Influence of *Pasteurella tularensis* on Enzymes Involved in Energy Metabolism in Tissues of Rats.* (26328)

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The observation that d-amino acid oxidase activity was suppressed markedly in livers and kidneys of tularemic rats(1) suggested the possibility that other enzyme systems, including those of the tricarboxylic cycle, might be influenced adversely during infection. Attention has been focused upon the activities of liver aconitase and liver fumarase which are among those that participate directly in energy metabolism, and which thus might be expected to reflect the influence of *Pasteurella tularensis* on fundamental metabolic mechanisms in its host.

Pinchot *et al.*(2) demonstrated that the effects of adrenal exhaustion occurring in tularemia could be somewhat alleviated by administration of cortisone. Their data have suggested the possibility that this is the result of temporary support of energy metabolism at nearly normal levels.

In view of their findings, studies also were conducted to determine the influence of administration of cortisone during the course of infection on rat liver aconitase and fumarase activity.

Materials and methods. Culture. Strain Sm of *P. tularensis,* which has a rat LD_{50} of approximately 3.5×10^3 organisms, was employed throughout this study. This culture was isolated in 1941 from a human ulcer by Dr. Lee Foshay, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.

Experimental methods. Aconitase and fumarase activity were determined according to the method of Racker(3). Cortisone acetate from Nutritional Biochemicals Corp., Cleveland, Ohio was administered in a mineral oil suspension by intraperitoneal injection.

The rats were maintained on a commercial high energy ration from Security Mills, Inc., Knoxville, Tenn. Test animals were fasted for 12 hours before each experiment but were allowed water *ad libitum.* Rats selected for inoculation received an infecting dose of one LD_{50}. These animals were killed by decapitation in appropriate numbers daily, following infection, for 4 days. After exsanguination, specimens of liver tissue were removed immediately to chilled buffer solution for preparation of homogenates.

All determinations were made in duplicate and enzyme activity was calculated on the basis of dry weight of the tissue employed.

Results. Aconitase activity. Enzyme activity was measured in liver homogenates of 26 normal and 36 tularemic rats. The latter were divided into 4 equal groups and aconitase activity was determined 24, 48, 72, and 96 hours following infection. Enzyme activity in livers of normal rats averaged 29.5 ± 1.1† units per mg dry weight of tissue, while activity in homogenates of tularemic livers was reduced within 24 hours after infection to an average of 21 ± 4.1 units. Thereafter, activity dropped more gradually until a level of 19.5 ± 2.1 units was reached at 96 hours following infection (Fig. 1). These changes did not result from inanition since no significant decrease in consumption of food and water by infected rats occurred prior to the fourth post-infection day.

Subsequently, cortisone acetate was administered to infected rats in concentrations of 5 mg per rat per day, starting at time of infection and for 96 hours thereafter. Once again, 36 rats were divided into 4 groups and liver aconitase activity was measured at 24 hour intervals. Enzyme activity dropped slightly after 24 hours but was maintained at 28 ± 2.4 units 48 hours after infection, whereupon it became depressed markedly after 72 hours (Fig. 1). Thus, cortisone

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† Mean ± Standard Error of Mean.

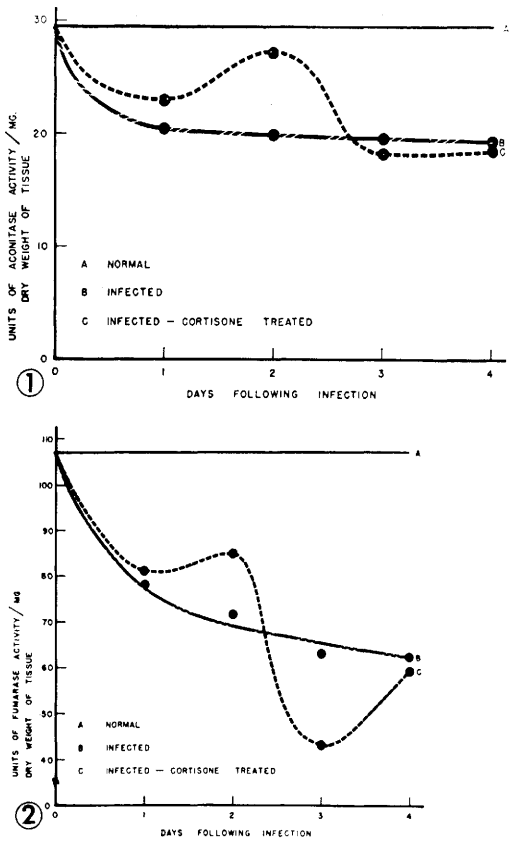

FIG. 1. Comparison of liver aconitase activity in normal and tularemic rats and in tularemic rats given cortisone at a rate of 5 mg/rat/day.

FIG. 2. Comparison of liver fumarase activity in normal and tularemic rats and in tularemic rats given cortisone at a rate of 5 mg/rat/day.

maintained aconitase activity at normal levels 48 hours following infection, compared to 19.7 ± 2.2 units in untreated animals, but subsequently failed to support activity at levels above those observed in infected control rats.

