An Endogenous Mediator of Depression of Amino Acids and Trace Metals in Serum during Typhoid Fever

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Concentrations of most amino acids and of zinc in serum were decreased during periods of incubation and illness in 11 volunteers who developed symptoms after an oral dose of 10^7 virulent Salmonella typhosa. When a 1.0-ml sample of sterile serum from volunteers who were ill with typhoid fever was injected into normal rats, it stimulated a prompt and significant depression of the concentration of zinc in the rats' sera and a flux of amino acids into their livers. These observations support the hypothesis that an endogenous factor (similar to endogenous pyrogen released by polymorphonuclear leukocytes) was present in the blood during typhoid fever and served as a mediator for the observed depression in zinc and amino acids in serum. The magnitude and pattern of infection-related depression in individual amino acids in serum may be, in part, a function of the amount of endogenous mediator released and of the rates of utilization of amino acids by tissues.

The period of symptomatic febrile illness during a generalized infectious process is accompanied by prominent catabolic events that lead to a negative balance in nitrogen and other elements in the body [1]. However, many other infection-related biochemical changes in the host appear to develop long before the onset of fever and symptoms of illness and to be anabolic rather than catabolic in nature. Little is known about the role of these very early biochemical responses of the host or the underlying mechanisms that initiate them. Total concentrations of amino acids were significantly altered in whole blood of volunteers infected with Salmonella typhosa (S. typhi) [2]. A similar depression in the total free amino acids in whole blood or serum has been observed during prospective studies in volunteers infected with Pasteurella tularensis [3], live, attenuated (vaccine strain) Venezuelan equine encephalitis (VEE) virus [4], or sandfly-fever virus [5]. Serum zinc was also decreased during the period of incubation and clinical illness of several bacterial and viral infections, while serum copper values became elevated during the latter stages of the same illnesses [5, 6].

Recent studies in experimental animals suggested that many early metabolic changes in the infected host were closely interrelated and could be regarded as "nonspecific" host responses initiated by the action of circulating, endogenous mediators that act on cells of the liver or other tissues. The use of a ^14C-labeled, nonmetabolizable amino-acid analog, 1-aminocyclopentane-1-carboxylic acid (cycloleucine) in rats exposed to Diplocooccus pneumoniae provided evidence for a flux of amino acids from serum to liver in infected animals and a subsequent increase in the rates of synthesis and release of serum proteins from liver [7]. Similarly, a marked flux of amino acids...
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into liver has been observed in rats injected with
an endogenous mediating substance released by
stimulated polymorphonuclear (PMN) leukocytes [8]. A similar mediator obtained from PMN leukocytes has been shown to cause a depression in serum zinc and iron [9-11], a movement of these trace metals into liver [12], an increase in serum copper, and the hepatic secretion of ceruloplasmin [12] and acute-phase globulins [13]. The mediator of these biochemical changes seemed analogous to the endogenous mediator of fever, a protein released by PMN leukocytes that is thought to act on temperature-regulating centers within the hypothalamus [14].

As part of long-term investigations into the efficacy of typhoid vaccines [15, 16], the opportunity arose to study volunteers infected with typhoid fever to determine whether a humoral endogenous factor appeared in the serum of man that would mediate the changes mentioned above in amino acids and trace metals of serum.

In addition, the study made it possible to compare serial responses of individual amino acids in serum during typhoid fever in man with similar measurements made during sandfly fever [5]. Markey's depressions in total free amino acids in plasma have been observed in experimentally induced Salmonella typhimurium [17] and distemper-virus [18] infections in beagles. The pattern of change in individual amino acids, however, appeared to vary with the particular microorganism that was used. With S. typhimurium infection of dogs, the most marked and consistent decreases were observed for alanine, glycine, proline, lysine, and threonine [17]. This finding raised the possibility that patterns of sequential changes in individual amino acids in serum would differ during S. typhosa infection in man from those described recently during sandfly fever [5].

**Materials and Methods**

**Subjects.** Thirty-one healthy male subjects participated on a voluntary basis and served as the nonimmunized control group for a study of vaccine efficacy. Volunteers were inmates of the Maryland House of Correction who, before participating, were fully informed of the nature and details (including risks) of the study. None of the subjects had a history of prior exposure to typhoid fever.

