significant difference was found between these 2 steroids at the dose levels employed. Further, Woodbury(4) has shown that chronic administration of diphenylhydantoin or phenobarbital with DOCA pellets caused an increase in the EST greater than the sum of the increases produced by the 2 drugs given separately. And so, it might be expected that treatment with DOCA would also prolong the anesthesia time in adrenalectomized mice. However, such an effect was not demonstrated.

Summary. The effects of the administration of cortisone acetate, hydrocortisone acetate and DOCA on the duration of pentothal anesthesia in adrenalectomized female mice were ascertained. Adrenalectomy induced a highly significant prolongation in the duration of pentothal anesthesia. Pretreatment of cortisone acetate and hydrocortisone acetate returned the anesthesia time to normal level in adrenalectomized mice, while DOCA and saline had no effect.

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Sulfate Fixation by Embryonic Chick Lung in Tissue Culture.* (20629)

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The authors have studied the synthesis of protein, nucleic acid, and collagen in chick embryonic tissue by a quantitative roller tube method(1-3). In view of the relationship of chondroitin to collagen, it was considered worth while to study the uptake of S^{35}-labeled sulfate under these conditions. Layton(4-7) has found a high rate of sulfate fixation after 45 hours' incubation with embryonic chick heart, skeletal muscle, liver, and bone in Tyrode's solution containing labeled sulfate. He suggested that the labeled sulfate was bound in the chondroitin sulfate of the connective tissue ground substance; cortisone added to the medium inhibited sulfate fixation by heart and skeletal muscle.

Methods. Roller tube cultures of 15-day chick lung were prepared as previously described(1) using 20-40 mg of tissue per tube. Tyrode's solution and 25% chick embryo extract were used as media. The medium was changed 3 times weekly, and incubation of the cultures were continued in embryo extract for as long as 6 weeks at 37°C. At the termination of incubation, the medium was poured off, the tissue was washed with water, and the tissue fragments were rubbed from the walls of the tubes in 5% trichloroacetic acid. The contents of 4-5 tubes were pooled, dehydrated, and delipidized. Details of the procedures have been described previously (1). In order to determine the incorporation of labeled sulfate into the tissue, sulfate§ was added to the nutrient medium in quantities of 0.002 millicuries per ml of medium. Uptake of the labeled sulfate was recorded as counts per minute per milligram‖ of the lipid-free

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§ The radioactive sulfate used in this investigation was supplied by the Oak Ridge National Laboratory on allocation by the U. S. Atomic Energy Commission.

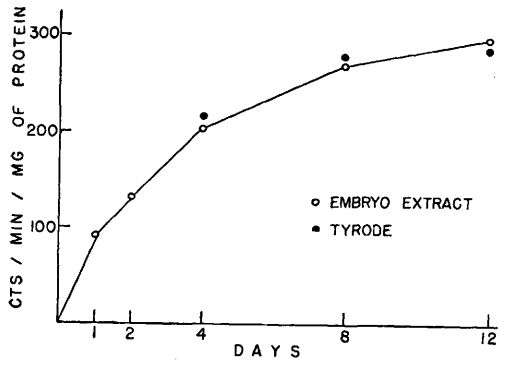

FIG. 1. Incorporation of S^{35}-labeled sulfate into protein of lung cultures in 25% embryo extract and in Tyrode's solution.

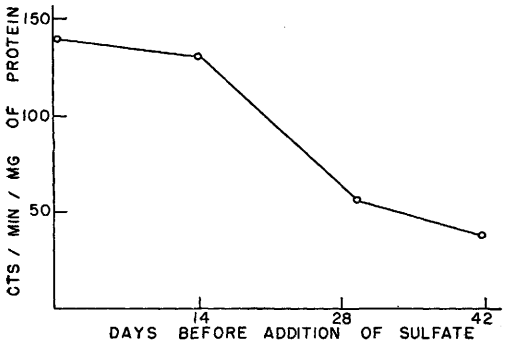

FIG. 2. Incorporation of S^{35}-labeled sulfate into protein of lung cultivated in 25% embryo extract for varying times prior to addition of the labeled sulfate. Incubation in presence of labeled sulfate continued for 2 days.

dry tissue; corrections for decay and self-absorption were made.

