Influence of Bacterial Infections on Glycolytic Enzyme Patterns in Rat Tissues

BRIJ M. MITRUKA

Section of Laboratory Animal Sciences, Yale University School of Medicine, New Haven, Connecticut 06510

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The course of bacterial invasion of a particular tissue varies according to a variety of factors such as synergic or antagonist actions between invaders, non-specific and specific host responses, and alterations in the host environment. Another determinant of the extent of a particular tissue involvement is the striking tendency of microorganisms to invade specific cells or to localize in a particular organ of the host(1,2). The alterations in the metabolic patterns of the infected tissue may be reflected in changes in the activities of some or all of the enzymes of the glycolytic pathways(3). In mammalian tissues, the activities of glycolytic enzymes may be in constant proportion to each other(4) and the rate of glycolysis may be regulated by several key enzymes which are on the strategic branching point of the metabolic pathway(5,6).

In view of these proposals, this study describes the change in patterns of several glycolytic enzymes in tissues of rats inoculated with either Salmonella typhimurium, Diplococcus pneumoniae, or Pseudomonas aeruginosa.

MATERIALS AND METHODS

Cultures. A virulent strain of S. typhimurium isolated from our rat colony, was used in this investigation. The median lethal dose (LD₅₀) was calculated by the method of Reed and Muench(7) 30 days after intraperitoneal administration in the rat and was found to be approximately 2 x 10⁷ cells per animal. A low-

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virulent strain of *P. aeruginosa* (LD$_{50}$ approximately $10^{11}$ cells) was obtained from the stock cultures of our diagnostic bacteriology laboratory. The organisms were maintained by weekly transfer on trypticase soy agar slants. Trypticase soy broth (TSB) was inoculated with organisms from an overnight culture. The highly virulent strain of *D. pneumoniae* (Type 3) which has the LD$_{50}$ for rat of approximately $5 \times 10^6$ cells, was grown in brain–heart infusion broth. After incubation for 16 hours at $37^\circ$C, the organisms were centrifuged and washed three times with sterile normal saline solution. The bacterial suspension was diluted to contain $10^6$ cells per milliter.

**Animals.** Ten-week-old male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 300–500 grams were maintained on a diet of a rat chow and water *ad libitum*. During the 2-week conditioning period, each animal was weighed and examined, and baselines of chemical values were determined. The animals were divided at random into four groups. One group of 42 animals was inoculated intraperitoneally with a 1-ml preparation of *P. aeruginosa*, the second group (46 animals) was inoculated with 1 ml *S. typhimurium* cell suspension, the third group (40 animals) received 1 ml *D. pneumoniae*. The fourth group of 40 animals was sham-inoculated with 1 ml sterile normal saline solution. A group of 20 animals was fasted for 48 hours (only water was given) to determine the effect of starvation on the levels of tissue enzymes. The animals were bled by heart puncture at various time intervals and samples of heart, liver, kidney, spleen, and skeletal muscles were quick-frozen in a dry ice–alcohol bath. The organs were homogenized and the number of organisms was determined by serial 10-fold dilution in sterile normal saline solution. A 0.1-ml amount of each dilution was spread on the surface of blood agar plates. After 24 hours of incubation, colonies were counted. Tissue pieces weighing 1 gram (wet weight) were stored for 1–2 weeks in plastic vials frozen at $-20^\circ$C until used for enzyme analysis.

**Tissue extraction.** The tissues were weighed rapidly and homogenization was begun on frozen tissue so that thawing occurred during tissue disintegration. The tissues were homogenized for 2 minutes at top speed in a Virtis 45 homogenizer (Virtis Research Equipment, Gardiner, NY) A chilled homogenization medium containing KCl, 0.15 M; KHCO$_3$, 0.05 M; NaH$_2$EDTA, 0.006 M (pH 7.6) was used throughout these studies. However, in some cases, a 10% tissue homogenate made with the dilution media were stored at $-20^\circ$C for 3–4 days. Glyceraldehyde phosphate dehydrogenase (GAPDH) activity was used as reference enzyme for the stability and reproducibility of the results(9). Enzyme determinations were made on an appropriate dilution of the extracts.