**Exposure.** On day 0 each volunteer was exposed by drinking 45 ml of milk that contained $10^5$ viable S. typhosa (Quailes strain), prepared in the manner described previously [15, 16].

**Clinical observation and treatment.** For 30 days after exposure, all subjects were examined daily for clinical abnormalities, elevation of body temperature, and the presence of S. typhosa in the stool. Any volunteer with an oral temperature in excess of 100 F was admitted to the research ward. When the temperature was 103 F or higher for 24 hr, a course of oral chloramphenicol was begun (3 g day for seven days). No antibiotic was given for the next seven days, and then chloramphenicol therapy was continued for another five days. Each subject admitted to the ward had daily blood cultures for S. typhosa and periodic laboratory determinations, including white blood-cell counts (WBC), and tests for hemoglobin and O-, H-, and Vi- (envelope) agglutinating antibodies. Criteria for diagnosis of overt typhoid fever included an oral temperature of 103 F or higher for 24 hr, a positive blood or stool culture, and the development of O-, H-, or Vi-agglutinating antibodies. In an ancillary study to be reported elsewhere, each hospitalized volunteer was given an oral dose of 3 g of L-tryptophan on the fifth day of hospitalization.

**Collection of blood specimens.** Preprandial blood samples were obtained from all volunteers between 6 AM and 8 AM on days -3, -1, and 0 before exposure to S. typhosa and on days 1 and 3 after exposure. Samples of blood were obtained from all volunteers admitted to the ward at 6 AM on days 1, 5, and 6 after admission and on days 27 and 30 after exposure to S. typhosa. Aliquots of serum were stored frozen at $-16 \text{ C}$ to permit later determination of serum Zn, Cu, lactic-acid dehydrogenase (LDH), LDH isoenzymes, creatinine phosphokinase, and glucose. Sulfosalicylic acid (100 mg/ml of serum) was added to another 3-ml aliquot of serum, mixed, centrifuged, and the supernatant fluid was frozen at $-16 \text{ C}$ until used for amino-acid analysis.

**Assay of serum for endogenous mediator.** A pool of sera obtained before exposure from all hospitalized volunteers and one of sera obtained on the first day of illness were passed through a Millipore filter to remove any bacteria present. Half of the pooled sera from the first day of illness was heated to 90 C for 30 min. Healthy
male rats (150-175 g, Fisher-Dunning strain) were injected sc with 1 μCi 100 g body weight of 14C-cycloleucine labeled in the carboxyl-carbon atom (New England Nuclear, Boston, Mass.). Twenty-four hours later one group of six rats was injected ip with 1 ml of 0.9% NaCl, a second group with 1 ml of serum obtained before exposure, a third group with 1 ml of serum from the first day of illness, and a fourth with 1 ml of heat-treated serum from the first day of illness. All rats were killed 6 hr later and the concentrations of Zn and Fe in serum and of 14C-cycloleucine in liver were determined by previously described techniques [6, 8].

Analytical methods. The amino-acid content of the protein-free filtrates of serum were analyzed by an automated procedure on ion-exchange resin [5]. Serum Zn, Fe, and Cu were measured in an atomic-absorption spectrophotometer [6]. Serum 14C-rl [19], creatinine phosphokinase [20, 21], and glucose [22] were determined by automated procedures. Isoenzymes of LDH were separated in a slab of gel with gradients of 4.5%, 6%, 8%, and 12% (Ortec, Inc., Oak Ridge, Tenn.) at pH 9.0; 0.75 st tris-sulfate buffer was used, and the gel was stained for 45 min at 37 C.

Calculations. The computations of data on amino acids were done by computer [5]. Hours of fever were calculated as the product of degrees F greater than 99 F multiplied by duration in hours. A difference between two means was considered significant at a probability value, P < 0.01, as determined by t test.

