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Enzyme Activities of the Livers of Mice Infected with *Salmonella typhimurium*

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The activities of the mouse liver enzymes tryptophan oxygenase, tyrosine aminotransferase, and pyruvate kinase were measured after infection with three dose levels of *Salmonella typhimurium* strain SR-11. Infection occurred in all groups as evidenced by an increase in bacterial numbers and by death of the animals. The activities of the enzymes increased in all groups during the course of the infection. The results obtained during infection are compared with those obtained after endotoxin injection.

Considerable progress has been made in recent years in describing the metabolic events that follow injection of gram-negative endotoxin into animals (13). Berry and Smythe (3) reported a decrease in liver tryptophan oxygenase (TO) activity in animals given endotoxin; this decrease was maximal at the time the animals succumbed. The decrease in the activity of this enzyme and death of the animals could be prevented by administration of cortisone at time of endotoxin injection (4); but the activities of some other enzymes were increased. Rapoport et al. (15) have shown that induction of TO occurs early in the course of pneumococcal infection but returns to normal levels 1 day after infection. Berry et al. (5) demonstrated that the activity of tyrosine aminotransferase (TAT) increases and suggested that the associated loss of glycogen in endotoxin-injected animals might be related to inability of these animals to utilize glyconeogenic intermediates. Snyder et al. (17) associated the increased activity of pyruvate kinase (PK) in endotoxin-injected animals with the depletion of liver glycogen.

LaNoue et al. (12) measured three gluconeogenic liver enzymes in rats infected with *Pseudomonas aeruginosa* and in animals injected with endotoxin. These investigators did not distinguish differences in the activity of either phosphoenol pyruvate carboxykinase or fructose-1,6-diphosphatase after infection or endotoxin injection. However, the activity of glucose-6-phosphatase in endotoxin-treated rats was about 80% of control animals, whereas the activity in infected animals dropped to less than one-half that of control animals.

The effect of infection with *Salmonella typhimurium* on the activities of host enzymes has not been measured and the significance of these biochemical events during infection with a gram-negative organism is unknown at this time. The purpose of this report is to relate the changes observed in the activity of the enzymes TO, TAT, and PK in mice infected with *S. typhimurium* to the results of prior studies with endotoxin.

MATERIALS AND METHODS

Preparation of organisms. *S. typhimurium* strain SR-11 was maintained by weekly transfer on Brain Heart Infusion-agar slants. Brain Heart Infusion broth (Difco) was inoculated with organisms from an overnight culture. After incubation for 12 hr at 37 C, the organisms were centrifuged and washed 3 times with 0.15 M NaCl. The bacterial suspension was adjusted to an optical density of 0.3 at 600 nm with a Bausch & Lomb Spectronic 20 colorimeter. Duplicate plate counts of this suspension showed 1.5×10^8 organisms/ml.

Inoculation of animals. Female Swiss-Webster mice (18 to 20 g; Rockland Farms, Boyertown, Pa.) were used. The mice were allowed to rest 1 week after arrival before experimental use. Infection was established by intraperitoneal injection of 0.5 ml of the appropriate dilution of the standardized culture.

Preparation of mouse liver. The animal was sacrificed by cervical dislocation, and the entire animal was immersed in 70% ethanol for about 1 min. The liver was removed aseptically and placed in a homogenizing tube containing sterile 0.15 M KCl. The organ was homogenized for 2 min and samples were taken for bacterial counts, enzyme determinations, and determination of dry weight or protein content.

Bacterial counts. The numbers of organisms were determined by serial 10-fold dilution of the liver homogenates in sterile 0.15 M NaCl. A 0.1-ml amount of each dilution was spread on the surface of SS agar

plates. After 24 hr of incubation, colonies were counted.

Liver dry weight and protein. Three milliliters of the homogenate was placed in a preweighed porcelain crucible and dried at 90 C for 16 hr. The crucibles were again weighed. Correction for the KCl in the homogenizing medium was made. Dry weight determinations were used for calculation of TO and TAT activity. Protein was measured by the Folin-Ciocalteau procedure described in Kabat and Mayer (9). This measurement was used in calculation of PK activity.

Enzyme assays. TO was assayed by the method of Knox and Auerbach (10) as modified by Berry and Smythe (3, 4). TAT was measured by the method of Rosen and Milholland (16). Both of these enzyme assays used liver homogenates. For determination of PK, the homogenates were centrifuged at 144,000 × *g* for 30 min, and the supernatant fluid was used as the source of enzyme in the assay procedure of Weber et al. (21).

