J. Biol. Chem., 1952, v196, 51.
2. ———, *ibid.*, 1952, v196, 69.
3. Gerarde, H. W., and Jones, M., *ibid.*, 1953, v201, 553.
4. Layton, L. L., *Cancer*, 1950, v3, 725.
5. ———, PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 570.
6. ———, *ibid.*, 1951, v76, 596.
7. Layton, L. L., and Sher, I., *Fed. Proc.*, 1953, v12, 237.

Received October 7, 1953. P.S.E.B.M., 1953, v74.

Beneficial Effects of Cortisone on Survival of Rats Infected with *D. pneumoniae.* (20630)

HARRY J. ROBINSON, RICHARD C. MASON, AND AMELIA L. SMITH.
From the Merck Institute for Therapeutic Research, Rahway, N. J.

It has been reported that acute bacterial infections result in adrenal enlargement suggestive of increased cortical function(1). It has also been demonstrated that adrenalectomy causes an increased sensitivity to a wide variety of stresses including that of acute infection(2). A number of investigators have shown that adrenalcortical extracts and 11-oxygenated steroids can restore the resistance of adrenalectomized animals to non-specific stress. Only a few reports, however, are available to indicate that cortical hormones can counteract the stress of acute bacterial infections either in adrenalectomized or intact animals(3,4). All previous evidence, on the contrary, suggests that massive doses of cortisone lower the resistance of both adrenalectomized and intact animals to infection(5-8). The data presented in this paper demonstrate that there is an optimal dose of cortisone which can enhance the resistance and survival of both adrenalectomized and intact rats infected with *Diplococcus pneumoniae.*

Methods and material. The rats used in this study were the MS-2 strain obtained from Manor Farms, Staatsburg, N. Y. They were fed a standard mixed natural food diet *ad libitum* and were housed in an air-conditioned animal room. The animals were infected intradermally with 0.1 cc of a 1 x 10^{-6} or 1 x 10^{-7} dilution of a 6 hour culture of *D. pneumoniae,* Type I. Each injection contained approximately 17 to 250 organisms as determined by plate counts on 5% blood in brain-heart agar. Blood samples for bacteremia studies were obtained either by a direct heart puncture or from tail blood collected in a pipette. Adrenalectomized animals were operated at least 5 days prior to infection and were maintained on 1.0% saline. Cortisone acetate (Cortone Merck) was administered subcutaneously as a micro-crystalline suspension in a single daily dose. The hormone injections were initiated 5 days before infection and the animals were treated and observed for a 10-day period following the infection. A total of 545 animals were used in this study of which 90 were adrenalectomized controls, 195 adrenalectomized treated, 115 intact controls, and 145 intact treated.

Results. It can be seen from Fig. 1 that adrenalectomy markedly decreased the survival rate of rats infected with *D. pneumoniae.* Cortisone, in doses ranging from 2.5 to 5.0 mg/rat/day, significantly increased the survival of both the adrenalectomized rats and the rats with intact adrenals. Maximal sur-

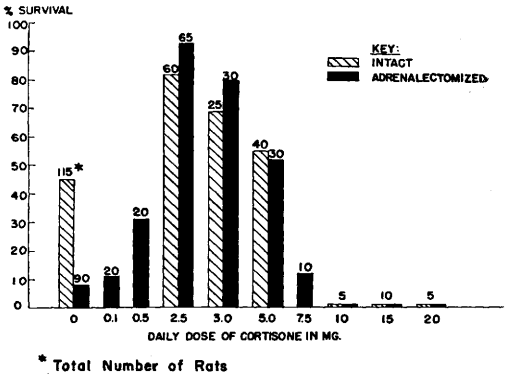

FIG. 1. Effect of Varied Doses of Cortisone on Survival of Rats Infected with *D. pneumoniae.*

TABLE I. Blood Culture Studies of Rats Receiving Cortisone 48 Hours Post Infection.

Daily dose (mg)	Mean No. of pneumococci per cc of blood			
	No. of animals	Adrenalectomized	No. of animals	Intact
0	40	14200 ± 3000*	59	9200 ± 4000
2.5	39	1100 ± 650	25	1600 ± 1100
15.0	19	240000 ± 155240	15	102500 ± 27821

* Stand. error.

vival occurred in both groups at the 2.5 mg dosage level. The resistance of adrenalectomized and intact rats was reduced when cortisone was given in doses of 10-20 mg/rat/day and the animals died with overwhelming pneumococcal sepsis. Blood culture studies revealed that the bacteremia was minimal at the 2.5 mg level in both the adrenalectomized and intact control groups (Table I).

Discussion. It is clear from the present study that under certain conditions, cortisone may significantly enhance host resistance to infection in both intact and adrenalectomized rats. The manner in which cortisone increases the resistance of the rat to infection is not known at present. However, several observations are of interest in speculating about the mechanisms concerned with the beneficial effects of intermediate doses of cortisone. First, the adrenal glands enlarge during infection and secrete increased amounts of adrenal cortical hormones, as judged by the fall in circulating eosinophiles, thymic atrophy, and the reduction of adrenal ascorbic acid and cholesterol. This response is presumed to be an attempt on the part of the host to counteract the stress of acute infection. The question arises as to whether or not the adrenal cortices are able to secrete sufficient quantities of hormone to meet the requirement of the host during severe infections. If not, perhaps additional amounts of hormone would aid in maintaining the defense mechanism at maximal efficiency. This view is supported by the results of the survival and blood culture studies reported in the present paper which show a maximal survival and minimal bacteremia at the optimal cortisone level of 2.5 mg/rat.

