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Serum Zinc, Iron, and Copper Concentrations during Typhoid Fever in Man: Effect of Chloramphenicol Therapy

(10) Robert S. Pekarek,[1] R. M. Kluge,[2] H. L. DuPont,[3] R. W. Wannemacher, Jr. R. B. Hornick, K. A. Bostian, and W. R. Beisel

In volunteers experimentally infected with *Salmonella typhi*, serum iron and zinc concentrations became significantly depressed and there was a concomitant rise in serum copper before the onset of overt clinical illness. However, after several days of fever and the initiation of chloramphenicol therapy, serum iron and zinc concentrations significantly increased. Additional studies—in volunteers with typhoid fever treated with chloramphenicol, in a volunteer with typhoid fever receiving cefazolin and gentamicin, and in untreated rhesus monkeys infected with *Salmonella typhimurium*—provided evidence that the increase in serum iron concentration during the febrile phase was the result of chloramphenicol therapy, whereas the increase in serum zinc concentrations was a disease-related phenomenon. The importance of trace-metal monitoring during infectious disease and chemotherapy is discussed.

Additional Keyphrase: *trace elements and infectious disease*

U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. 21701; and The Division of Infectious Diseases, University of Maryland School of Medicine, Baltimore, Md. 21201.

These investigations were conducted in conjunction with a continuing study of the pathogenesis, diagnosis, prophylaxis, and therapy of infectious disease. These tests were governed by the principles and rules for medical volunteers established by the Declaration of Helsinki. The Human Experimentation Committee at the University of Maryland has approved this study and both oral and written consent were obtained from each volunteer. The investigations were also supervised by the Commission on Epidemiological Survey of the Armed Forces Epidemiological Board. In conducting the research described in this report, the investigators adhered to the "Guide for the Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

[1] Present address: USLA, ARS, Human Nutrition Laboratory, 2420 2nd Ave. N., P. O. Box D, University Station, Grand Forks, N. D. 58201.

[2] Present address: Division of Infectious Diseases, Department of Medicine, University of Florida College of Medicine, Gainesville, Fla. 32610.

[3] Present address: Division of Infectious Diseases, University of Texas, Medical School, Houston, Tex. 70025.

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Significant alterations in host zinc, iron, and copper metabolism occur during various acute and chronic infectious diseases (*1–3*). Prospective studies in man demonstrated that such alterations begin early in the incubation period after exposure to either bacterial or viral organisms (*4, 5*). As shown in laboratory animals, these infection-induced changes represent a rapid redistribution of the metals within the various tissues of the host (*6, 7*).

Beside infection-induced alterations in trace-metal metabolism, various drugs also can produce significant changes. For example, administration of chloramphenicol produces increased serum iron concentrations and an increase in the saturation of transferrin, even in the absence of such recognized but infrequent complications as aplastic anemia or other forms of hematopoietic toxicity (*8, 9*). Because the serum trace-metal profiles induced by various infectious diseases, in conjunction with other clinical data, may have potential diagnostic and prognostic usefulness, it is important to establish and differentiate the alterations produced by a particular infectious illness from those that may be induced by chemotherapy.

Therefore, as an ancillary and incidental part of a continuing series of investigations into the efficacy of new experimental typhoid vaccines (*10, 11*), the opportunity arose to study the sequential effects of typhoid fever on zinc, iron, and copper concentrations in the serum of experimentally infected volunteers. In addition, these investigations also afforded the opportunity to study the effect of chloramphenicol treatment on these trace-metals in serum, to see if particular patterns of concentration could provide useful criteria for screening potential toxic effects of chemotherapy during an infectious illness. For further comparison, we sequentially determined serum trace-metal concentrations in untreated rhesus monkeys infected with *Salmonella typhimurium*.

Materials and Methods

Study 1. Nineteen healthy men, inmates of the

Maryland House of Correction, Jessup, Md., participated in this study on a volunteer basis. Before they participated, the subjects were fully informed as to the purposes, details, risks, and discomforts of the study; each man was advised that he could withdraw from the study at any time; and written and oral consents were obtained from each volunteer. The protocol was reviewed and approved by the Human Experimentation Committee of the University of Maryland Medical School, and the study was conducted in accord with the Declaration of Helsinki. None of the volunteers had a history of prior immunization or exposure to typhoid fever.