**Enzyme assays.** Glucokinase ( GK), (EC 2.7.1.2); phosphofructokinase (PFK) (EC 2.7.1.11); pyruvate kinase (PK) (EC 2.7.1.40); phosphoglucomutase (PGM) (EC 2.7.5.1); D-fructose-1, 6-diphosphatase 1-phosphohydrolase (FDPase) (EC 3.1.3.11); glucose-6-phosphate dehydrogenase (GPDH) (EC 1.1.1.49); lactic dehydrogenase (LDH) (EC 1.1.1.27); glyceraldehyde dehydrogenase (GLYPDH) (EC 1.1.1.8); glyceraldehydesphosphate dehydrogenase (GAPDH) (EC 1.2.1.12); phosphohexoseisomerase (PHI) (EC 5.3.1.9); aldolase (ALD) (EC 4.1.2.b); and enolase (ENO)
(EC 4.2.1.11) were measured by the methods described by Shonk and Boxer(9). Isocitric dehydrogenase (ICDH) (EC 1.1.1.42) was measured by the method of Ochoa(10). Glucose-6-phosphatase (G6Pase) (EC 3.1.3.9) was measured according to the method of Weber and Cantero(11). Pyruvate carboxylase (PYC) (EC 6.4.1.1.) was assayed by the method of Scrutton et al.(12), and phosphoenolpyruvate carboxykinase (PEPC) (EC 4.1.1.32) was measured by the method of Nordlie and Lardy(13).

All enzyme activities were expressed in terms of international units. One unit of any enzyme is defined as that amount of enzyme which catalyzes the transformation of 1 $\mu$mole of substrate per minute.

Isotope techniques with liver slices in vitro. Tissue slices were prepared with a Stadie-Riggs hand microtome and approximately 200 mg of slices per milliliter of high potassium medium(14) were incubated in a Warburg flask. The incubation medium (4 ml) contained 20 $\mu$moles per milliliter of glucose, 2 $\mu$Ci of glucose-1-$^{14}$C (specific radioactivity 7.8 $\mu$Ci per $\mu$mole) or glucose-6-$^{14}$C (specific radioactivity 8.2 $\mu$Ci per $\mu$mole) (New England Nuclear Corp., Boston, MA) and Krebs-Ringer bicarbonate buffer (pH 7.4)(15). The specific radioactivity of glucose in the incubation medium was 0.1 $\mu$Ci per $\mu$mole. For carbon dioxide absorption a filter paper roll soaked with 2.5 N KOH was placed in the center well. The flasks were gassed initially for 5 minutes with 5% CO$_2$ and 95% O$_2$ mixture and then shaken in a reciprocating water bath at 37°C. All flasks were equilibrated for 10 minutes, the manometer stopcocks were closed and the flasks were shaken 90 minutes longer. The reaction was terminated by injecting 0.5 ml 0.2 M H$_2$SO$_4$ from the sidearm into the medium. Shaking was continued for 20 minutes more to collect the liberated CO$_2$. All $^{14}$CO$_2$ measurements were made by placing the filter paper and washings from the center well into a standard low potassium counting vial. A 15-ml scintillation solution prepared by mixing 378 ml absolute synthetic ethanol with 600 ml toluene containing 0.4% PPO (2,5-diphenyloxazole) and 0.0015% POPOP (1,4-bis-2, 5-phenyloxazolyl)-benzene. The sample was shaken vigorously and counted in a Packard liquid-scintillation spectrometer (Packard Instrument Company, La Grange, IL). The counting efficiency was about 52% and a background about 60 counts per minute (cpm). Trapping efficiency of $^{14}$CO$_2$ was 95.5% as determined with a standard solution of NaH$^{14}$CO$_3$.

The contents of the Warburg flask were centrifuged and radioactivity in lactic acid was measured by the methods as described by Wenner and Weinhouse(16). The tissue slices separated from the medium were digested in KOH for glycogen and fatty acid determinations. The total radioactivity in glucose and glycogen in the incubation mixture was determined by preparation, crystallization, and radio-assay of phenylglucosazone(14). Long-chain fatty acids were isolated from the alcoholic KOH digest of the tissues by acidification and extraction into petroleum ether.

Chemical methods. Initial and final glucose concentrations of the incubation media were determined by using Nelson's adaption of the Somogyi method(17). Glycogen concentrations in tissues were determined by the method of Kemp
et al. (18) and lactic acid according to Barker and Summerson (19). Protein concentrations of tissue homogenates were determined with a modified biuret reagent (20).

