mycin had higher blood urea levels than when aureomycin was not fed.

Summary. The effect of feeding crystalline aureomycin hydrochloride on digestion and nitrogen retention was determined in balance trials with six steers. Feeding 0.2 g aureomycin daily caused a marked reduction in the digestibility of crude fiber. It also reduced the digestibility of dry matter and of nitrogen-free extract. When 0.6 g of aureomycin was fed daily to steers, it produced a marked anorexia and a fetid diarrhea within 48 to 72 hours. This condition persisted for several days after the aureomycin feeding was discontinued. Continued feeding of 0.2 g aureomycin daily, produced somewhat milder digestive disturbances.

Received December 26, 1950. P.S.E.B.M., 1951, v76.

Loss of Protection by Vaccination Following Cortisone Treatment in Mice with Experimentally Induced Tuberculosis. (18465)

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Cortisone administered in single or repeated doses has been shown to reduce the amount of circulating antibody(1-3). Cortisone has also been reported to decrease the tuberculin reactivity of guinea pigs sensitized to the tubercle bacillus(4,5) and to cause a spread of the disease in guinea pigs(6). Also studies in the mouse, using both a chronic and acute tuberculosis infection showed that the enhancing effect of cortisone was most marked in the chronic infection(7). In the present study, in addition to acute and chronic infection, the effect of cortisone on mice vaccinated with heat-killed cells was also investigated. Our results are in essential agreement with those of D'Arcy Hart and Rees(7) and in addition indicated that the protective effect of vaccination is abolished by cortisone.

Materials and methods. 1. *Strain of organisms.* Two substrains of *Mycobacterium tuberculosis*, human type, H37Rv, were used. The first, possessing the original characteristics of the strain as obtained from the Trudeau Laboratories, was highly virulent for mice. The second, obtained after 2 years of serial weekly passage in Dubos Tween-albumin medium, showed reduced virulence for mice. The highly virulent substrain has been maintained by serial passage in mice. 2. *Preparation of vaccine and vaccination of mice.* Vaccine was prepared from the original strain grown in Dubos Tween-albumin medium for 14 days at 37°C. The culture was adjusted to a turbidity permitting 65% transmission of light at 650 mμ* and subjected to free-flowing steam for one hour. Sterility controls of such suspensions showed no growth after 2 months of incubation at 37°C. Mice† weighing 12 g were injected intraperitoneally with 0.5 ml amounts of vaccine administered 3 times per week for a period of 2 weeks. 3. *Infection of animals.* Test animals were infected by intravenous inoculation of 0.25 ml amounts of culture in Dubos medium adjusted to permit 70% transmission of light at 650 mμ. Mice dying during the course of the

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* On a Coleman Spectrophotometer Model 6A.

† Barckman, IS-32 mice were used throughout the course of these experiments.

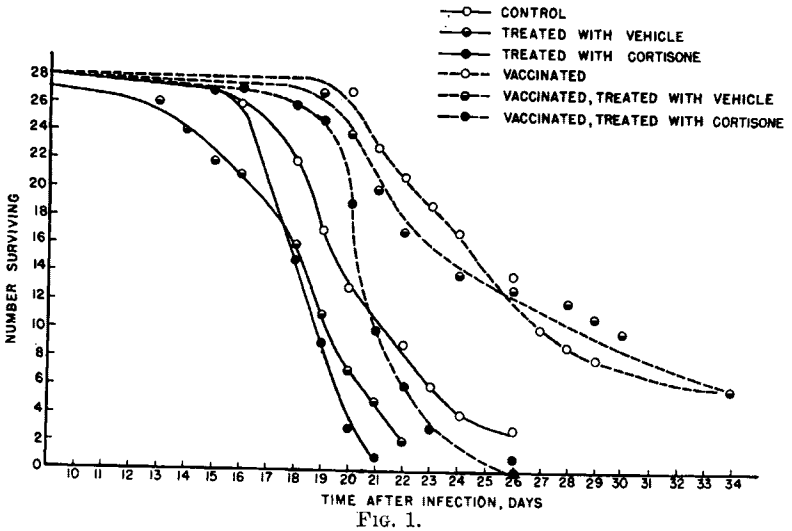

FIG. 1.
Effect of cortisone on tuberculosis in vaccinated mice.

experiment as well as those surviving the tests were autopsied and examined to confirm tuberculosis as the cause of death. 4. *Drug administration.* Mice were injected once daily with 0.1 mg of cortisone acetate‡ available as a five-fold aqueous dilution of a stock containing 5 mg of cortisone acetate per ml of vehicle. The vehicle contained 0.4% Tween 80, 0.5% sodium carboxymethyl cellulose, 1.5% benzyl alcohol and 0.9 sodium chloride.