Results

Clinical manifestations. Eleven of the 31 exposed volunteers (35%) were admitted to the research ward, had typical cases of typhoid fever, required therapy, and were included in the detaiiled studies described below. The median incubation period for the eleven subjects was nine days with the highest fever on days +10 and +11 (figure 1). The average total fever hours (figure 1) for the group was 265 ± 34 hr (mean ± SEM). Bacteremia was present in 73%: S. typhosa was detected in the stools of 82%; it was cultured as early as day 1 and as late as day 29 after exposure. Leukopenia developed in 91% of the volunteers; 91% developed significant titers of H antibody, 73% titers of O antibody, and 45% titers of Vi antibody to S. typhosa.

Biochemical changes. The amount of Zn in serum on day 3 was significantly depressed to 87% ± 5% of the mean concentration before exposure, with a maximum decrease to 77% ± 6% of baseline on day 13. Serum Zn did not return to control concentrations until day 30 after exposure to the disease (figure 1). In contrast, concentrations of Cu in serum were significantly elevated on day 9 before exposure, with maximal increments to 161% ± 13% of the baseline value on day 14. Levels of Cu in serum were still significantly above controls on day 30.

Mean concentrations of LDH in serum were significantly increased to 115% ± 4% of mean concentrations before exposure on day 1, increased to a maximum of 224% ± 12% of control on day 14, and were still significantly elevated on day 30 (figure 1). When the LDH activity was expressed in Wrobelwski units [19], the values increased from a preexposure control average of 178 ± 17 units to 377 ± 56 units on day 9. As illustrated in figure 2, the major increase in serum LDH activity appeared to be in bands 1, 2, and 3 of the isoenzyme pattern. No significant changes were observed in concentrations of creatinine phosphokinase or glucose (fasting) in serum.
before exposure. By day 3 after exposure, despite the fact that the total amount of amino acids in serum was not significantly reduced, the concentrations of alanine, proline, aspartate, and glutamate were significantly lower than their respective control values. A prolonged period of depression in the concentration of serum alanine, glycine, proline, threonine, glutamate, and glutamine occurred on days 9–14. While concentrations of some of these amino acids were still
on Zn and Fe in serum or on hepatic content of cycloleucine of recipient rats.

Discussion

In the present study, the percentage of exposed, nonimmunized volunteers who developed typical typhoid fever and the length of the median incubation period were in excellent agreement with previously reported observations [15, 16] with a similar infecting dose of 0.5 bacteria of the Quaiies strain of S. typhosa. Furthermore, the clinical findings, incidence of bacteremia and positive stool cultures, leukopenia, response to treatment, and increases in titer of antibody were all in agreement with previously reported observations [2, 15, 16]. The volunteers who did not develop typhoid fever were not studied for biochemical changes or for the presence of a mediator in their serum.