Calculations. The data obtained by chemical and isotope studies were used to calculate the net changes in glucose and glycogen contents of the liver slices. Initial and final concentrations (90 minutes after incubation) of labeled glucose in the incubation mixture were measured and glucose uptake was calculated by the following methods (21).

\[
\text{Glucose uptake} = (\text{initial glucose}) - (\text{final glucose}) \times \left(\frac{\text{(specific activity of glucose C)}}{\text{(specific activity of initial glucose C)}}\right).
\]

Net change (glucose) = (glucose output) - (glucose uptake).

Net change (glycogen) = (final glycogen) - (initial glycogen).

Net \(^{14}\text{CO}_2\) production via pentose-phosphate shunt was estimated by subtracting the \(^{14}\text{CO}_2\) yield from the metabolism of glucose-6-\(^{14}\text{C}\) from that of glucose-1-\(^{14}\text{C}\) as described by Ashmore et al. (14).

RESULTS

Bacteria in rat tissues. Blood samples drawn from rats by cardiac puncture and samples from homogenized tissues were plated on blood agar plates to determine the distribution of bacteria after injection of pure cell suspensions. Salmonella typhimurium pure culture inoculation resulted in a spread of the organisms in liver, kidney, spleen, and heart muscles by 3 hours postinjections (Table 1). The concentration of the organisms in these tissues reached a peak by 12 hours postinoculation. All the animals died by 80 hours with S. typhimurium infections. In the group of animals inoculated with P. aeruginosa, liver, kidney, and heart muscle had large numbers of organisms at 12 hours postinoculation which gradually declined by 72 hours. None of the animals in the group died and by 1 week postinoculation, a low number of viable P. aeruginosa were isolated from liver and spleen homogenates. The animals inoculated with D. pneumoniae had low numbers of organisms in the liver, spleen, and kidney, but lungs and heart muscle showed rapid growth of the pneumococci. The organisms were found in heavy concentrations in the tissues by 24 hours postinoculation. All the animals died by 80 hours after the inoculation with D. pneumoniae organisms. No organisms were found in the tissues of the group of animals which were sham-inoculated with sterile saline solution. These animals were killed at the same time intervals as the inoculated animals.

Effect of bacterial infection on the key glycolytic enzymes in tissues. Samples of liver homogenates from 48-hour fasted rats showed 30–50% lower activities of GK, PFK, and PK than those of normally fed animals. As compared to liver enzymes in uninfected fed animals, the activities of these key glycolytic enzymes were 100–150% higher in rats infected with S. typhimurium (Fig. 1). Pseudo-
*monas aeruginosa* infections caused smaller increments in liver GK, PFK, and PK. However, no enzyme activity was detected above that of normal homogenates when equal numbers of organisms were added to uninfected liver homogenates.

The activities of GK in spleen, kidney, heart, and skeletal muscle were increased by 40–100% in the *S. typhimurium*-infected group of animals; whereas in the group of animals infected with *P. aeruginosa*, the activity of GK was not altered significantly in these tissues. The activity of PFK was likewise increased by 30–50% in rat tissues infected with *S. typhimurium* but the other bacterial infections tested caused less pronounced changes in PFK activities in these tissues. Pyruvate kinase activity was elevated 2- to 3-fold in spleen and skeletal muscles infected with *S. typhimurium* bacteria. However, the PK activity in kidney and heart muscles was elevated by 50–60% in animals inoculated with *S. typhimurium* or *P. aeruginosa*. Since PK was very active in most tissues, this enzyme was measured in rat tissues during various stages of development of the infections. Generally there was an elevation in PK activity at the early stages of infection. Heart, spleen, kidney, skeletal muscle, and serum had 40–170% higher levels of PK by 8 hours postinoculation (Fig. 2), whereas maximal increase in the activity of liver PK were found at 24 hours postinoculation in the *P. aeruginosa*-infected animals. *Diplococcus pneumoniae* infection caused only a 40% increase in PK activity in the liver in contrast to a 100–150% rise in PK activity in the other tissues of *D. pneumoniae*-infected animals.