Experimental. The data of a typical experiment are presented graphically in Fig. 1. Six groups of 28 mice each were used. Groups 1, 2 and 3 were not vaccinated; 4, 5 and 6 were vaccinated. All mice were challenged with culture of the virulent substrain 2 days after vaccination. Animals in groups 2 and 5 were treated with 0.1 mg of cortisone administered once daily 6 days per week beginning on the day following infection and those in groups 3 and 6 were treated with the same amount of vehicle as used for the cortisone treated animals. The 2 remaining groups, 1 and 4 served as untreated controls. The experiment was terminated 34 days after infection.

Among the non-vaccinated animals, Group 1, the untreated controls showed an average survival time of 19.7 days. Treatment with vehicle or cortisone reduced the average survival time approximately 1½ days. Vaccination prolonged survival time to 25 days and this prolongation of survival time was not significantly affected by treatment with vehicle alone. However, treatment of vaccinated mice with cortisone depressed the survival time to 20.5 days a figure approaching that observed for the non-vaccinated controls. To confirm tuberculosis as the cause of death, lungs from a majority of the mice in each group were examined histologically and in addition spleens, livers and kidneys from a smaller number of animals were examined. After staining by Hematoxylin-Eosin, Ziehl-Nielsen and Gram, sections were examined microscopically. The numerous lesions in the lungs and the minimal ones in the other tissues showed many acid fast rods unaccompanied by other organisms. Thus in non-vaccinated animals, cortisone had no effect on the outcome of the rapidly fatal infection that could not be explained by a slightly toxic effect of the vehicle in the presence of infection. Under these conditions the animals may have had insufficient time to develop a significant degree of immunity. When however, animals were protected by vaccination cortisone overcame the advantage conferred by vaccination.

2. *Effect of cortisone on infection of low*

‡ Cortone Merck a brand of Cortisone Acetate was used in these experiments.

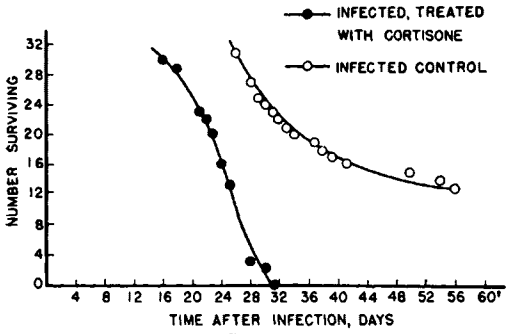

FIG. 2.

Effect of cortisone on low virulence tuberculosis in mice.

virulence. The effect of cortisone on an infection of reduced virulence was studied in two trials. In the first 2 groups of 32 mice each were infected with the substrain of reduced virulence. The mice in one group were treated with 0.1 mg of cortisone administered subcutaneously once daily 6 days per week and the other group served as the infected controls. The data for this test, which was continued for 56 days, are shown graphically in Fig. 2. The median survival time for the infected controls was 40 days while treatment with cortisone reduced the average survival time to 24 days. The untreated controls began to die on the 25th day and at the termination of the experiment on the 56th day 40% were still alive. The cortisone treated animals began to die on the 17th day and all were dead by the 31st day.

In the second trial groups of 32 mice each again were used and the experiment was continued for 35 days. None of the infected control animals died within the experimental period whereas only 50% of the animals treated with cortisone survived.

Discussion. Enhancement of tuberculosis during the hyperadrenal state has been observed in man and experimental animals. The mechanisms by which cortisone enhances the infectious process in experimental tuberculosis have not yet been completely elucidated. The results of the present study suggest that one of the mechanisms by which cortisone may alter the course of this disease is by interference with the immune response. In rapidly progressive tuberculosis where the role of acquired immunity would be expected to be minimal, cortisone did not exert a significant effect on the course of the disease. However, when vaccinated animals were treated with cortisone, the prolonged survival conferred by vaccination was lost.

The results obtained with slowly progressive tuberculosis are in harmony with the hypothesis that cortisone affects the immune response. The slowly progressive disease would permit the animal to acquire immunity. Here the enhancing effect of cortisone on the progress of the infectious process was highly significant; in one of the experiments presented, all the cortisone-treated animals were dead by the 32nd day, whereas more than 40% of the infected controls were still alive at the 56th day after infection.

This interpretation of the present findings is consistent with observations indicating that treatment with cortisone suppresses the amount of circulating antibody.

The data presented are in agreement with the observations of D'Arcy Hart and Rees that cortisone markedly exacerbated chronic tuberculosis infection in the mouse but are not completely in accord with their findings that cortisone treatment enhanced the acute type of infection. This difference may be related to the high dose of cortisone employed by these investigators, 0.5 mg as compared with 0.1 mg used in the present study.

Summary. 1. Treatment with cortisone overcame the beneficial effect of vaccination in mice infected with a highly virulent strain of *M. tuberculosis,* human type, but did not significantly enhance the lethality in non-vaccinated mice. 2. Treatment with cortisone increased the susceptibility of mice to infection with *M. tuberculosis* of reduced virulence.

Received December 27, 1950. P.S.E.B.M., 1951, v76.