After a three day pre-exposure control period, each volunteer ingested 10^5 viable *Salmonella typhi* (Quailes strain) in 45 ml of milk as previously described (*10, 11*). Each subject was examined daily for increase of body temperature, the presence of *S. typhi* in the stool, and any other clinical signs of illness for a period of 30 days after the exposure. Individuals developing an oral temperature in excess of 100 °F were promptly admitted to the research ward. The criteria for diagnosis of overt typhoid fever and the initiation of antibiotic therapy have been described (*10, 11*). Chloramphenicol (3 g/day) was administered orally for seven days. Chloramphenicol therapy was stopped for the next seven days and then resumed for an additional five days. One individual received cefazolin (1 g intramuscularly every 6 h) and gentamicin (80 mg intramuscularly every 8 h) for 24 h. The two drugs were stopped for the next 24 h, then resumed for an additional five days. Thereafter the individual was started on the above chloramphenicol regimen.

After overnight fasting, venous-blood samples were collected daily between 6 and 7 a.m. from all subjects during the pre-exposure period and for 14 days after exposure. In those volunteers who became clinically ill and were hospitalized, blood samples were obtained for an additional seven days.

Serum zinc, iron, and copper concentrations were determined by atomic absorption spectroscopy (*5*). Serum transferrin and α_2-macroglobulin concentrations were determined by an automated immunoassay, by use of a nephelometric technique as described by Ritchie et al. (*12*).

Study 2. As an incidental part of another ongoing study on vaccine efficacy, 25 healthy men, having no history of prior exposure to typhoid fever, participated on a voluntary basis and served as the nonimmunized control group. Again, the volunteers were inmates of the Maryland House of Correction, and informed consent was obtained from each subject.

Individual exposure to *S. typhi* (Quailes strain), daily examinations, criteria for diagnosis of typhoid fever, and chloramphenicol treatment were the same as described above. In those individuals who developed overt illness, blood samples were obtained on the day of onset and admission to the research ward and on days 3, 5, 8, 12, 15, 16, 20, and 24 after admission, for serum iron determinations. Serum iron concentrations were determined by an automated colorimetric technique (*13, 14*).

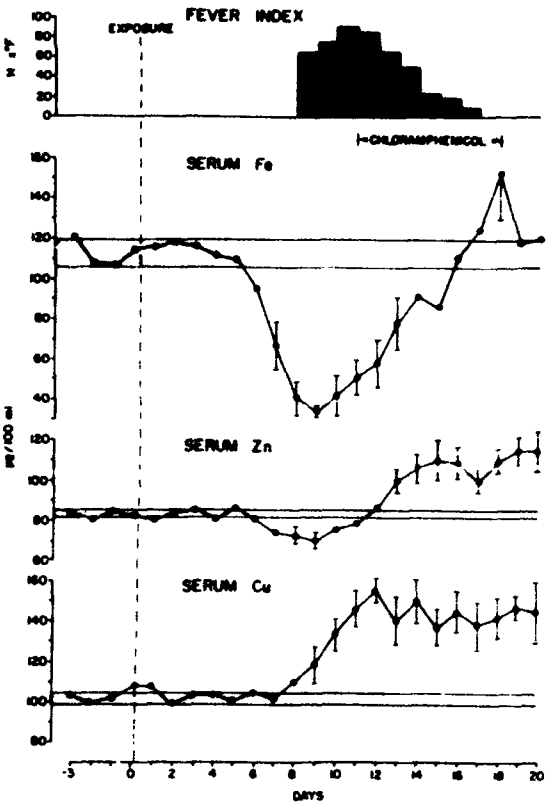

Fig. 1. Sequential changes in serum iron, zinc, and copper concentrations during typhoid fever in eight men treated with chloramphenicol

Shaded horizontal bands represent pre-exposure mean ±SE. Values significantly ($P < 0.05$) different from the pre-exposure mean are represented by brackets indicating ±SE

Monkey study. Two adult female rhesus monkeys were inoculated intravenously with 1×10^{10} viable *S. typhimurium* (MIT strain) suspended in saline. Core body temperatures were recorded hourly by a Honeywell Model 15 recorder via a copper-Constantan thermocouple surgically implanted in the para-spinal lumbar musculature so that the sensing tip rested just beneath the posterior abdominal peritoneum.