**TABLE 1**
**Spread of Organisms in Rat Tissues after Intraperitoneal Injection of 10⁸ Bacterial Cells**

| Organism       | Time after injection (hours) | Viable bacteria inb | Heart muscle |
|----------------|-----------------------------|---------------------|-------------|
|                |                             | Liver    | Spleen    | Kidney | |
| *Salmonella typhimurium* | 3  | ++      | +       | +      | +    |
|                | 6  | +++     | +++     | +++    | +++   |
|                | 12 | ++++    | ++++    | ++++   | ++++  |
|                | 24 | ++++++   | ++++++  | ++++++ | ++++++ |
|                | 72 | ++++++   | ++++++  | ++++++ | ++++++ |
| *Pseudomonas aeruginosa* | 3  | +       | +       | +      | +    |
|                | 6  | +       | +       | +++    | +    |
|                | 12 | +++      | +++     | ++++   | +    |
|                | 24 | ++++     | +++     | ++++   | +    |
|                | 72 | +        | ++      | +      | +    |
|                | 168| +        | +       | —      | —    |
| *Diplococcus pneumoniae* | 3  | +       | +       | +      | +    |
|                | 6  | +       | +       | +      | +    |
|                | 12 | +++      | +++     | +++    | +++  |
|                | 24 | ++++     | +++     | +++    | +++  |
|                | 72 | ++++++   | ++++    | ++++   | ++++ |

* All animals died by 80 hours postinjection.

b Viable bacteria were recorded as (+) meaning presence of less than 10⁸ colonies, (++) less than 10⁹, (+++) less than 10³, (++++) more than 10⁷, and (—) absence of colonies, per gram of tissue on blood agar plates. Values given are the averages of three animals at each time period.
It was interesting to note that kidney, spleen, heart, and serum of P. aeruginosa-infected animals showed similar changes in PK activity as those observed in the tissues of S. typhimurium-infected animals.

Effects of bacterial infections on certain liver dehydrogenases. The activity of some dehydrogenases were compared in the liver tissues infected with bacteria. The animals inoculated with S. typhimurium showed a 400% increase in the activities of LDH and GAPDH and an 800% increase in ICDH activities (Fig. 3). In contrast, P. aeruginosa-infected liver had a 680% increase in GPDH activity, a 200% elevation in LDH and a 100% increase in GAPDH. Other soluble dehydrogenases in liver showed 30-100% decreases in enzyme activities. The animals inoculated with D. pneumoniae showed significant elevations in LDH (900%) but GPDH activity was diminished by 80% and GLYPDH by 30%.

An examination of the ratios of key gluconeogenic and glycolytic enzymes are presented in Table 2. The ratio of the activity of G6Pase to GK is approximately 7, whereas the ratio was 0.6 in tissues infected with S. typhimurium and 2.8 in tissues infected with P. aeruginosa. Similar patterns of changes in the ratios of FDPase to PFK and PEPC + PYC to PK were observed in the uninfected and infected livers of the animals.

Metabolism of G-6-P in liver. The metabolic fate of G-6-P in the liver cell depends upon the activity of four enzymes, G6Pase, PGM, GPDH, and PHI, which channel it into four different metabolic pathways. Assay of the G-6-P metaboliz-
ing enzymes in homogenates of infected and uninfected liver tissues revealed that G6Pase, which channels G-6-P into the circulation as blood glucose, was markedly diminished as a result of bacterial infection (Table 3). The activity of PGM, which channels G-6-P into glycogen, was increased 3-fold in S. typhimurium infections but decreased considerably in P. aeruginosa infections. On the other hand, the activity of PHI, which routes G-6-P into an energy generating pathway, was increased 3-fold in acute S. typhimurium infections but decreased (40%) with the chronic infectious process. The metabolism of G-6-P via the pentosephosphate shunt (GPDH) for nucleoprotein synthesis was increased 7-fold in P. aeruginosa infection.

Metabolism of labeled glucose in rat liver slices. A 200-mg liver slice sample from uninfected or 72-hours postinoculated animals was incubated with \(^{14}\)C-glucose in order to correlate the results of enzyme changes in the liver with those of glucose utilization in vitro. Initial concentrations of glycogen in S. typhimurium-infected tissues were similar to those of 48-hour fasted rats (Table 4).

