotrophin were used and when injections were given at 6-hour intervals in guinea-pigs, no effects were observed on the extent of the lesions. In rabbits, however, using relatively high doses of corticotrophin in the same frequency, the progress of the disease appeared to be no more rapid than when cortisone was given in 1/10 of the dose (Wanzer, Morgan and Smith, 1954).

With specific reference to murine tuberculosis there have been few studies concerned with the influence of corticotrophin. In one report it was stated that when a dose of 0·5 mg. of corticotrophin was administered in saline, the survival of mice infected with *Myco. tuberculosis* (H37Rv) did not vary from that observed in infected but untreated animals (Youmans and Youmans, 1954). In another experiment (Swedberg, Dahlstrom and Luft, 1951) it was considered that the survival time of mice infected with a virulent strain of *Myco. tuberculosis* was reduced significantly. In this latter study, however, mice receiving control injections alone were not used. In a preliminary study (Hobby, Auerbach and Lenert, 1955) in white mice infected intracerebrally with the virulent Vallee strain of *Myco. tuberculosis* and given 1·2 units of corticotrophin in 5 per cent beeswax thrice weekly, there was a reduction in survival time from 43 to 23 days and the inflammatory process in the meninges progressed to a state of necrosis. Necrosis was also observed in animals receiving cortisone and hydrocortisone, but not in infected animals not receiving hormones.

As previously suggested the apparent differences noted here may therefore reflect a species variation in adrenal response to corticotrophin. In some of the experiments quoted, however, it is clear that little or no effect was observed because of inadequate and ephemeral concentrations of the hormone in the blood. The present findings confirm this and support the idea that adrenal stimulation by corticotrophin is a purely quantitative phenomenon, varying in degree from species to species.

That there is a qualitative variation in adrenal output naturally and in response to corticotrophin in different species has been demonstrated by Bush (1953). Kass *et al.* (1955) have shown that variations may occur in adrenal output in response to infection within the same species according to the susceptibility of the strain of the host. The significance of output of various steroids under varying conditions from the adrenal is as yet unknown nor is it yet certain that an undefined steroid is not the active principle in the enhancement of infection which is mediated through the adrenal.

Although no data are available concerning the nature of the adrenal secretion in mice, the predominant adrenal steroid secreted in the rat and the rabbits is corticosterone. It has been demonstrated that either hydrocortisone or corticosterone has been the predominating steroid released in all the animals thus far studied. Corticosterone appears to be relatively ineffective as an anti-inflammatory agent (Long and Spensley, 1954). Cortisone and hydrocortisone exert effects in mice qualitatively similar to those of corticotrophin. It is thus suggested that hydrocortisone might be produced by the murine adrenal gland.

The results obtained in animals receiving cortisone in the present study agree with those reported previously (Hart and Rees, 1950; Solotorovsky, Gregory and Stoerk, 1951). In one report (Engbaek, Friis and Teilum, 1952) a 10-fold increase was observed in the populations of *Myco. tuberculosis* in the lungs of mice receiving cortisone; no rise occurred in the spleens of the same animals. In most species the same phenomenon is reported, a notable exception being the guinea-pig. In this animal, several workers (Le Maistre and Tompsett, 1951; Weimer *et al.*, 1953) found that neither cortisone nor corticotrophin caused any adverse effects on the disease.

It is of interest that hydrocortisone in the same dose exerted a far more marked effect on populations than cortisone. This observation merits further study as this method might prove useful in assessing derivatives of this series of steroids for their ability to enhance infection as distinct from their many other properties.

The mechanism of the effects noted remains unclear. The hormones may have altered the internal environment in such a way that extraordinarily high populations of tubercle bacilli were attained without causing early death. Such an environmental change could take the form simply of protecting host tissue against microbial influence and hence passively encouraging unrestricted bacterial proliferation. From the present experiments it seems likely that the situation is more complicated and that some inhibitory mechanism of the host is also being affected by the hormone. Otherwise it is difficult to explain why the same stabilization of the census noted in the untreated animals also occurred, although at a higher level, in the animals maintained on hormone. This population stabilization occurred at various levels depending on the organ or hormone involved.

The absolute height of the census of tubercle bacilli in certain experimental groups was remarkable and shows that the population in the fatal untreated infection is considerably lower than the maximal attainable level. Presumably, as mentioned above, this phenomenon represents a protective effect of hormone on the host tissues. Similar observations have been reported for the effects of cortisone on an influenza B viral infection in the chick embryo or in tissue culture (Kilbourne and Horsfall, 1951; Kilbourne and Tateno, 1953). In this situation, apparently simpler because no known factors of host resistance to infection have been found, it was proposed that the hormone postponed the death of the cell and hence higher populations could be tolerated.