RESULTS

Relationship between infectious dose and tissue numbers. To obtain quantitative data that might reflect the severity of infection, groups of mice were inoculated with 7.5×10^1, 7.5×10^4, and 7.5×10^6 organisms. Groups of eight mice were sacrificed at various intervals and the numbers of organisms in the liver were evaluated (Fig. 1). In a separate experiment, whole body counts were done as described by Berry et al. (10). The animals were sacrificed, skinned, and eviscerated. The carcass was homogenized in a blender, and bacterial numbers were determined (Fig. 2). In both experiments, a relationship between infecting dose and kinetics of in vivo growth of the organism was apparent. Only a few animals inoculated with 7.5×10^6 organisms survived longer than 3 days after infection, whereas few of the animals inoculated with 7.5×10^4 organisms survived longer than 8 days. The numbers of organisms (about 2×10^8) in the tissues were maximal at the time of death. The number of organisms recovered from animals inoculated with 7.5×10^1 organisms was lower than that obtained from groups injected with the larger doses. However, some of these animals survived the infecting dose and their lower counts resulted in the lower mean value. Microbial counts on the whole body tended to be slightly higher than liver counts.

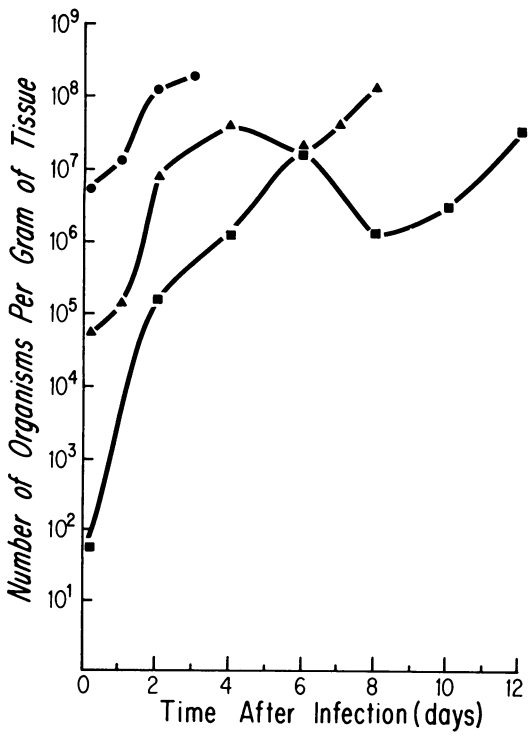

FIG. 1. *Viable bacterial counts of mouse liver after injection with Salmonella typhimurium strain SR-11. Infected with 7.5 × 10⁶ (●), 7.5 × 10⁴ (▲), 7.5 × 10¹ (■). Each point represents mean value for eight mice.*

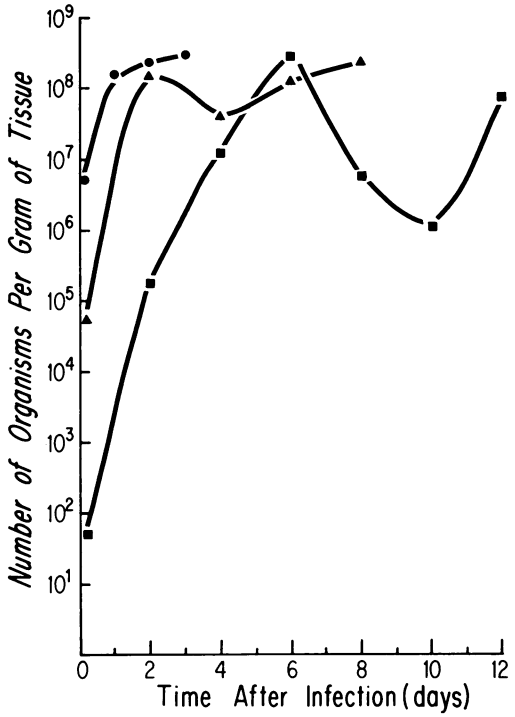

FIG. 2. *Viable body counts of mice infected with Salmonella typhimurium strain SR-11. Infected with 7.5 × 10⁶ (●), 7.5 × 10⁴ (▲), 7.5 × 10¹ (■). Each point represents mean value for eight mice.*