![Graph showing pyruvate kinase activity in tissues of rat infected with bacteria. Shaded areas indicate normal range. Mean values for uninfected tissues (UI) were 98 units per milliliter of serum and 29, 385, 124, 50, 38 units per gram of liver, skeletal muscle, heart, spleen, and kidney tissues, respectively. These values were used as controls to calculate percentage change. Each point represents the average of determinations from tissues of seven rats with the range indicated by the bars.](image-url)
| Enzymes                        | Uninfected | Infected* with | Infected* with |
|-------------------------------|------------|----------------|----------------|
|                               | Activity   | S. typhimurium | P. aeruginosa  |
|                               | Ratio      | Activity Ratio| Activity Ratio|
| Glucose-6-phosphatase         | 12.8 (11.7-14.4) | 2.6 (1.7-3.9) = 0.6 | 7.0 (5.2-9.1) = 2.8 |
| Glucokinase                   | 1.8 (1.7-2.0) | 4.3 (4.0-4.5)  | 2.5 (2.2-2.7)  |
| Fructose-1, 6-diphosphatase   | 15.6 (14.2-17.3) | 13.8 (10.2-16.4) = 3.3 | 15.7 (14.6-17.1) = 5.6 |
| Phosphofructokinase           | 2.4 (2.2-2.7) | 5.1 (4.9-5.4)  | 2.8 (2.6-3.1)  |
| Phosphoenolpyruvate carboxylase | 6.8 (5.9-8.4) | 4.4 (2.5-5.4)  | 7.6 (6.1-9.4)  |
| Pyruvate kinase               | 25 (25-27)  | 63 (58-70)     | 38 (35-40)     |
| Pyruvate carboxylase          | 13.0 (9.8-16.0) | 10.3 (6.7-13.2) = 0.16 | 11.4 (10.6-12.5) = 0.30 |
| Pyruvate kinase               | 25 (24-27)  | 63 (58-70)     | 38 (25-40)     |

* Samples were taken 72 hours postinoculation.

* Expressed as micromoles of substrate utilized per hour at 37°C per gram of protein. The assays were carried out under optimal substrate and cofactor conditions at pH 7.6.

* Each value represents the mean of four determinations from separate animal tissues, values in parentheses represent the range.
| Tissue infected with | G6Pase Activity<sup>a</sup> | G6Pase Activity % | PGM Activity | PGM Activity % | GPDH Activity | GPDH Activity % | PHI Activity | PHI Activity % |
|----------------------|-----------------------------|-------------------|--------------|--------------|---------------|---------------|--------------|----------------|
| None                 | 6.7<sup>b</sup>             | 100               | 20           | 100          | 3.0           | 100           | 110          | 100            |
|                      | (6.1-7.3)                   |                   | (15-25)      |              | (2.5-3.4)    |               | (99-112)     |                |
| S. typhimurium       | 1.3                         | 20                | 60           | 300          | 2.6           | 87            | 364          | 331            |
|                      | (1.2-1.5)                   |                   | (55-65)      |              | (2.3-2.9)    |               | (349-380)    |                |
| P. aeruginosa        | 4.0                         | 60                | 2.0          | 10           | 22            | 733           | 63           | 57             |
|                      | (3.7-4.5)                   |                   | (1.8-2.1)    |              | (20-25)      |               | (57-70)      |                |

<sup>a</sup> Activity = micromoles of substrate utilized per minute at 37°C per gram of wet tissue. G-6Pase, glucose-6-phosphatase; PGM, phosphoglucosemutase; GPDH, glucose-6-phosphate dehydrogenase; PHI, phosphohexose isomerase.

<sup>b</sup> Each value represents the average of four determinations with the range given in parentheses.
Fig. 3. Effects of bacterial infections on the relative change of rat liver glucose-6-phosphate dehydrogenase (GPDH), lactic dehydrogenase (LDH), glyceraldehyde phosphate dehydrogenase (GAPDH), glycerophosphate dehydrogenase (GLYPDH), and isocitric dehydrogenase (ICDH). Mean values (± standard error of the mean) of these enzymes in uninfected tissues are, 3 (±0.5), 136 (±13), 65 (±2.3), 59 (±3.8), and 22.4 (±2.9) units per gram of tissues, respectively. The range is indicated by each bar and numerals represent the mean values. Each value represents the average of determinations from tissues of four rats.

**TABLE 4**

**Metabolism of Glucose by Uninfected and Infected Rat Liver Slices**

| Tissues infected with | Glycogen \(^b\) | Glucose \(^b\) |
|-----------------------|-----------------|-----------------|
|                       | Initial | Net change | Output | Net change |
| None                  |     |           |     |           |
| Fed\(^c\)             | 128    | -96       | 65   | 16        |
|                       | (120–138) | (-105–(-82)) | (57–71) | (14–19) |
| Fasted (for 48 hours) | 0.6    | 4.2       | 22   | -3.1      |
|                       | (0.4–0.9) | (3.6–4.9) | (17–30) | (-0.9–(-5.4) |
| *S. typhimurium*\(^c\) | 1.1    | -0.5      | 26   | -54       |
|                       | (0.8–1.4) | (-0.9–(-0.1) | (21–32) | (-40–(-26) |
| *P. aeruginosa*\(^c\) | 4.3    | 1.6       | 60   | 10        |
|                       | (3.9–4.9) | (1.2–2.1) | (50–68) | (5–14) |

\(^a\) Glucose was added at a concentration of 20 \(\mu\)moles per milliliter.