In view of the rapidity of the effect of the steroids in the present studies it is unlikely that interference with acquired resistance played a significant rôle in bringing about the increase of the populations of tubercle bacilli. A direct effect of the hormones on tubercle bacilli *in vivo* cannot be excluded, but *in vitro* experiments concerned with this problem have yielded negative results.

SUMMARY

A study has been made of the influence of corticotrophin, cortisone and hydrocortisone on microbial populations (H37Rv) in the lungs and spleens of albino mice.

When these hormones were given the populations greatly increased, the survival time was shortened and the lesions in the tissues were modified. It also

appeared that the adrenal response to corticotrophin in the mouse was a function of the dose of the hormone and the method of administration, and that the method used might prove of value in assessing new derivatives of the adrenocortical steroids for their enhancing action on infective processes.

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REFERENCES

BATTEN, J. C. AND MCCUNE, R. M. JR.—(1957) *Brit. J. exp. Path.*, **38**, 424.
BUNN, P. AND DROBECK, BERYL—(1952) *Amer. Rev. Tuberc.*, **66**, 175.
BUSH, I. E.—(1953) *J. Endocrin.*, **9**, 95.
ENGBAEK, H. C., FRIIS, T. AND TEILUM, G.—(1952) *Acta path. microbiol. scand.*, **31**, 317.
FENNER, F., MARTIN, S. P. AND PIERCE, CYNTHIA H.—(1949) *Ann. N.Y. Acad. Sci.*, **52**, 751.
HART, P. D'A AND REES, R. J. W.—(1950) *Lancet*, ii, 391.
HOBBY, G. L., AUERBACH, O. AND LENERT, T. F.—(1955) Presented at the Annual Meetings of the American Trudeau Society, May 1955 (in press).
JOHNSON, J. R. AND DAVEY, W. N.—(1954) *Amer. Rev. Tuberc.*, **70**, 623.
KASS, E. H., INGBAR, S. H., LUNGDREN, M. M. AND FINLAND, M.—(1951) *J. Lab. clin. Med.*, **37**, 780.
Idem, HECHTER, O., MOU, T. W. AND LURIE, M. B.—(1955) *Arch. intern. Med.*, **96**, 397.
KILBOURNE, E. D. AND HORSFALL, F. L. JR.—(1951) *Proc. Soc. exp. Biol., N.Y.*, **76**, 116.
Idem AND TATENO, I.—(1953) *Ibid.*, **82**, 274.
LE MAISTRE, C. AND TOMPSETT, R.—(1951) *Amer. Rev. Tuberc.*, **64**, 295.
LONG, D. A. AND SPENSLEY, P. C.—(1954) *Lancet*, i, 645.
LURIE, M. B., ZAPPASODI, P., DANNENBERG, A. M. AND SWARTZ, I. B.—(1951) *Science*, **113**, 234.
MCCUNE, R. M. JR., DINEEN, P. A. P. AND BATTEN, J. C.—(1956) *Ann. N.Y. Acad. Sci.*, **65**, 91.
Idem AND TOMPSETT, R.—(1956) *J. exp. Med.*, **104**, 737.
Iidem AND MCDERMOTT, W.—(1956) *Ibid.*, **104**, 763.
PIERCE, CYNTHIA, H., DUBOS, R. J. AND SCHAEFER, W. B.—(1953) *Ibid.*, **97**, 189.
SHWARTZMAN, G.—(1950) *Proc. Soc. exp. Biol., N.Y.*, **75**, 835.
SMITH, J. M., MURPHY, J. S. AND MIRICK, G. S.—(1951) *Ibid.*, **78**, 505.
SOLOTOROVSKY, M., GREGORY, F. J. AND STOERK, H. C.—(1951) *Ibid.*, **76**, 286.
SOUTHAM, C. M. AND BABCOCK, V. I.—(1951) *Ibid.*, **78**, 105.
SPEIRS, R. S. AND MEYER, R. K.—(1949) *Endocrinology*, **45**, 403.
SWEDBERG, B., DAHLSTROM, G. AND LUFT, R.—(1951) *Acta Endocr., Copenhagen*, **6**, 215.
WANZER, S. H., MORGAN, T. E. AND SMITH, D. T.—(1954) *J. Bact.*, **67**, 264.
WEIMER, H. E., BOAK, RUTH A., BOGEN, E., DRUSCH, HELENE, E., MILLER, J. N., MOSHIN, JEAN R. AND CARPENTER, C. M.—(1953) *Amer. Rev. Tuberc.*, **68**, 31.
YOUMANS, G. P. AND YOUMANS, ANNE S.—(1954) *Ibid.*, **69**, 790.