

## 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 depressed during periods of incubation and illness in 11 volunteers who developed symptoms after an oral dose of  $10^5$  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 signifi-

cantly 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 depressed during the periods 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].

Received for publication January 3, 1972, and in revised form March 23, 1972.

We thank the Warden and his staff, and the men at the Maryland House of Correction, Jessup, Maryland, who volunteered as subjects for these experiments. The technical assistance of Miss K. A. Bostian and Mr. R. E. Dinterman is gratefully acknowledged.

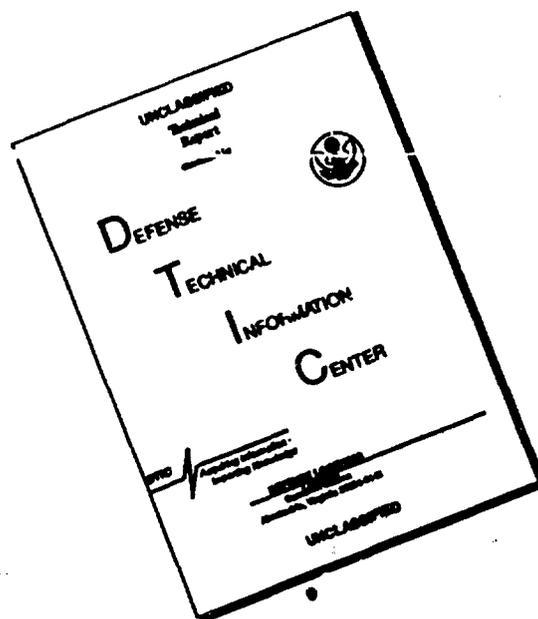
Experiments involving humans reported in this paper were governed by the principles, policies, and rules for medical volunteers established by U. S. Army Regulation no. 70-25 and by the Declaration of Helsinki.

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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  $^{14}\text{C}$ -labeled, nonmetabolizable amino-acid analog, 1-amino-cyclopentane-1-carboxylic acid (cycloleucine) in rats exposed to *Diplococcus 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]. Marked 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 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 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  $\mu$ Ci/100 g body weight of  $^{14}$ C-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  $^{14}$ C-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  $^{14}$ H [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 M 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 detailed 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  $265 \pm 34$  hr (mean  $\pm$  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  $87\% \pm 5\%$  of the mean concentration before exposure, with a maximal decrease to  $77\% \pm 6\%$  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 rose significantly to  $122\% \pm 12\%$  of mean concentrations on day 9 before exposure, with maximal increments to  $161\% \pm 13\%$  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  $115\% \pm 4\%$  of mean concentrations before exposure on day 1, increased to a maximum of  $224\% \pm 12\%$  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  $178 \pm 17$  units to  $377 \pm 56$  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.

The sequential changes in total amino acids in serum during typical typhoid fever are presented in figure 1 and table 1. On day 1 after exposure to *S. typhosa*, the total concentration of amino acids in serum was significantly depressed to  $84\% \pm 6\%$  of its average value before exposure; by day 3 it had returned to baseline concentrations. However, by day 9 the total concentration of amino acids in serum was again significantly decreased to  $85\% \pm 4\%$  of values before exposure, remained significantly depressed through day 14, and had not returned completely to control concentration by day 30.

The concentrations of individual amino acids in serum are presented in table 1. The concentrations before exposure are in good agreement with normal values reported by others [5, 23, 24]. On day 1 after exposure, the concentrations of alanine, glycine, proline, aspartic and glutamic acids, isoleucine, leucine, and valine were all significantly lower than their respective mean values