Daily before and after exposure, venous blood samples were obtained at 8:00 a.m., before feeding. Serum zinc, iron, and copper concentrations were determined by atomic absorption spectrophotometry as previously described (*5*). Infection was confirmed by positive blood cultures and the development of agglutinating antibodies.

Results

Nine of the 19 exposed volunteers in the first study were admitted to the research ward with typical typhoid fever. These individuals all required chemotherapy and were included in the study.

Figure 1 illustrates the sequential serum iron, zinc,

Table 1. Effect of Typhoid Fever in Eight Volunteers on Serum Transferrin and α_2-Macroglobulin Concentrations

Days	Daily postexposure mean concns	
	Transferrin	α_2-Macroglobulin
	mg/100 ml[a]	
Control	283 ± 7	339 ± 13
Postexposure		
0	275 ± 11	324 ± 24
1	298 ± 20	348 ± 31
2	301 ± 18	349 + 27
3	294 ± 17	347 ± 22
4	302 ± 16	351 ± 25
5	293 ± 16	329 ± 25
6	289 ± 19	339 ± 25
7	273 ± 22	332 ± 28
8	267 ± 16	333 ± 22
Onset of illness		
9	253 ± 9[b]	330 ± 22
10	256 ± 16	333 ± 24
11	252 ± 14[b]	342 ± 28
12	243 ± 17[b]	303 ± 24
13	246 ± 15[b]	316 ± 30
14	246 ± 16[b]	302 ± 55
15	242 ± 18[b]	297 ± 34
16	258 ± 22	339 ± 48
17	255 ± 24	315 ± 78
18	269 ± 28	362 ± 57
19	245 ± 13[b]	308 ± 36
20	233 ± 14[b]	292 ± 40

[a] Mean ± SE.
[b] Values significantly different from control ($P < 0.05$).

and copper concentrations in eight of the subjects who, with the development of clinical illness, received chloramphenicol. Both serum iron and zinc concentrations were significantly decreased from baseline just before overt febrile illness began, reaching maximal depressions on day 9. With the onset of fever, serum copper concentrations significantly increased and remained elevated throughout the sampling period. By day 10, both serum iron and zinc concentrations began to increase slowly, just before chloramphenicol therapy was started. By day 13 (second day of therapy), serum zinc concentrations had increased significantly above baseline. Similarly, serum iron concentrations became significantly increased by day 18 (seventh day of therapy).

As shown in Table 1, serum transferrin concentrations became significantly depressed with the onset of illness and remained below baseline throughout the study. By contrast, no significant changes were observed in concentrations of α_2-macroglobulin.

Because chloramphenicol causes increases in serum iron concentrations (*8, 9*), serial samples were obtained from nine of 16 volunteers who developed typhoid fever in Study 2, to see if a relationship existed between therapy and serum iron concentrations.

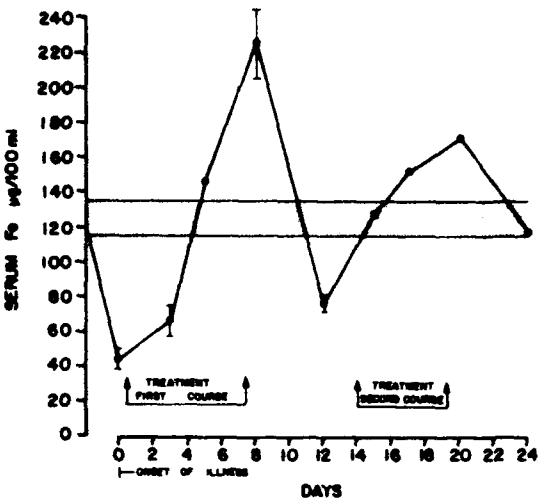

Fig. 2. Effect of chloramphenicol treatment on serum iron conconcentrations during typhoid fever in man

Values significantly ($P < 0.05$) different from the pre-exposure mean (*shaded band*) are represented by brackets indicating ±SE

As shown in Figure 2, serum iron concentrations were first depressed early in the illness. However, with the administration of chloramphenicol for seven consecutive days, serum iron concentrations began to increase, becoming significantly elevated after the seventh day of therapy. With cessation of treatment, the values decreased abruptly, a pattern identical to that observed in Study 1. When the second course of chloramphenicol was started, serum iron values again began to increase and returned to baseline when the treatment ended.