Infection and TO activity. Mice infected with

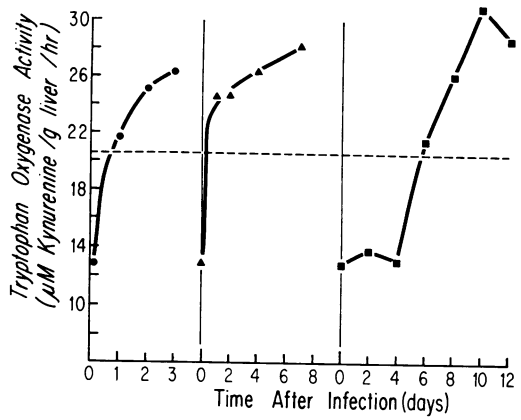

FIG. 3. *Changes in liver tryptophan oxygenase activity of mice infected with Salmonella typhimurium strain SR-11. Infected with 7.5 × 10⁶ (●), 7.5 × 10⁴ (▲), 7.5 × 10¹ (■). Fasted animals indicated by dashed line. Each point represents mean value for eight mice.*

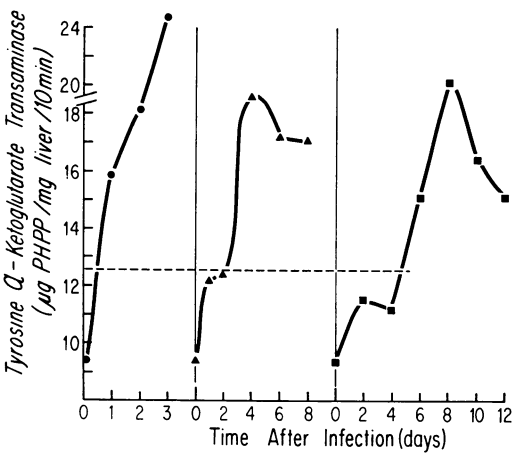

FIG. 4. *Changes in liver tyrosine aminotransferase activity of mice infected with Salmonella typhimurium strain SR-11. Infected with 7.5 × 10⁶ (●), 7.5 × 10⁴ (▲), 7.5 × 10¹ (■). Fasted animals indicated by dashed line. Each point represents mean value for eight mice.*

the three different doses of *S. typhimurium* all showed an increase in TO activity over control animals (Fig. 3). Animals infected with the higher inoculum showed a greater increase in TO activity after inoculation than was obtained in those infected with lower doses of organisms. Regardless of the infecting dose, the liver enzyme activity increased to maximal activity about the last experimental day.

Infection and TAT activity. Measurement of TAT activity yielded results similar to experiments on TO (Fig. 4). Enzyme activity increased

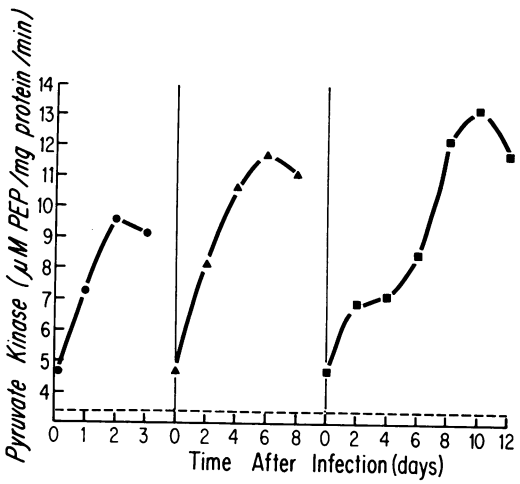

FIG. 5. *Changes in liver pyruvate kinase activity of mice infected with Salmonella typhimurium strain SR-11. Infected with 7.5 × 10⁶ (●), 7.5 × 10⁴ (▲), 7.5 × 10¹ ■. Fasted animals indicated by dashed line. Each point represents mean value for eight mice.*

after infection with maximal activity obtained at about the time the animals succumb to infection.

Infection and PK activity. As was observed in experiments on TO and TAT, the level of PK activity increased in mice infected with *S. typhimurium* (Fig. 5). The activity of the enzyme was higher in mice infected with the smaller numbers of *S. typhimurium*.