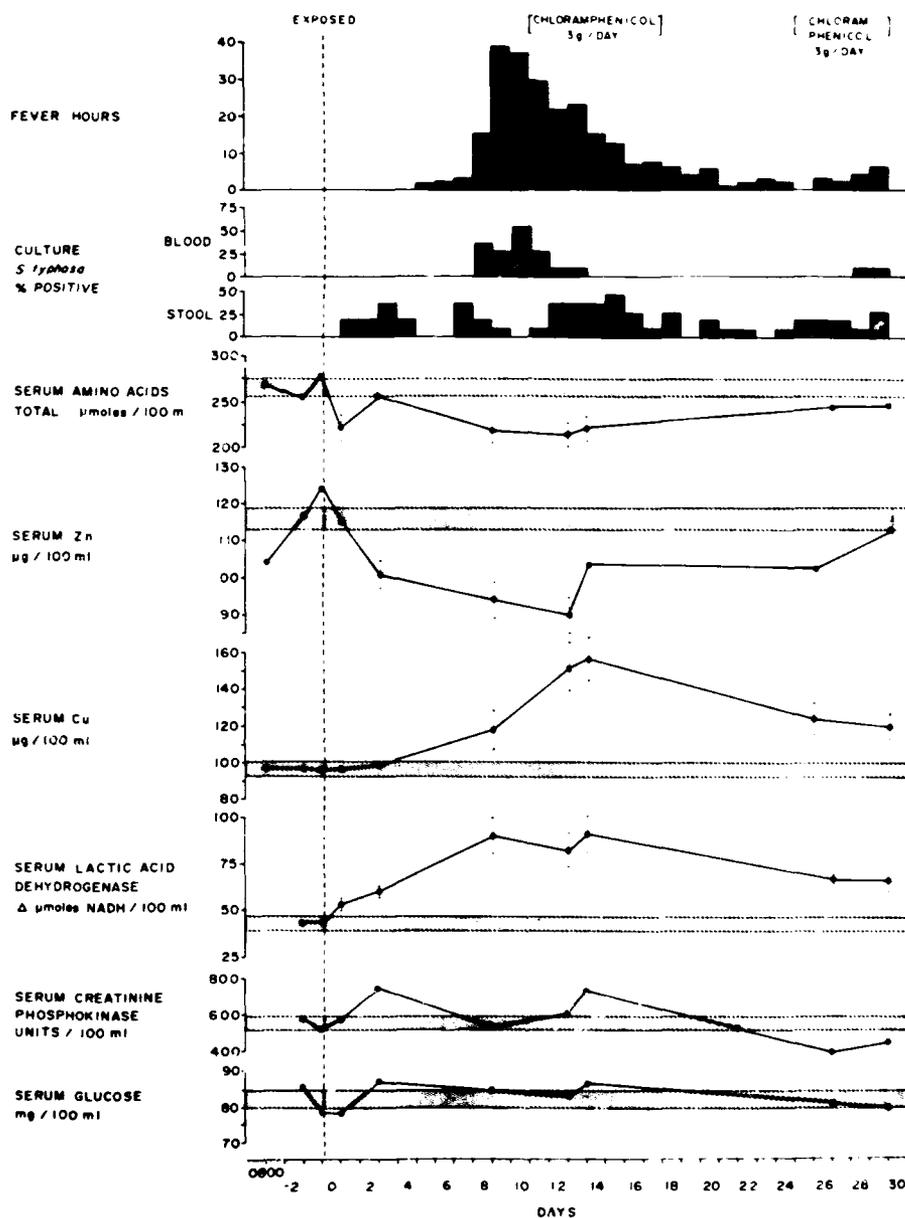
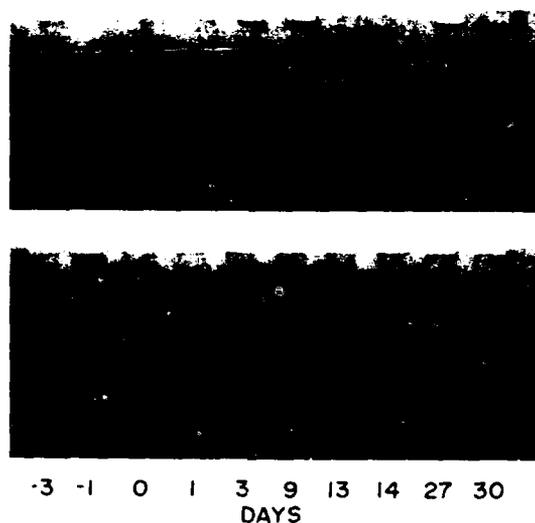


Figure 1. Sequential changes during typhoid fever in 11 men. Points with vertical lines indicate mean  $\pm$  SEM of values significantly different ( $P < .01$ ) from the mean data obtained before infection. The shaded area is the mean  $\pm$  SEM of the values before exposure.

