

Mechanisms of Pathogenesis in *Listeria monocytogenes* Infection

III. Carbohydrate Metabolism

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ABSTRACT

Several enzymes and metabolites concerned with carbohydrate metabolism were examined in mice infected with *Listeria monocytogenes*. Liver glycogen and glucose decreased parallel to severity of infection. The concentration of glucose in the blood fell to abnormally low levels with a hypoglycemia being most evident at 72 hr. There was a significant decrease in the activity of hepatic uridine diphosphate glucose-glycogen transglucosylase. This decrease in enzymatic activity correlated with the rate of glycogen depletion. Phosphorylase activity declined in a similar fashion, contraindicating enhanced glycogenolysis as the mechanism responsible for glycogen depletion. Although glucose-6-phosphatase decreased throughout the infection period, it did not appear to be the major metabolic defect causing hypoglycemia in *Listeria*-infected mice. Further distortion of carbohydrate metabolism was indicated by findings of increased levels of pyruvate and lactate in the blood of infected animals.

Depletion of carbohydrate reserves with subsequent alterations of the glycogen cycle enzymes has been reported in a wide variety of infections and pathological conditions. Berry and Smythe (3) reported glycogen changes in liver and muscle in mice infected intraperitoneally with 10^5 cells of *Salmonella typhimurium*. Glycogen loss appeared to parallel the release of endotoxin in the infected mice. Lindell et al. (9) reported a significant loss of glycogen from the liver, brain, and muscle in mice infected with *Staphylococcus aureus*. Slein and Logan reported that liver glycogen is depleted in weaning Dutch rabbits after intravenous injection of *Bacillus anthracis* toxin (16). These investigators noted a marked increase in blood sugar within 15 min after administration of toxin, and they suggested a direct relationship between hyperglycemia and degradation of liver glycogen. During hypoglycemia in rats challenged with anthrax toxin, liver glycogen is depleted (7). Singh et al. (14) reported a marked depletion of hepatic glycogen during experimental tuberculosis in guinea pigs. The results of their *in vivo* study on glycogen synthesis from glucose in the liver of tuberculous guinea pigs indicated that the depletion of hepatic glycogen was a result of sup-

pressed glycogenesis. Subsequent studies were performed on liver enzymes directly associated with the synthesis and breakdown of glycogen (15). A substantial inhibition in the activities of phosphorylase, uridine diphosphate glucose (UDPG)-glycogen transglucosylase, and phosphoglucomutase was observed in tuberculous guinea pig liver. During the course of *Coxiella burnetii* infection in guinea pigs, there is a rapid depletion of liver glucose and glycogen (12). Altered enzymatic patterns were indicated by the diminution of UDPG-glycogen transglucosylase activity along with a corresponding increase in phosphorylase levels.

Data presented in the previous paper (19) suggested a possible derangement of carbohydrate metabolism in mice infected with *Listeria monocytogenes*. This report describes additional experiments designed to elucidate further the nature of the defect. It was hoped that an investigation of some of the principal enzymes and metabolites concerned with carbohydrate metabolism might contribute to a better understanding of the mechanisms of metabolic response in listeriosis.

MATERIALS AND METHODS

Bacteria, animals, and infection procedures. *L. monocytogenes* strain A4413 and white female Swiss

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Webster mice (CD-1 strain, pathogen-free, from the Charles River Mouse Farms, North Wilmington, Mass.) weighing 16 to 18 g, as previously described (19), were employed for these studies. Infection, sacrifice, removal of tissue, and homogenization were as described previously (19).

Preparation of protein-free filtrates. Clear filtrates of mouse liver were obtained by the method of Paretzky (*personal communication*). A 1-g amount of fresh mouse liver was homogenized with 25 ml of 5% perchloric acid. The homogenate was centrifuged at $9,750 \times g$ for 15 min, and the supernatant fluid was filtered through acid-washed Whatman no. 42 filter paper into a cold 50-ml volumetric flask and brought to volume with cold deionized water. A 5.0-ml sample was removed and neutralized with 0.5 N KOH to a phenolphthalein end point. A second 5-ml sample was removed and neutralized in a centrifuge tube with the previously determined amount of 0.5 N KOH. The tube was chilled for 30 min and centrifuged at $4,340 \times g$ for 15 min. The supernatant fluid was decanted into a 100-ml volumetric flask, and the precipitate was washed three times with 10 ml of ice-cold deionized water. The supernatant fluids were combined and made to 100 ml.