Bacterial contribution to enzyme activity. To determine whether the increased liver enzyme activity was a direct contribution of the infecting organism, organisms (equivalent in number to that found in infected tissue) were added to uninfected liver homogenates. No enzyme activity was detected above that of the normal homogenate.

DISCUSSION

The investigation of changes in host enzyme activity after infection with *S. typhimurium* did not confirm prior studies on TO which showed an inhibition of this enzyme after injection of endotoxin (3, 4). But the observed increases in TAT and PK are compatible with reported data (5, 17).

The apparent paradox in activity of TO after infection with *S. typhimurium* and after injection with heat-killed cells derived from the same organism might be attributed to differences in cell inoculum. In other experiments (3, 4), 10^{10} heat-killed organisms were used, whereas the largest inoculum used to produce infection was 7.5×10^6 organisms. Viable microbial counts of tissue show

that at the time of maximum enzyme activity approximately 10^{10} live organisms were present in the tissue. Since some organisms would die in the tissues, one might assume that amounts of endotoxin sufficient to cause inhibition of TO were present. However, host detoxification may have reduced the amount of endotoxin to levels below that required for inhibition of TO.

The use of three different infectious doses was intended to measure host response to infection leading to death at different rates. The decline of host enzyme activity between day 10 and 12 in animals with the smallest inoculum suggests differences in the pathogenesis of these complex infections.

The initial inoculation of an amount of *S. typhimurium* that represents an amount of endotoxin insufficient to cause death or inhibit enzyme activity might have resulted in production of a state of tolerance. However, studies by Greisman et al. (8) have clearly demonstrated that tolerance is not demonstrable during infection.

Both TO and TAT are induced by cortisone, and stimulation of glucocorticoid secretion might have induced these enzymes. Berry and Smythe (4) showed that administration of cortisone either before or at the time of endotoxin injection prevents a decrease in TO. Rapoport et al. (14) measured the excretion products after tryptophan administration to human volunteers infected with *S. typhimurium*. The finding of increased conversion of tryptophan to kynurenine and other products in the TO pathway corroborate our findings, by direct enzyme assay, that the activity of TO is increased during infection. These workers suggest that an increase in glucocorticoid secretion is followed secondarily by an induction of TO and an increase in the rate at which tryptophan enters the kynurenine pathway. The suggestion that glucocorticoid secretion is responsible for induction of TO and TAT is not, however, applicable as an explanation for the increase in PK activity since PK activity is not increased by injection of cortisone (20).

The increase in activity of TAT during infection with *S. typhimurium* is consistent with published work on the effect of endotoxin on this enzyme (5). The increase in PK activity observed during infection is compatible with other work from our laboratory which shows an increase in PK activity after endotoxin injection (17).

The enzymes TO and TAT can be induced by starvation, and some of the increase in enzyme activity may be related to failure of the animals to eat. Inanition has been shown to be produced in mice infected with *S. typhimurium* or injected with endotoxin (2, 18). The role of starvation for more than 48 hr could not be determined because mice will not survive without food for the time periods used in this study. However, Berry and Smythe (2) have shown that mice infected with 10^5 *S. typhimurium* consume 28% of a nutritive solution 24 hr after inoculation. By the second day, they consume 80% as compared with controls. Thus, one would not expect starvation to be responsible for the rise in enzyme activity. In our experiments, the PK enzyme activity of infected mice increases, an effect opposite that caused by starvation (11).

These and other studies show that alterations in host metabolism do occur during infection with *S. typhimurium* and after injection of endotoxin. It is clear from the data presented that not all of the metabolic effects produced by endotoxin injection can be demonstrated in the infected animal. The reasons for this are unclear and require further study.

The depletion of carbohydrate stores in animals infected with *S. typhimurium* or injected with endotoxin has been described (6). The increase in activity of TAT during infection and after endotoxin injection suggests that glyconeogenic intermediates can be produced but that the host is unable to convert them into carbohydrate (7). Weber et al. (19) have shown the importance of PK control during glyconeogenesis. The ratio of the sum of phosphoenol pyruvate carboxykinase and pyruvate carboxylase activity to PK activity is 0.04. For glyconeogenesis to occur, the activity of PK must decrease and phosphoenol pyruvate carboxykinase and pyruvate carboxylase must increase. Thus, if PK control is lost, a net synthesis of glycogen cannot be obtained. The importance of these changes and the mechanisms by which they are produced are unknown and require additional study.

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