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 re-

spective 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



**Figure 2.** Sequential changes of isoenzymes of lactic dehydrogenase (LDH) in serum in two typical cases of typhoid fever. A 0.05-ml sample of serum was used for each determination. The band on the bottom of the gel is LDH<sub>1</sub> and the next two are LDH<sub>2</sub> and LDH<sub>3</sub>. LDH<sub>1</sub> and LDH<sub>3</sub> are too faint to be seen.

below their control values at days 27 and 30 after exposure, the differences from baseline were not significant. The amount of alanine in serum was maximally depressed to 64% - 4% of its average value before exposure and remained depressed for a longer period than any other amino acid studied. In contrast to other amino acids, the concentrations of both phenylalanine and tryptophan in serum were significantly elevated on days 13 and 14 after exposure to *S. typhosa*, while the concentration of phenylalanine was increased on day 9. The ratio of phenylalanine to tyrosine was significantly elevated on day 3, increased to a maximum of 174% - 17% of its average control value on day 9, and was still significantly elevated on day 30.

When pooled sera obtained from volunteers on the first day of illness from typhoid fever was injected into rats, it rapidly stimulated a significant reduction in concentrations of Zn and Fe in the sera of the rats and a significant accumulation of cycloleucine in their livers (figure 3). Heat-treated serum from the same pool or serum obtained before exposure had no significant effect

on Zn and Fe in serum or on hepatic content of cycloleucine of recipient rats.

### Discussion

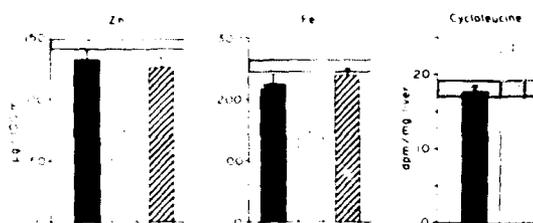
In the present study, the percentage of exposed, nonimmunized volunteers who developed typical typhoid fever and the length of the median incubation period were in excellent agreement with previously reported observations [15, 16] with a similar infecting dose of  $10^8$  bacteria of the Quail's strain 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 [5]. 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*.