By contrast, the one volunteer in Study 1 who was initially started on cefazolin and gentamicin therapy did not show any such increase and decrease in serum iron concentrations during the sampling period (Figure 3). However, like the rest of the individuals who developed clinical illness, his serum zinc concentration did increase after the initial depression.

In the two untreated rhesus monkeys infected with *S. typhimurium*, serum iron and zinc concentrations decreased abruptly and significantly with the initiation of this infection (Figure 4). Although serum iron concentrations reached baseline values on days 8 and 9, no significant increases in serum iron concentrations were observed in either monkey. However, like the volunteers with typhoid fever in Study 1, both monkeys had significant increases in serum zinc concentrations, beginning on day 11, which closely corresponded in timing to that seen in the volunteers (Study 1, Figure 1). Serum copper concentrations rose to extremely high values during the course of this infection (Figure 4).

Discussion

The data from these prospective clinical studies demonstrate that typhoid fever in man produces significant depressions of serum iron and zinc concen-

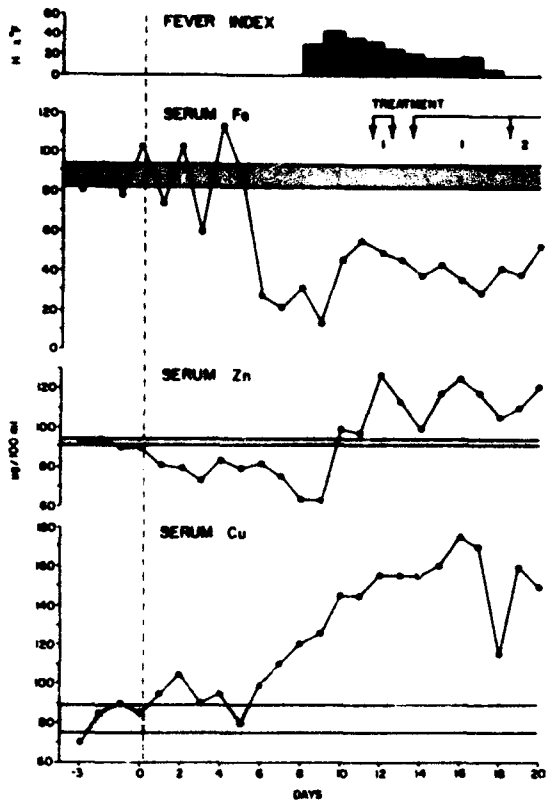

Fig. 3. Sequential changes in serum iron, zinc, and copper concentrations during typhoid fever in one subject initially treated with a split course of cefazolin and gentamicin (treatment No. 1) followed by chloramphenicol (treatment No. 2)