Liver and blood metabolites. Liver glycogen in the perchloric acid extract was analyzed with anthrone by the method of Kahan (6). Reducing carbohydrate in the perchloric acid extract was measured colorimetrically as described by Nelson (11). Blood glucose was determined colorimetrically at $540 m\mu$ after the addition of arsenomolybdate as described in Sigma Technical Bulletin No. 14 (Sigma Chemical Co., St. Louis, Mo.). After the addition of lactic dehydrogenase and β -nicotinamide adenine dinucleotide (NAD), the amount of reduced β -NAD ($NADH_2$) formed was measured spectrophotometrically at $340 m\mu$ and taken as a measure of the amount of lactic acid originally present in blood (Sigma Technical Bulletin No. 825-UV, Sigma Chemical Co., St. Louis, Mo.). After the addition of lactic dehydrogenase and excess β -NADH₂, the amount of β -NADH₂ converted to β -NAD was measured spectrophotometrically at $340 m\mu$ and taken as a measure of the amount of pyruvic acid originally present in blood (Sigma Technical Bulletin NO. 725-UV, Sigma Chemical Co., St. Louis, Mo.).

Liver enzymes. UDPG-glycogen transglucosylase in liver homogenates was assayed by the method of Leloir and Goldemberg (8). Liver phosphorylase was assayed by the method of Sutherland (17). Glucose-6-phosphatase was determined in liver homogenates according to the method of Shull et al. (13). The amount of inorganic phosphorus released was determined by the method of Fiske and SubbaRow (4).

RESULTS AND DISCUSSION

The maintenance of normal levels of intermediary metabolites of carbohydrate metabolism is to a great extent dependent upon their production by the liver. In attempting to attribute the cause of death to specific biochemical lesions, it seemed appropriate to examine levels of hepatic glucose

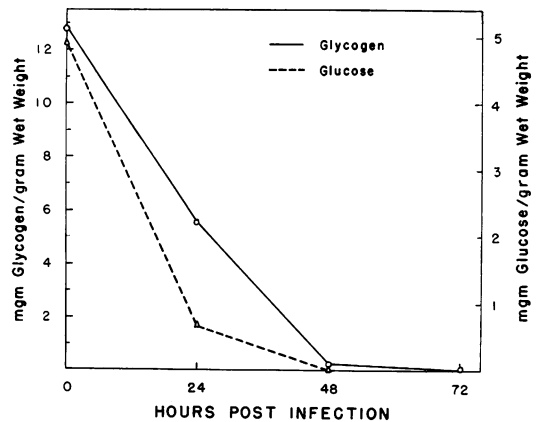


FIG. 1. Glycogen and glucose content in filtrates of mouse liver during *Listeria monocytogenes* infection. The results represent mean values from analyses performed in duplicate, each employing pooled liver extracts from three mice at each time interval after infection.

and glycogen during infection. Figure 1 presents graphically the changes in liver glycogen and glucose observed in mice at different times after infection. Liver glycogen decreased to 42 and 2% of normal values at 24 and 48 hr, respectively. Liver glucose likewise showed a decrease to 16% of normal at 24 hr and was completely depleted at 48 hr.

Glycogen content has been shown to vary under a multiplicity of conditions (2, 3, 9, 12, 14, 16). Several investigators have attempted to correlate glycogen levels with altered activities of the enzymes of the glycogen cycle. Singh et al. (15) observed that the decrease in UDPG-glycogen transglucosylase activity was of sufficient magnitude to account for the depletion of hepatic glycogen during experimental tuberculosis in guinea pigs. The nature of the enzymatic defect was presumed to be due to substantial lowering of total measurable activity. The metabolic abnormality responsible for depleted levels of glycogen in *C. burnetii* infection included both the suppressed activity of UDPG-glycogen transglucosylase and enhancement of glycogenolysis by increased levels of phosphorylase activity (12). It was hoped that parallel studies on the enzyme systems involved in the synthesis and breakdown of glycogen would contribute to an understanding of the mechanism of metabolic response of mice to experimental listeriosis. Figure 2 shows that a significant inhibition of UDPG-glycogen transglucosylase occurs in the liver homogenates of *Listeria*-infected mice. The diminution in enzyme activity parallels the observations on the glycogen levels shown previously in Fig. 1. The in vivo suppression of UDPG-glycogen trans-