The data from these experiments demonstrate that the concentrations of most individual, free amino acids and Zn in serum were significantly reduced during the early incubation period of infection with S. typhosa and throughout the course of illness. In an earlier study of sandfly fever, it was postulated that the observed decrease in amino acids in plasma was related to an increased flow of amino acids into tissues such as the liver, with subsequent use of amino acids for an increased synthesis of new protein [51]. Studies performed in the rat supported this postulate, indicating that the induction of bacterial infection or the administration of a mediator substance produced by PMN leukocytes led to (1) an increased synthesis of new protein [51]. Studies performed in the rat supported this postulate, indicating that the induction of bacterial infection or the administration of a mediator substance produced by PMN leukocytes led to (1) a flux of amino acids into liver, (2) an acute depression of Zn and Fe in serum, and (3) an increased synthesis of acute-phase serum proteins by the liver [7, 8, 11]. Similar acute metabolic changes could be induced in normal rats by the injection of either sterile serum obtained from infected donor rats or of a mediator substance produced by rat, rabbit, or monkey leukocytes [8, 11, 25]. The mediating factors of serum and PMN leukocytes were found to be heat labile [8, 11]. The present study provided the first evidence in man that several infection-related metabolic changes not associated with the presence of fever were mediated by some circulating endogenous substance(s). This evidence is based on the fact that...
Table 1. Sequential changes in individual amino acids in sera of humans during infection with Salmonella typhosa.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Before exposure (mmole/liter)</th>
<th>Day after exposure (percentage of amount before exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Alanine</td>
<td>380.8 ± 17.7</td>
<td>73.7 ± 6.7</td>
</tr>
<tr>
<td>α-Amino-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>butyric acid</td>
<td>13.2 ± 2.4</td>
<td>86.0 ± 10.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>41.6 ± 9.8</td>
<td>109.8 ± 8.1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>83.4 ± 5.3</td>
<td>94.2 ± 4.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>30.0 ± 4.4</td>
<td>80.0 ± 8.6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>136.0 ± 7.5</td>
<td>76.6 ± 7.1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>481.9 ± 29.0</td>
<td>97.1 ± 15.9</td>
</tr>
<tr>
<td>Glycine</td>
<td>257.5 ± 11.6</td>
<td>86.6 ± 8.7</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>74.1 ± 3.9</td>
<td>92.7 ± 11.8</td>
</tr>
<tr>
<td>Histidine</td>
<td>74.0 ± 3.1</td>
<td>108.5 ± 10.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>61.1 ± 3.0</td>
<td>86.0 ± 6.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>121.6 ± 4.7</td>
<td>87.6 ± 6.9</td>
</tr>
<tr>
<td>Lysine</td>
<td>1110.0 ± 6.1</td>
<td>90.2 ± 6.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>21.4 ± 1.5</td>
<td>111.8 ± 9.4</td>
</tr>
<tr>
<td>Ornithine</td>
<td>54.5 ± 4.0</td>
<td>97.9 ± 5.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>482.2 ± 13.8</td>
<td>89.7 ± 7.8</td>
</tr>
<tr>
<td>Proline</td>
<td>282.5 ± 16.4</td>
<td>81.0 ± 9.9</td>
</tr>
<tr>
<td>Serine</td>
<td>153.8 ± 8.6</td>
<td>88.3 ± 7.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>161.3 ± 10.9</td>
<td>94.6 ± 9.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>45.4 ± 4.7</td>
<td>107.6 ± 8.6</td>
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<tr>
<td>Tyrosine</td>
<td>49.2 ± 2.5</td>
<td>93.5 ± 7.0</td>
</tr>
<tr>
<td>Valine</td>
<td>212.0 ± 11.0</td>
<td>85.5 ± 6.8</td>
</tr>
<tr>
<td>Total</td>
<td>2915.4 ± 108</td>
<td>84.4 ± 6.3</td>
</tr>
</tbody>
</table>

* Mean ± SEM of 11 unvaccinated volunteers.

† Italicized numbers are means with a significant difference (P < .01).
Mlediator of Serum Biochemical Changes

related biochemical changes in man, when evaluated along with evidence obtained in laboratory animals, suggested that a mediator substance might have been present early in the incubation period or during clinically inapparent infections. Changes in concentrations of Zn or amino acids in serum were detected before the appearance of symptoms in volunteers infected with P. tularensis [3], sandfly-fever virus [5], or attenuated VEE virus [4, 6], as well as in men who exhibited an asymptomatic VEE infection. If these early, infection-related biochemical changes were in fact related to the presence of a mediator, it might be possible to detect such a mediator during bacterial and viral infections of man, but before the appearance of fever.

Changes observed in serum LDH isoenzymes during the incubation period of typhoid fever in these volunteers provided additional support for a potential role of circulating leukocytes in a control mechanism responsible for initiating early, nonspecific metabolic responses of the host. Experimental data recently obtained by Hale and Woodward [26] indicated that LDH activity in the serum of rats was increased after the in-vivo phagocytosis of infected particles of latex; this increase in activity appeared to be due to those specific LDH isoenzymes generated by PMN leukocytes. In the volunteers exposed to S. typhosa, the activity of LDH in serum, mainly from isoenzymes 1, 2, and 3, was increased on day 1 after ingestion of infectious bacteria. Since human PMN leukocytes contain LDH primarily as isoenzyme fractions 1, 2, and 3 [27], it may be postulated that the increase in LDH activity in serum during the incubation period of typhoid fever is related to the phagocytic activity of leukocytes, an activity believed to initiate the release of endogenous mediators of trace-metal depression and of increased amino-acid flux [12]. Direct evidence concerning this possibility was not obtained in the present study. Leukocyte pyrogen can be released in vitro by human leukocytes or monocytes when they are stimulated by phagocytosis or exposed to endotoxin [28].