Shaded horizontal bands represent the pre-exposure means ±SE for this subject

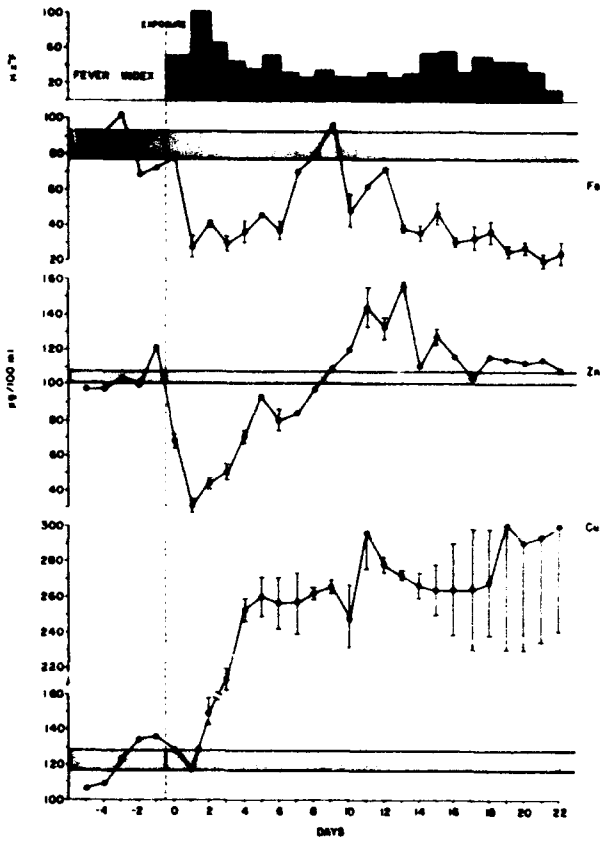

Fig. 4. Sequential effect of *Salmonella typhimurium* infection on serum iron, zinc, and copper concentrations in rhesus monkeys

Values significantly ($P < 0.05$) different from the pre-exposure mean (*shaded bands*) are represented by brackets indicating ±SE

trations just before and with the onset of febrile illness, accompanied by a concomitant rise in serum copper concentrations (Figure 1). Serum transferrin concentrations decreased significantly with the onset of febrile illness and remained depressed (Table 1). These initial alterations in host trace-metal metabolism are typically seen during an inflammatory or infectious process and have been previously documented in other prospective studies in volunteers with experimentally induced bacterial or viral infections (*4*, *5*, *7*).

However, as the disease course progressed and chloramphenicol treatment was begun, notable differences in the trace-metal values became evident. While serum copper concentrations remained significantly increased, as they normally do during infection or inflammation, serum iron and zinc concentrations also increased significantly. Because chloramphenicol has been shown to induce increases in serum iron concentrations (*8*, *9*), the increase in serum iron concentrations observed in the present study (Study 1) was also suspected to be drug-related. The changes in serum iron observed in Study 2 tend to support this, since the elevations closely corresponded in timing and duration with the chloramphenicol regimen. However, the most direct evidence was in the absence of serum iron increases in the volunteer (Figure 3) who was initially treated with cefazolin and gentamicin and in the monkeys infected with *S. typhimurium*.

Although the observed increase in serum iron concentrations appears to be drug-induced, the significant increase in serum zinc concentrations appears to be a disease-related phenomenon. This increase in serum zinc was observed in all the volunteers several days after the development of classified typhoid fever (Figures 1 and 3) and after a week of untreated illness in monkeys infected with *S. typhimurium* (Figure 4). Wannemacher et al. (*15*), reported that serum zinc concentrations decreased before the onset of clinical illness in volunteers with typhoid fever and remain depressed throughout the study. However, samples were not obtained each day, and there was a lapse in sampling between days 13 and 25 postexposure. Thus a transient increase in serum zinc concentration during this period would have been missed.

The reason for the delayed increase in serum zinc concentrations during systemic infections with *Salmonella* has yet to be determined. Although the α_2-macroglobulin protein fraction normally accounts for between 30–40% of the zinc bound in serum (*16*), we

saw no significant changes in the concentration of the α_2-macroglobulin during typhoid fever (Table 1). Further studies would be in order to determine how the increased amounts of zinc might be bound to—or associated with—the α_2-macroglobulin, albumin, or both in serum.

Because alterations in serum trace-metal concentrations may play an important role in the diagnosis of disease and disease etiology, it is important to define the profiles produced or complicated by the administration of various drugs. Monitoring of trace metals may not only be useful in determining the course of a particular disease, but also may serve as a sensitive screening procedure to aid in averting the various ramifications of drug-induced toxicity.

In this regard, it has been shown that marked increases in serum iron concentrations generally precede erythropoietic depression or toxicity (*8, 9*). In the present study, where chloramphenicol was administered in two short-term courses, the effects of the treatment on the hematopoietic system were extremely minimal. Serum iron concentrations became significantly elevated only on the last day (seventh day) of the first course, returning quickly to normal or below normal with cessation of the drug. The chloramphenicol regimen described herein appears optimal, resulting in complete recovery from disease in all volunteers and no toxic after-effects.

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