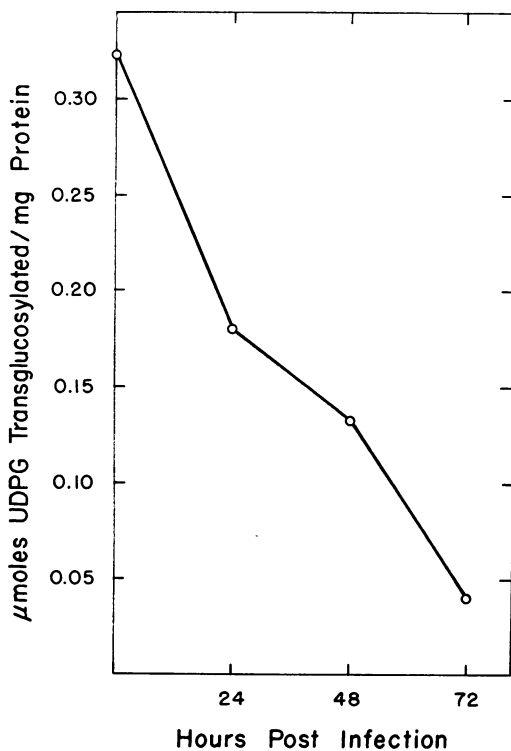


FIG. 2. Activity of mouse hepatic UDPG-glycogen transglucosylase during *Listeria monocytogenes* infection. The results represent mean values for determinations performed in duplicate, each employing pooled liver homogenates from three mice at each time interval after infection. The reaction mixture contained 0.15 ml of 25% liver homogenate (w/v, in 0.25 M sucrose containing 0.001 M ethylenediaminetetraacetate), 20 μmoles of tris(hydroxymethyl)-aminomethane (Tris)-maleate buffer (pH 7.5), 3 mg of glycogen, 1 μmole of glucose-6-phosphate, and 1 μmole of UDPG, in a total volume of 3 ml. The tubes were incubated for 15 min at 37 C.

glucosylase in listeric mouse liver could result in depletion of liver glycogen as a consequence of decreased hepatic glycogenesis.

The levels of glycogen in liver are also controlled by phosphorylase, which catalyzes the phosphorylytic cleavage of the α -glucosidic 1,4 linkages of glycogen to α -glucose-1-phosphate. The activity of mouse hepatic phosphorylase during *L. monocytogenes* infection is presented in Fig. 3. The pattern of decline bears a close resemblance to that reported for UDPG-glycogen transglucosylase, with the diminution of activity being most pronounced at 72 hr. The decrease in phosphorylase activity appears to rule out the possibility that hepatic glycogen depletion during listeriosis occurs as a result of increased glyco-