A second fact which may be of interest in regard to the possible mechanism of action in cortisone on infection is the ability of the hormone and the adrenal cortical extracts to increase the phagocytosis of particulate matter by the fixed histiocytes of the reticulo-endothelial system(9-11). It should be emphasized, however, that these reports have dealt with the phagocytosis of thorium dioxide, India ink, and carboxy cellulose. Their relationship to our studies with pneumococci is at present only speculative.

A third factor of possible significance is the action of cortisone in counteracting the effects of certain toxins. Cortisone has been reported to produce a sense of well-being in patients critically ill with pneumococcal pneumonia (12) and in animals to counteract the lethal effect of toxins and pyrogens(13,14). Thus, doses of cortisone sufficient to offset partially or completely the toxemia associated with the infection, but not great enough to suppress the inflammatory reaction or other important defense mechanisms, may be an important factor in enhancing the survival of animals suffering from acute bacterial infection. This would seem to be of particular importance in view of the marked susceptibility of adrenalectomized animals to toxins and noxious agents in general. It may also play an equally significant role in animals with intact adrenals.

It should be emphasized that at present there is no method available for determining the optimal requirement of cortisone in any given infection or stress. It is apparent that the optimal dose will vary with numerous factors including the animal species, the infectious agent, the pathology of the lesion, and the duration of the stress. A dose of 2.5 mg of cortisone may be "pharmacological" when administered to a normal, noninfected rat while the same dose may be within the "physiological" range when given to an infected animal.

Summary. The data demonstrate that cortisone in the dosage range of 2.5-5.0 mg/day can enhance the survival of intact and adrenalectomized rats subjected to the stress of ex-

perimental pneumococcal infection. The results of bacteremia studies suggest that optimal doses of cortisone operate by enhancing host resistance to the infectious agent. On the other hand, doses larger than 5 mg per rat per day lower the resistance of rats to this infection. Hence, in cortisone therapy, as in the case with certain other hormones, *optimum dosage* is important and this may vary with different conditions.

1. Lurie, M. B., Abramson, S., Heppleston, A. G., and Allison, M. J., *Am. Rev. Tuberc.*, 1949, v59, 198.

2. Perla, D., and Marmorston-Gottesman, J., *Natural Resistance and Clinical Medicine*, 1941, Chap. 18, p475, Boston: Little, Brown and Co.

3. Overman, R. R., Bass, A. C., Davis, A. K., and Golden, A., *Am. J. Clin. Path.*, 1949, v19, 907.

4. Robinson, H. J., Mason, R. C., and Smith, A. L., *Fed. Proc.*, 1953, v12, 362.

5. Robinson, H. J., *The Suprarenal Cortex*, Proceed. of the Colston Research Society's Fifth Symposium, 1952, p105, London: Butterworths.

6. Shwartzman, G., *Am. J. Path.*, 1951, v27, 714.

7. Kass, E. H., Ingbar, S. H., Lundgren, M. M., and Finland, M., *J. Lab. and Clin. Med.*, 1951, v37, 780.

8. Mogabgab, W. J., and Thomas, L., *ibid.*, 1950, v36, 968 (in Soc. Proc.).

9. Gordon, A. S., and Katsh, G. F., *Ann. New York Acad. Sci.*, 1949, v52, 1.

10. Stoerk, H. C., *ibid.*, 1953, v56, 742.

11. Timiras, P. S., *Acta Endocrinol.*, 1953, v12, 11, (Supplement 11).

12. Kass, E. H., Ingbar, S. H., and Finland, M., *Ann. Int. Med.*, 1950, v33, 1081.

13. Lewis, L. A., and Page, I. H., *Endocrinol.*, 1948, v43, 415.

14. Robinson, H. J., Smith, A. L., and Mason, R. C., to be published.

Received September 4, 1953. P.S.E.B.M., 1953, v84.

Properties of Bovine Anti-Hemophilic Factor. (20631)

THEODORE H. SPAET AND BEVERLY G. KINSELL.

From the Department of Medicine, Stanford University School of Medicine, San Francisco, Calif.

The production of a purified and concentrated preparation of human anti-hemophilic factor (AHF) has been hampered by the marked instability of this substance. Such rapid deterioration of plasma AHF occurs with storage either at room or refrigerator temperature, that more than half the activity may be lost within 24 hours. Moreover, AHF is largely destroyed in the process of plasma fractionation. Cohn's fraction I is the most potent plasma derivative presently available; yet various lots of this preparation contain only 8-35% of the anti-hemophilic activity represented in fresh normal plasma(1).

Although some success has been reported in the stabilization of human AHF(2), another approach would be the preparation of an intrinsically stable AHF derived from animal sources. Bovine plasma is known to possess anti-hemophilic activity, and potent fractions have been prepared from it(3). Ac globulin, which is unstable in human blood is stable in bovine blood(4). Accordingly, it was hoped that the AHF of bovine plasma might show a stability corresponding to that of the bovine Ac globulin. This has proved to be the case.

Methods and materials. Collection of blood. All blood specimens were collected in untreated glass containers. One part of 0.1 molar potassium oxalate to 9 parts of blood served as the anticoagulant. Beef, hog, and sheep blood were obtained from freshly slaughtered animals. The plasma was separated within several hours of collection, and was stored in deep freeze until ready for use. Unless otherwise indicated, the AHF content of human plasma was tested within one hour of blood collection. *Prothrombin* was determined by the method of Ware and Stragnell(5) with slight modifications in the case of serum specimens as previously described (6). *Thrombin evolution* was determined in recalcified plasma according to the technic of Pitney and Dacie(7). *Assay of AHF* was accomplished *in vitro* by testing the ability of the unknown specimen to induce prothrombin