It was determined that cortisone administered as described above was the optimal amount for enhancement of enzyme activity.

Fumarase activity was measured in liver homogenates of 20 normal animals. Sixteen infected rats were divided into 4 groups and enzyme activity was determined at 24 hour intervals as described above. Fumarase activity in normal rats averaged 108.2 ± 3.3 units per mg dry weight of liver homogenate. The function of this enzyme in infected animals was found to be reduced 24 hours after infection to 78.3 ± 4.1 units while at 48 hours activity was 72.2 ± .1 units. After 72 hours it dropped to a low value of 62.9 ± 4.5 units and remained at that level through the 96 hour period (Fig. 2).

In a fourth experiment, each of 12 infected rats was treated with cortisone acetate as previously described. These also were divided into 4 groups and fumarase activity again determined. Treatment with cortisone maintained fumarase activity at 84.0 ± 9.5 units 48 hours after infection, a level slightly higher than in infected-untreated rats (Fig. 2). Activity after 72 hours was reduced to 43.1 ± 4.0 units, a level much lower than that observed in the infected-untreated rats at the same time interval. However, fumarase function subsequently increased and at 96 hours was comparable to that in the infected control group.

Discussion. The reduction in activity of liver aconitase and fumarase during the course of infection suggests that depression of energy metabolism is responsible for some of the pathology observed in tularemia. Also, our observation that the activity of these enzymes can be supported temporarily at nearly normal levels by treatment with cortisone suggests adrenal involvment, since the adrenals are known to exert control over carbohydrate metabolism.

Evidence for this concept is supplied by Pinchot and his co-workers(3) who observed that adrenal exhaustion accompanied by a pronounced drop in body temperature occurred several hours prior to death in tularemic rats. Treatment with adrenal cortical hormone did not relieve adrenal exhaustion or reduce mortality but the animals were better able to maintain body temperature and seemed more active than the controls.

From both of these studies, it appears that adrenal damage was too extensive to be compensated adequately by therapeutic use of cortisone. However, it is well recognized that *P. tularensis* exhibits a high degree of invasiveness resulting in the parasitism of nearly all body tissues. Thus, disturbances in other vital metabolic functions may occur as a result of infection which also contribute

to the severe symptoms noted in tularemia.

Nevertheless, impairment of normal energy yielding reactions seems to be a critical factor influencing the course of infection. An analogy to this is provided by Gilfillan *et al.* (4,5) who studied the adverse influence of *Salmonella pullorum* on liver enzymes of the tricarboxylic acid cycle in baby chicks. They, likewise, concluded that many of the symptoms of the infection were referable to disturbances in this particular metabolic pathway.

Although these comparisons are made between 2 unrelated hosts and 2 unrelated bacterial species, there appears to be an underlying similarity in fundamental pathology in each instance. Gilfillan and his collaborators(5) have suggested the possible role of endotoxins in producing the abnormalities they observed. The work of Berry *et al.*(6) substantiates this concept. They demonstrated that mice poisoned with endotoxin of *Salmonella typhimurium* were depleted of nearly all carbohydrate. However, poisoned mice given a protective dose of cortisone maintained carbohydrate levels 2 to 3 times as great as that of the poisoned controls. Quite possibly, similar effects may be produced in rats by action of an endotoxin of *P. tularensis* which has been demonstrated and characterized by Ormsbee and Larsen(7) and which appears to be related to those of other gram-negative bacteria.

An evaluation of the effects of specific endotoxins on host metabolism would be most desirable in studies of tularemic infection.

Summary. Aconitase and fumarase activity in liver tissue of tularemic rats was demonstrated to be reduced by more than 33% below normal values at height of infection. Administration of cortisone in a concentration of 5 mg per rat per day during infection supported aconitase activity at nearly normal levels for 48 hours but failed to do so beyond that time. Liver fumarase activity of rats treated with cortisone was maintained at only slightly higher levels than observed for the controls. The data presented suggest extensive adrenal involvement in tularemic rats which can be alleviated only partially and temporarily by daily administration of cortisone.

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Connective Tissue V. Comparison of Synthesis and Turnover of Collagen and Elastin in Tissues of Rat at Several Ages.* (26329)

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Previous work from this laboratory has shown that collagen of several tissues of rat increased with age while elastin content remained relatively constant(1). Since there are variations in rates of synthesis and turnover of collagen in several tissues of growing rats(2), it becomes important to study the synthesis and turnover of collagen and elastin in tissues of rats as a function of age.

Methods. Female rats, Wistar strain, 5 weeks, 8 months and 2 years of age, respectively, were injected intraperitoneally with saline containing uniformly labeled C^{14}-lysine (7.8 μc per 100 g body weight). Groups of 3-6 rats were sacrificed at 1, 3, 10, 20, 30 and 40 days, respectively, after injection.

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