\(^b\) All values are expressed in micromoles per gram of wet weight tissues per 90 minutes and represent the average of four determinations with the range given in parentheses; values were calculated as described in Materials and Methods.

\(^c\) Food and water were given *ad libitum* to experimental animals.
Slightly higher amounts of glycogen were found in the tissues from *P. aeruginosa*-infected rats. Glycogen changes during incubation were small in the fasted and infected animal tissues. In the liver slices from normally fed rats, glycogen concentration decreased and the contents of glucose in the medium increased by 16 μmoles per gram of tissue. There was considerable net utilization of glucose in the incubation mixture containing *S. typhimurium*-infected tissues.

The amount of glucose-1-14C utilized was accounted for in CO₂ (3.0%), lactic acid (20%), and fatty acids (5.1%) in normal animal liver slices whereas a considerable percentage increase in CO₂ was found in the samples from fasted animals (Table 5). In order to estimate the percentage of glucose utilized via pentosephosphate shunt, 14CO₂ produced from glucose-6-14C was determined. The results obtained by using glucose-1-14C and glucose-6-14C in tissue slices show that in normally fed rat liver 20% of glucose utilized was converted into glycogen, 20% glycolized in lactic acid and fatty acid and 1.3% (14CO₂ yield from glucose-1-14C—14CO₂ from glucose-6-14C) entered the pentose-phosphate shunt. Liver slices from the fasted animals catabolized 5-6% of glucose by the shunt. Glycogenesis was lower by 6.6% in the fasted liver tissues. The liver slices infected with *S. typhimurium* had an increase in glycolysis of 44% as compared to that of normal fed animals. In *Pseudomonas*-infected tissues, the utilization of 14C-glucose by the pentose-phosphate shunt was considerably increased as indicated by the production of 14CO₂ (39%) while glycogen synthesis was decreased to 4.0%.

Direction of metabolic flux. Based on the enzyme levels of glycolysis and gluconeogenesis and metabolism of G-6-P in liver, the direction of metabolic flux in uninfected and infected tissues was estimated. The activities of some of the key enzymes in the glycolytic pathway were compared on a percentage basis taking the values found in uninfected liver arbitrarily as 100% (Fig. 4). These relative activities result in the net flow of carbon along the pathways shown by the dark lines. Thus, in *S. typhimurium*-infected tissue the major operative pathway was the energy-yielding glycolytic pathway and the pathway of gluconeogenesis was essentially inhibited; however, oxidation through the shunt was only slightly affected. In *D. pneumoniae*-infected liver, the major pathway was glycolysis while the pentose-phosphate shunt and glycogen synthesis were inhibited and gluconeogenesis unaffected. Although the tissues infected with *P. aeruginosa* exhibited a reduced rate of gluconeogenesis and glycolysis (30-40%), the direct oxidative pathway was markedly increased and glycogen synthesis was inhibited.

**DISCUSSION**

Several studies have been reported on alterations of enzyme levels in tissues by a variety of physiologic, nutritional, and hormonal manipulations and by administration of various pharmacologic agents (3,6,22,23). However, relatively few investigations have been performed to evaluate the changes in key enzymes of a metabolic pathway during infectious disease states. In this study attempts were
| Tissues infected with | Glucose uptake | Glucose-1-C\(^14\) | Glucose-6-C\(^14\) |
|----------------------|----------------|------------------|------------------|
|                      | μmoles\(^b\)  | CO\(_2\) μmoles | Lactic acid μmoles | Fatty acid μmoles | CO\(_2\) μmoles | Glycogen μmoles |
| None                |               |                  |                   |                  |                   |                   |
| Fed\(^c\)           | 47 (43-51)    | 1.4 (1.2-1.7)    | 3.0 (8.5-10)      | 9.4 (20)         | 2.4 (2.1-2.8)    | 0.8 (0.5-0.9)    | 1.7 (8.6-10)    | 9.4 (20)       |
| Fasted (for 48 hours)| 26 (22-32)    | 3.7 (3.2-4.5)    | 14 (4.7-5.6)      | 5.1 (20)         | 1.8 (1.5-2.3)    | 6.8 (1.8-2.7)    | 2.2 (3.5)       | 8.4 (3.5)      | 13 (2.9-4.0)  |
| S. typhimurium\(^e\)| 60 (55-65)    | 0.6 (0.4-0.9)    | 1.0 (31-35)       | 33 (55)          | 8.4 (7.5-9.4)    | 14 (0.2-0.5)     | 0.4 (13-16)     | 0.7 (14)       | 23 (13-16)    |
| P. aeruginosa\(^e\)| 50 (45-54)    | 22 (21-24)       | 44 (9.2-11)       | 10 (20)          | 2.5 (2.1-2.9)    | 5.0 (2.3-2.8)    | 2.5 (1.1-2.8)   | 5.0 (2.0)      | 4.0 (1.1-2.8) |