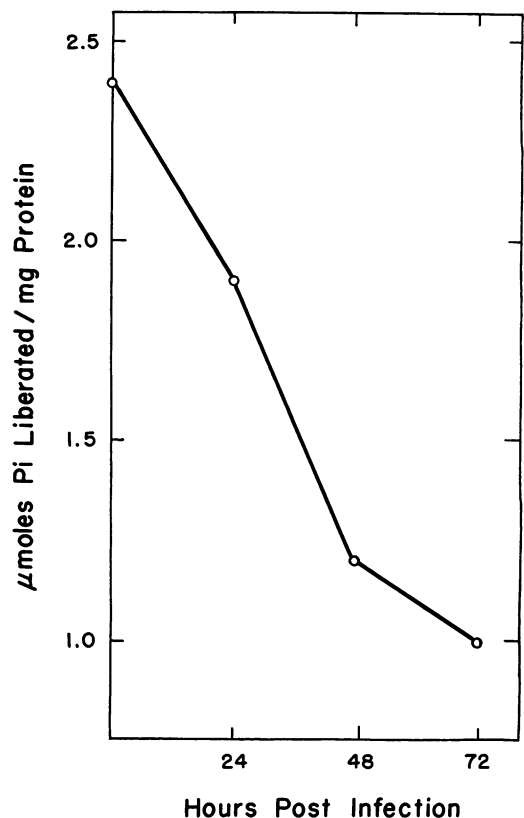


FIG. 3. Activity of mouse hepatic phosphorylase during *Listeria monocytogenes* infection. The results represent mean values from determinations performed in duplicate, each employing pooled liver homogenates from three mice. The standard assay mixture contained 36 μmoles of glucose-1-phosphate, 80 μmoles of NaF, 1.04 μmoles of adenosine monophosphate, 3.6 mg of glycogen, 50 μmoles of Tris-maleate buffer (pH 6.4), and 0.2 ml of 25% homogenate (w/v, in 0.25 M sucrose, 0.10 M NaF, and 0.05 M Tris-maleate buffer, pH 6.5), in a total volume of 1.1 ml. The reaction mixture was incubated for 20 min at 30 C.

genolysis. This speculation is in accord with the findings of Villar-Palasi and Larner (18) that tissue levels of glycogen correlate with enzymes of the glycogen cycle.

Levels of blood glucose in infected mice fall to abnormally low levels at 24 and 48 hr, with a hypoglycemia being most evident in the acute stages of infection (Fig. 4). The maintenance of a constant supply of blood glucose is to a great extent dependent on the conversion of glucose-6-phosphate to glucose by the hepatic enzyme glucose-6-phosphatase. It seemed appropriate, in conjunction with the data on hepatic and blood glucose, to examine this enzyme during infection.

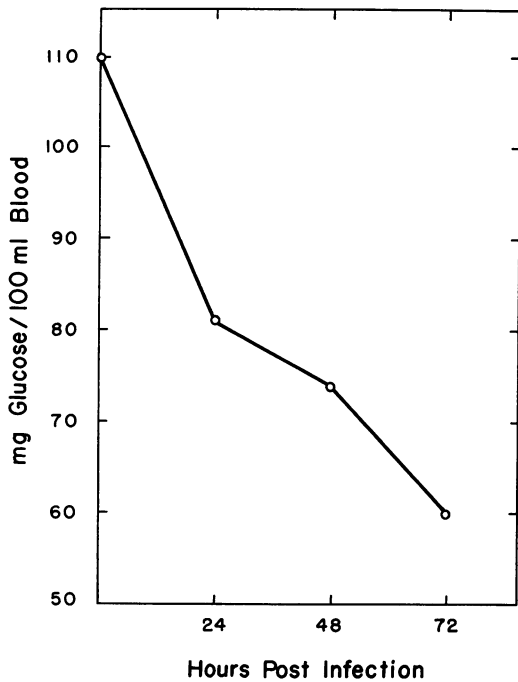


FIG. 4. Levels of blood glucose in *Listeria*-infected mice. The results represent mean values from analyses performed in duplicate on blood pooled from 8 to 12 mice.

Figure 5 reveals a slight decrease in activity after 24 hr, which became more pronounced in the later stages of infection. *Listeria* infection may in part depress glucose production by lowering the activity of glucose-6-phosphatase. The hypoglycemia would result from the decreased capacity of the liver to convert glucose-6-phosphate to glucose. Because of the parallelism observed with glucose and glycogen depletion in conjunction with the depressed activity of the major glycogenolytic enzyme phosphorylase, the inhibition of glucose-6-phosphatase as the major metabolic defect causing hypoglycemia in *Listeria*-infected mice is contraindicated. Liver glycogen would be unavailable to contribute to blood sugar by way of glucose-1-phosphate and glucose-6-phosphate. In addition, if the defect were at the glucose-6-phosphatase level, then a build-up of glycogen rather than a sharp depletion might be expected. These observations lend further credibility to the possibility that the enzymatic defect is at the UDPG-glycogen transglucosylase level and secondarily affects other enzymes.