Most of the copper present in serum is bound tightly to ceruloplasmin, a protein synthesized within the liver [29]. Generalized infections in man and experimental animals are typically accompanied by an increase in concentrations of ceruloplasmin and copper [30]. The marked rise
in serum copper during typhoid fever could most likely be explained by an increased synthesis of ceruloplasmin. A similar increase in concentrations of ceruloplasmin and copper in serum has been observed in rats injected with the endogenous mediator derived from PMN leukocytes [12]. These indirect observations support the postulate that the increased flux of amino acids into liver and increased rates of synthesis of serum proteins observed during generalized bacterial infections of laboratory animals is a phenomenon that also occurs in man.

Experimental endotoxemia in man, sandfly fever or typhoid fever, was accompanied by elevated concentrations of growth hormone [31, 32, 33]. Such increases in growth hormone were generally found to be relatively small in magnitude and brief in duration. While growth hormone could account, in part, for certain metabolic changes in the human host, studies by Winnacker suggested that an endogenous mediator obtained from peritoneal leukocytes of monkeys would stimulate a release of growth hormone in normal recipient monkeys [32]. Furthermore, preliminary studies in experimental animals suggest that the leukocytic mediator will stimulate a depression of trace metals and flux of amino acids in hypophysectomized or adrenalectomized rats (Robert S. Pekark, unpublished observation).

The concentration of each amino acid in serum represents, at any given time, the algebraic product relating rates of efflux and influx into serum of that amino acid from various compartments of the body as well as from dietary gains or excretory losses. Altered amino-acid metabolism during infection must certainly be influenced by the catabolic changes which accompany a febrile illness. The losses of nitrogen in the body during a generalized infection represent relatively stereotyped host responses that are dependent primarily on factors such as the presence and magnitude of fever, the degree of anorexia, the nutritional status of the host, and the duration of illness; thus, the catabolic response during an infectious illness is relatively independent of specific etiology [1]. But alterations in metabolism of amino acids during an infection must, in addition, be influenced importantly by factors other than those which produce a negative nitrogen balance. Altered concentrations of many individual amino acids in serum were observed to begin well in advance of fever, anorexia, or symptoms when investigated in sandfly fever [5] and in the present study of typhoid fever. Further, a comparison of changes observed in individual amino acids revealed many differences between the responses during sandfly fever and those during typhoid fever. In volunteers exposed to sandfly-fever virus, the concentrations of all amino acids in serum were depressed during the incubation and illness phases of this infection; the branched-chain amino acids (leucine, isoleucine, and valine) were decreased to a greater degree and for a longer period of time than other amino acids [5]. In contrast, concentrations of alanine, glycine, proline, threonine, glutamate, and glutamine in serum were depressed during the incubation and illness phases in volunteers exposed to S. typhosa, and the magnitude and duration of depression was greater for alanine than for the other amino acids. The changes in amino acids in serum during typhoid fever in man are similar to those reported for amino acids in sera of dogs infected with S. typhimurium [17]. Bacterial infections of man and experimental animals appear to stimulate a far greater outpouring of acute-phase serum glycoproteins by the liver than do viral infections [34]. The rate of hepatic utilization of individual amino acids can be markedly affected by the activity and requirements of several biochemical processes, such as an increase in gluconeogenesis or ketogenesis, as well as synthesis of both cellular and serum proteins.

Recent observations during studies in experimental animals indicate that more endogenous mediator is released into the serum during an infection with S. typhimurium than one due to D. pneumoniae (Robert W. Winnemacher, Jr., unpublished observation). Based on these data it would appear that the infection-related pattern of change in a certain individual amino acid in serum may be a function of the effects of both the endogenous stimulation of amino-acid flux and the rate of utilization by tissues of that amino acid. These patterns of change can vary with the stage of the infection and the type of infecting organism. Thus, it is not surprising to find that the alterations in the pattern of amino acids in sera of volunteers infected with S. typhimurium is markedly different from that observed in volunteers exposed to sandfly-fever virus.

To evaluate the diagnostic value of these differences, it will be necessary to study a number of
different types of viral, bacterial, and rickettsial infections and to develop methods for quantitation of endogenous mediator(s) present in serum.

References