\(^a\) The incubation medium contained 20 μmoles glucose per milliliter and the specific radioactivity of glucose was 0.1 μCi per μmole (approximately 5 × 10\(^6\) cpm per μmole.)

\(^b\) All values are expressed in micromoles per gram of wet weight tissues per 90 minutes and represent the average of four determinations with the range given in parentheses; values were calculated from the initial and final concentrations of labeled glucose in the incubation mixture as described in Materials and Methods.

\(^c\) Food and water were given ad libitum to experimental animals.
made to correlate the pathogenicity of bacteria with host response as indicated by relative changes in the patterns of tissue enzymes. Although our understanding of the molecular aspects of host defense mechanisms against early stages of bacterial invasion is not clear, it seems that the metabolic activity of the host cell changes in relation to the degree and type of infection. The use of three separate organisms of different pathogenic characteristics was intended to examine the host response to various levels of infections.

The rise in enzyme levels in tissues from *S. typhimurium*-infected rats was presumably due to host response to acute bacterial infection as evidenced by rapid increase in bacterial numbers and by death of the animals. Elevation of PK enzyme activity at the early stages of infection was in correlation with high bacterial concentration at 6 hours postinoculation. However, the increased enzyme activity was not directly contributed to by the bacteria as indicated by our data on rat tissues and those reported by Snyder(24) with experimental mice.

The examination of ratios of key enzymes of glycolysis and gluconeogenesis indicated an increased rate of glycolysis in host liver in response to acute bacterial infection. The effect of *S. typhimurium* infection may be a modifying action directed at the strategic metabolic points where metabolic flow is determined. In order to estimate metabolic flux, levels of liver dehydrogenases were used as an indicator of relative changes in oxidation, glycolytic and pentose-phosphate pathways due to bacterial infection. In the acute *S. typhimurium* infection, significant elevations in LDH, GAPDH, and ICDH in liver indicated marked eleva-

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**Fig. 4.** Comparison of carbohydrate metabolism in uninfected and infected rat livers. The activities were compared on a percentage basis, taking the values found in uninfected liver arbitrarily as 100%. (1), glucose-6-phosphatase; (2), phosphoglucomutase; (3), glucose-6-phosphate dehydrogenase; (4), phosphoglucoisomerase; (5), D-fructose-1,6-diphosphatase 1-phosphohydrolase; (6), phosphofructokinase; (7), lactic dehydrogenase. The relative activities of metabolic pathways are indicated by the thickness of the arrows. The X sign indicates absence of enzyme activity.
tions in glycolysis and oxidative processes of the tissues. However, increases in the activities of these enzymes may be partially due to nonspecific host response to bacterial infection(2).

The alterations in enzyme levels with acute bacterial illness in animals may be complicated to some extent by nutritional changes in animals caused by infectious disease(5,22,25). Our studies show that the effects of starvation were opposite to those of *S. typhimurium* infections since the activities of GK, PFK, and PK enzymes were decreased by 30–50% and the gluconeogenic enzymes were increased by 50% in tissues of rats fasted for 48 hours (unpublished data). However, some increments in LDH enzyme levels may be contributed by starvation of the animals during the infection since the activity of LDH was found to be increased in liver tissues from 48-hour fasted rats (unpublished data).