Results. Fig. 1 presents data showing the incorporation of radioactive sulfate into lung tissue cultivated in 25% embryo extract and in Tyrode's solution. An initial rapid uptake is followed after 8 days by a slower rate of incorporation in both media.

Tissues incubated in embryo extract at 4°C did not take up the S^{35}. There was no incorporation of the sulfate in lung cultivated in media containing 0.0005 M dinitrophenol, 0.005 M fluoride, 0.001 M cyanide, or 0.001 M azide, all of which inhibited proliferation of the tissue. There was no uptake of the sulfate in embryo extract or in plasma protein at either 37°C or 4°C in the absence of tissue.

‖ The authors wish to express appreciation to the Department of Physiology for the use of the Geiger-Müller counter.

The incorporation of sulfate into cultivated lung tissue of varying ages is shown in Fig. 2. Roller tube cultures of lung were grown in 25% embryo extract for varying times up to 42 days; medium containing labeled sulfate was added for the final 2 days of cultivation only. After 2 weeks the uptake of sulfate decreased very rapidly, bearing out previous observations(2,3) that the numbers of active cells decrease after a 2-week period, while there is a concomitant increase of extracellular protein.

The addition of 50 μg/ml of cortisone acetate¶ to the embryo extract medium did not change appreciably the incorporation of sulfate into the tissue (Fig. 3), even though a slight inhibition of the proliferation of the tissue fragments occurred for a 2-day period initially. The use of tissue from cortisone-treated embryos(3) presented similar results when cultivated in a medium containing cortisone, although here the initial inhibition of growth was prolonged to 8-10 days.

Summary. Chick embryonic lung tissue cultivated *in vitro* in the presence of sulfate labeled with S^{35} fixed sulfate in the protein of the tissue. Lung tissue which was not proliferating did not incorporate sulfate. Cultures of lung older than 2 weeks had a greatly reduced ability to fix sulfate. The presence of cortisone in the medium did not alter the incorporation of sulfate into the tissue.

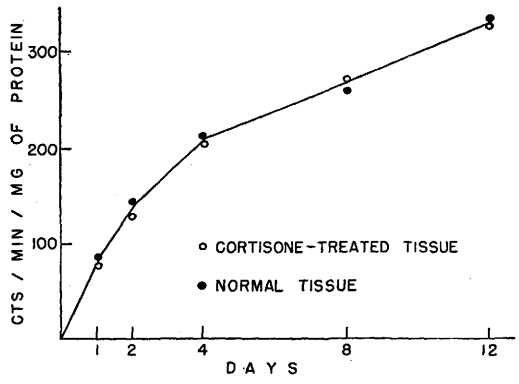

FIG. 3. Incorporation of S^{35}-labeled sulfate into normal and cortisone-treated lung cultivated in presence of cortisone acetate (50 μg per ml of the medium).

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

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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-

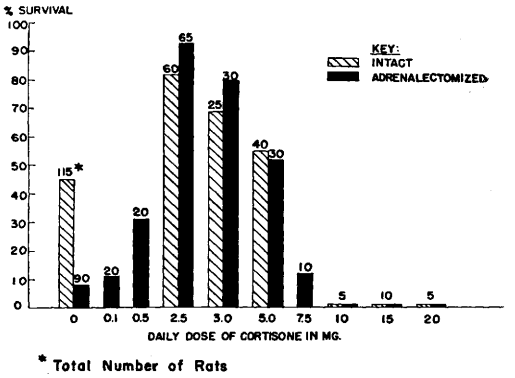

FIG. 1. Effect of Varied Doses of Cortisone on Survival of Rats Infected with *D. pneumoniae.*