Amino acid	Before exposure ( $\mu$ mole/liter)	Day after exposure (percentage of amount before exposure)						
		1	3	9	13	14	27	30
Alanine	380.8 $\pm$ 17.7*	73.7 $\pm$ 6.7*	83.5 $\pm$ 5.1	64.1 $\pm$ 4.4	69.8 $\pm$ 4.4	72.7 $\pm$ 6.2	88.4 $\pm$ 10.3	83.5 $\pm$ 6.7
$\alpha$ -Amino- butyric acid	13.2 $\pm$ 2.4	86.0 $\pm$ 10.9	101.7 $\pm$ 13.8	121.5 $\pm$ 15.2	127.9 $\pm$ 20.7	112.3 $\pm$ 16.7	83.4 $\pm$ 10.7	110.5 $\pm$ 23.0
Arginine	41.6 $\pm$ 9.8	104.8 $\pm$ 8.1	109.8 $\pm$ 7.3	100.8 $\pm$ 11.4	93.6 $\pm$ 7.4	95.4 $\pm$ 7.1	105.6 $\pm$ 10.0	93.9 $\pm$ 6.3
Asparagine	83.4 $\pm$ 5.3	94.2 $\pm$ 4.1	101.3 $\pm$ 4.5	89.1 $\pm$ 5.8	92.4 $\pm$ 6.7	88.6 $\pm$ 7.8	102.1 $\pm$ 8.7	95.3 $\pm$ 6.3
Aspartic acid	50.0 $\pm$ 4.4	80.0 $\pm$ 8.6	61.5 $\pm$ 9.5	90.8 $\pm$ 8.0	94.5 $\pm$ 14.7	88.2 $\pm$ 11.3	104.1 $\pm$ 8.7	103.0 $\pm$ 13.5
Glutamic acid	136.0 $\pm$ 7.5	76.6 $\pm$ 7.1	88.3 $\pm$ 5.3	86.1 $\pm$ 4.8	80.8 $\pm$ 8.0	82.1 $\pm$ 9.0	95.7 $\pm$ 7.0	90.0 $\pm$ 6.5
Glutamine	481.9 $\pm$ 29.0	97.1 $\pm$ 15.9	105.2 $\pm$ 11.0	87.9 $\pm$ 6.1	80.6 $\pm$ 8.4	81.9 $\pm$ 7.8	100.0 $\pm$ 18.2	86.8 $\pm$ 13.2
Glycine	257.5 $\pm$ 11.6	86.6 $\pm$ 8.7	95.9 $\pm$ 3.9	84.5 $\pm$ 7.7	81.9 $\pm$ 7.0	83.4 $\pm$ 7.7	90.9 $\pm$ 10.1	88.0 $\pm$ 8.4
Half-cystine	74.1 $\pm$ 3.9	92.7 $\pm$ 11.8	103.0 $\pm$ 5.3	94.8 $\pm$ 5.2	92.4 $\pm$ 6.5	90.4 $\pm$ 7.1	112.0 $\pm$ 13.5	105.8 $\pm$ 7.0
Histidine	74.0 $\pm$ 3.1	108.5 $\pm$ 10.7	125.4 $\pm$ 18.6	102.9 $\pm$ 19.1	92.9 $\pm$ 21.9	90.5 $\pm$ 11.4	86.1 $\pm$ 10.3	91.8 $\pm$ 10.1
Isoleucine	61.1 $\pm$ 3.0	86.0 $\pm$ 6.8	102.3 $\pm$ 5.9	101.7 $\pm$ 6.6	90.5 $\pm$ 10.5	90.2 $\pm$ 10.0	96.2 $\pm$ 12.5	101.5 $\pm$ 11.2
Leucine	121.6 $\pm$ 4.7	87.6 $\pm$ 6.9	103.3 $\pm$ 5.0	103.6 $\pm$ 6.0	100.4 $\pm$ 10.0	102.3 $\pm$ 9.7	98.3 $\pm$ 11.0	104.6 $\pm$ 8.2
Lysine	111.0 $\pm$ 6.1	90.2 $\pm$ 6.5	117.9 $\pm$ 12.0	93.4 $\pm$ 9.0	84.6 $\pm$ 6.2	109.3 $\pm$ 14.0	80.0 $\pm$ 15.4	96.3 $\pm$ 8.7
Methionine	21.4 $\pm$ 1.5	111.8 $\pm$ 9.4	102.8 $\pm$ 6.2	85.2 $\pm$ 9.2	88.0 $\pm$ 10.9	94.7 $\pm$ 11.7	96.3 $\pm$ 17.8	98.8 $\pm$ 14.5
Ornithine	54.5 $\pm$ 4.0	97.9 $\pm$ 5.1	109.3 $\pm$ 8.5	109.3 $\pm$ 10.9	85.8 $\pm$ 6.9	92.7 $\pm$ 10.5	100.9 $\pm$ 12.3	104.4 $\pm$ 16.1
Phenylalanine	48.2 $\pm$ 1.8	89.7 $\pm$ 7.8	105.8 $\pm$ 4.5	140.4 $\pm$ 10.7	145.0 $\pm$ 24.1	126.3 $\pm$ 12.9	103.4 $\pm$ 12.2	126.4 $\pm$ 22.4
Proline	282.5 $\pm$ 16.4	81.0 $\pm$ 9.9	85.8 $\pm$ 5.4	72.6 $\pm$ 5.3	74.5 $\pm$ 7.8	72.8 $\pm$ 6.3	84.4 $\pm$ 10.6	94.4 $\pm$ 7.5
Serine	153.8 $\pm$ 8.6	88.3 $\pm$ 7.1	98.3 $\pm$ 6.6	100.9 $\pm$ 8.6	97.9 $\pm$ 12.7	98.8 $\pm$ 7.2	103.3 $\pm$ 7.4	106.1 $\pm$ 6.5
Threonine	161.3 $\pm$ 10.9	94.6 $\pm$ 9.2	133.4 $\pm$ 35.0	77.5 $\pm$ 9.8	81.9 $\pm$ 8.9	86.6 $\pm$ 11.2	99.2 $\pm$ 8.6	96.7 $\pm$ 9.5
Tryptophan	45.4 $\pm$ 4.7	107.6 $\pm$ 8.6	109.2 $\pm$ 7.7	93.6 $\pm$ 5.3	116.3 $\pm$ 9.0	186.2 $\pm$ 20.8	146.6 $\pm$ 40.0	97.4 $\pm$ 5.5
Tyrosine	49.2 $\pm$ 2.5	93.5 $\pm$ 7.0	91.7 $\pm$ 5.0	82.3 $\pm$ 6.6	94.1 $\pm$ 9.6	93.8 $\pm$ 9.0	91.0 $\pm$ 9.4	98.6 $\pm$ 7.2
Valine	212.0 $\pm$ 11.0	85.5 $\pm$ 6.8	100.1 $\pm$ 5.1	95.0 $\pm$ 5.4	95.0 $\pm$ 7.6	94.1 $\pm$ 7.2	101.0 $\pm$ 10.5	103.6 $\pm$ 7.6
Total	2,915 $\pm$ 108	84.4 $\pm$ 6.3	96.8 $\pm$ 3.6	84.6 $\pm$ 4.2	83.5 $\pm$ 5.6	86.1 $\pm$ 5.0	95.1 $\pm$ 7.1	93.1 $\pm$ 4.7