The investigations of enzymes of G-6-P metabolism in liver homogenates and isotope studies with liver slices showed that 69% glucose was utilized in infected tissues to form lactic acid and fatty acids as compared to fed or fasted controls in which 25–27% of glucose utilized was found in these products. This indicates about a 40% increase in glycolysis in the infected tissues. A significant rise in PHI levels in *S. typhimurium*-infected tissue supports the evidence of increased glycolysis in these tissues. A net decrease in glucose content of incubation medium containing infected tissues and an 80% decrease in G6Pase enzyme in tissue homogenates suggest a considerable decrease in gluconeogenesis. However, a larger portion of glucose taken up by the infected liver slices was utilized for biosynthesis of glycogen as evidenced by the increased glycogen contents of the tissues and the activities of PGM enzyme. The initial glycogen contents of infected tissues were similar to those of livers in 48-hour fasted animals as reported previously(25).

The growth of pneumococcal bacteria was rather rapid in lung and heart muscle of the experimental animals indicating specificity of the organism to these tissues. However, at the terminal stages of illness more than 10⁸ cells per gram were found in liver, spleen, heart, and lung tissues of animals. A considerable increase in the activities of PHI, ALD, GK, PFK, PK, and LDH enzymes indicated increased glycolysis in liver tissues of rats inoculated with *D. pneumoniae* organisms. Although PK enzyme in spleen and kidney was increased at early stages of infection, the increments in PK levels in liver tissues were relatively small at 72 hours postinoculation. The significance of these observations is not known at this time.

The activities of GPDH and PGM enzymes were inhibited in the tissues of rats infected with pneumococcal bacteria. These results suggest that the gluconeogenesis and pentose-phosphate shunt are considerably decreased in the infected tissues.

In animals inoculated with *P. aeruginosa*, chronic infection occurred as evidenced by the presence of low numbers of organisms at 168 hours postinoculation. The activities of GK, PFK, and PK enzymes in spleen, kidney, heart, and skeletal muscles increased with variable range in the infected tissues. The effects on
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Gluconeogenesis and glycolysis in liver tissues due to *P. aeruginosa* infection were less pronounced as indicated by the ratio of the activities of the key enzymes. The alterations in patterns of hepatic enzymes caused by parenchymal cell injury in chronic inflammatory disease were reported to be lower (26). However, a 7-fold increase in GPDH enzyme level and a 39% increase in 14C-glucose metabolism in infected liver tissues indicated marked stimulation of pentose-phosphate shunt. A marked increase in GPDH activity due to *P. aeruginosa* may be due to compensatory and regenerative processes by means of supplying NADPH and ribose-5-phosphate.

The results of these studies suggest that the patterns of key glycolytic enzymes follow synchronous behavior under the stress of bacterial infections. Although the mechanism of enzyme changes due to bacterial infection is not known at this time, it is clear that the enzyme changes are due to host response to bacterial activity. The influence of bacterial action on the controlled processes in the tissues may be mediated through endocrine changes during acute or chronic adaptation (6,27). Further studies are in progress which may elucidate the mechanisms of specific changes in host cell metabolism in relation to *in vivo* metabolic activity of infective agents.

SUMMARY

The effects of *S. typhimurium*, *P. aeruginosa*, and *D. pneumoniae* on the glycolytic enzyme patterns in rat tissues were examined by measurement of activities of several key enzymes in the metabolic pathways. The animals inoculated with *S. typhimurium* showed a 30–100% increase in glucokinase and phosphofructokinase of spleen, kidney, heart, and skeletal muscle; whereas *P. aeruginosa* infections caused less pronounced changes. Pyruvate kinases activities in these tissues were elevated by 40–170% at the early stages of infection. Liver homogenates from animals infected with *S. typhimurium* demonstrated a 100–150% increase in key glycolytic enzymes and a 20–80% decrease in key gluconeogenic enzymes; while the activities of lactic dehydrogenase, glyceraldehyde phosphate dehydrogenase, isocitric dehydrogenase, phosphoglucomutase, and phosphohexose isomerase were increased by 4- to 8-fold. In contrast, *P. aeruginosa*-infected liver had a 2- to 8-fold increase in glucose-6-phosphate dehydrogenase, lactic dehydrogenase, and glyceraldehyde phosphate dehydrogenase. *Diplococcus pneumoniae* infections caused a 10-fold elevation of liver lactic dehydrogenase but the activities of other dehydrogenases were diminished. The combination of isotope and enzymatic investigation of metabolic routes indicated that the metabolic flux in *S. typhimurium*- or *D. pneumoniae*-infected liver tissues was increased toward the direction of glycolysis. *Pseudomonas aeruginosa* caused a major shift toward the direction of the pentose-phosphate shunt. The results suggest that the glycolytic enzymes in rat tissues follow a synchronous behavior pattern which are adaptive in response to bacterial infections.
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