One cannot rule out the possibility that the failure to maintain blood glucose levels during infection results from an impairment of gluconeogenesis. The most likely explanation would ap-

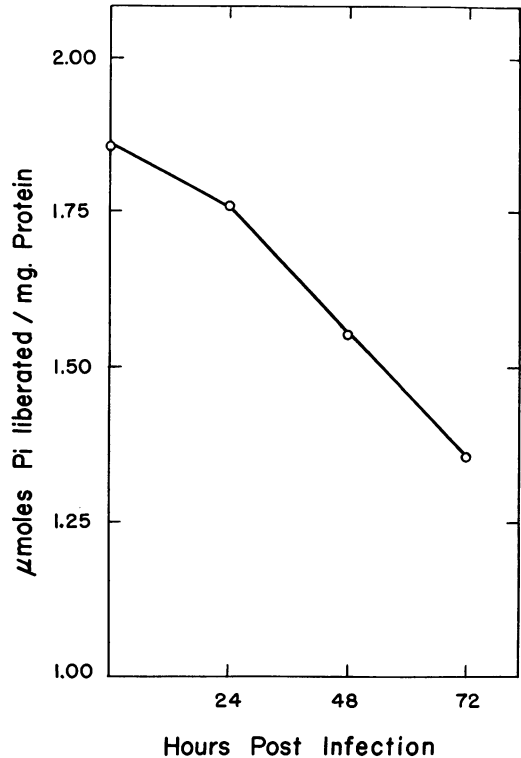


FIG. 5. Activity of mouse hepatic glucose-6-phosphatase during *Listeria monocytogenes* infection. The results represent mean values from determinations performed in duplicate, each employing pooled liver homogenates from three mice. The assay mixture contained 0.2 ml of 25% liver homogenate (w/v, in 0.25 M sucrose), 20 μmoles of Tris-maleate buffer (pH 6.5), and 25 μmoles of glucose-6-phosphate (pH 6.5), in a total volume of 0.5 ml. The mixture was incubated for 30 min at 37 C.

pear to be that the pathogen inhibits gluconeogenesis by indirectly affecting the rate-limiting enzymes, fructose diphosphatase and phosphoenolpyruvate-carboxy kinase. Under conditions of carbohydrate deprivation or fasting, amino acids from tissue protein serve as the chief gluconeogenic substances. Data presented previously (19) suggest that partially depleted hepatic protein levels in the listeric mice are not available for conversion to carbohydrate at a rate sufficient to maintain normal blood sugar concentration. The diminution in food and water intake shown in the previous paper (19) must also be considered as a factor contributing to abnormal levels of blood sugar.

Lactate and pyruvate levels in the blood are altered in a variety of physiological and experimental states associated with glycolytic as well as Krebs cycle derangements (1, 5). After inocula-

TABLE 1. Blood lactic and pyruvic acid levels in mice infected with *Listeria monocytogenes*

Time postinfection	Pyruvate ^a	Lactate ^a
hr	mg/100 ml	mg/100 ml
0	1.05	9.4
24	1.39	13.8
48	5.40	35.5
72	2.89	21.6
96	—	18.0

^a The results represent mean values from analyses performed in duplicate employing blood pooled from 8 to 12 mice.

tion of a toxic cellular component of *L. monocytogenes* into rabbits, McIlwain et al. (10) noted increases in both lactate and pyruvate. Table 1 shows that the content of lactate and pyruvate in the blood of mice infected with *L. monocytogenes* increased throughout the period of infection. Maximal values are obtained at 48 hr. It seems likely that the accumulation of lactic and pyruvic acids during infection may be caused by reduced activity of the enzymes metabolizing pyruvic acid. This would include both pyruvate decarboxylase and glutamic-pyruvate transaminase. Since thiamine pyrophosphate is essential in the transformation of pyruvate to acetate, dietary deficiency of thiamine may in part be responsible for the failure of the liver to assimilate pyruvate efficiently into the Krebs cycle sequence of reactions. The severity of the biochemical aberrations as well as the number of organisms in the infected liver increase progressively throughout the infection. Although acute necrosis is probably the predominant factor responsible for the gross changes, the primary underlying biochemical lesions remain to be identified. The present experiments do not preclude the possibility of the derangement being at the level of hormonal secretion, since endocrine dysfunction could obviously lead to many of the observed metabolic disturbances.

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