\* Mean  $\pm$  SEM of 11 unvaccinated volunteers.† Italicized numbers are means with a significant difference ( $P < .01$ ).



**Figure 3.** Effect of injection of 1 ml of 0.9% NaCl (□), sera taken before infection (■), unheated sera from the first day of illness (▨), or heated (90 C, 30 min) sera from the first day of illness (⊞), from volunteers exposed to *Salmonella typhosa* on Zn and Fe in serum and distribution of hepatic  $^{14}\text{C}$ -cycloleucine of recipient rats. Hepatic  $^{14}\text{C}$ -cycloleucine content of liver from rats injected with heated sera from first day of illness was not significantly changed from values found in controls that received 0.9% NaCl. Each value is the mean  $\pm$  SEM of six rats. Saline controls are expressed as mean  $\pm$  SEM.

serum obtained from volunteers at the onset of symptomatic typhoid fever contained mediator substance(s), which, when injected into rats, would depress the concentrations of Zn and Fe in serum and stimulate an accumulation of cycloleucine (a nonmetabolizable amino-acid analog) in the liver of recipient normal rats. The endogenous mediator present in the sera of volunteers who were ill with typhoid fever was heat labile; in this respect it appeared to be similar to the endogenous mediator of fever that is released from phagocytizing human leukocytes or macrophages [14].

The present study was designed, in part, to determine whether a humoral mediator was present in the sera of volunteers who were ill with typhoid fever. We reasoned that the mediator, if present, would attain its highest concentration during the early phase of fever. Since the mediator was present in serum obtained before administration of chloramphenicol, antibiotic therapy could not be responsible for the appearance of the mediator in human serum or for the metabolic effects of the mediator in normal rats. Because of the design of the present study, no data were obtained for the time interval between inoculation with *S. typhosa* and appearance of the mediator in serum, nor was it determined whether mediator circulated during the inapparent infection in nonsymptomatic volunteers who developed an antibody response to the infecting organism. However, earlier studies of infection-

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 infused 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. Leukocytic 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 Winnaeker 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. Pekarek, 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. Wannemacher, 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. typhosa* 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.

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