

① 16 Jul 79 (12) 6

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Redistribution of Zinc within Burned and Burned Infected Rats (40765)

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Burns covering 30% or more of the total body surface place a severe metabolic demand on the host, a demand that often exceeds even that imposed by some infections (1). Though burns elicit alterations in amino acid, trace metal, and protein metabolism akin to those observed during infection, injury, and inflammation (1-3), there is a recent report by Oh *et al.* which indicates that burns might differ from other inflammatory stresses in that zinc does not accumulate in the liver despite a significant decrease in serum zinc (4). The absence of zinc sequestration by the liver in burned animals, might be the result of leakage from the wound or a differential deposition within the wound itself (5). The latter possibility is intriguing in that, during myocardial infarction, zinc appears to accumulate preferentially at the site of the damage in organelles associated with biosynthesis, (7) and there is evidence that added zinc aids in wound healing in zinc deficient animals (8).

Another reason to reinvestigate whether hepatic zinc accumulation occurs in burned animals is that hepatic zinc accumulation has always preceded or accompanied

acute-phase globulin synthesis during inflammation and infection, even in zinc deficient animals (9-11). Since burned rats are known to exhibit increased synthesis of acute-phase globulins (2), the absence of enhanced zinc uptake by the liver would indicate that this apparent association between zinc influx to the liver and increased acute-phase globulin synthesis is, indeed, merely coincidental and neither essential nor causal.

Infection is often a complication of a burn injury and infection per se is accompanied by both hypozincemia and enhanced hepatic uptake of zinc. We therefore also studied burned-infected rats. Reduced food intake is common in infections, but not always a component of burn injury, thus we examined the effect of fasting as well as *ad libitum* food intake on zinc distribution in burned and burned-infected rats.

**Materials and methods.** Male albino rats (180-200 g) were used in all studies (Holtzman Co., Madison, Wis.). In the first study there were three groups of rats: control, burned, and burned-infected; food was available *ad libitum* to all groups. Eight rats per group were killed on Days 1, 2, 3, 4, and 7 postburn. In the second study there were four groups of rats: fed control, fasted control, fasted-burned, and fasted-burned-infected; food was removed from the last three groups just prior to scalding and/or infection.

A 30% total body surface, full thickness burn of the dorsum was achieved by immersing anesthetized, shaved rats which had been placed in a mold to define the extent of injury in boiling water for 10 sec (12). No resuscitation was carried out. Infection was accomplished by placing 1 ml of a 16-hr broth culture of *Pseudomonas aeruginosa* on the burn followed by swabbing to dis-

<sup>1</sup> Presented at the 63d annual meeting, Federation of American Societies for Experimental Biology, Dallas, Texas, 1979.

<sup>2</sup> The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. In conducting the research described in this report the investigators adhered to the "Guide for 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.

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tribute the organisms over the entire burned surface. A clinical isolate of *P. aeruginosa* was used, strain 12-4-4, and the culture was adjusted to yield  $10^8$  organisms and inoculation of the animals took place within 2 hr of burning. Blood samples from burned and burned-infected rats were cultured in trypticase-soy broth to assess the presence of bacteremia.

At scheduled intervals the rats were anesthetized by the intraperitoneal injection of 0.5–1 mg sodium pentobarbital/25 g body wt, and the body cavities opened and bled from the hepatic vein. The livers were then perfused with physiologic saline and samples of liver and kidneys were taken for zinc determinations. The tissues were solubilized by the addition of an equal volume of 25% tetramethylammonium hydroxide (13) followed by incubation at a 37°C shaking water bath until the particulate matter had entirely dissolved. Serum and tissue zinc was determined by atomic absorption spectrophotometry. Serum albumin was measured by bromocresol green reagent (14). Analysis of variance was used to assess statistical significance.

**Results.** In the first study where food was provided *ad libitum*, burned rats exhibited a significant decrease in serum zinc concentration 3 days postburn and this hypozincemia persisted through Day 7. There was no detectable bacteremia in burned rats

during the week of the study. Burned-infected rats displayed significant hypozincemia on Day 2 with an additional diminution on Day 3 and an even further decline on Day 7. From Days 3 through 7, the burned-infected rats had a significantly lower serum zinc concentration than burned rats (Fig. 1). Three of eight rats had detectable bacteremia on Day 3, and all had blood cultures positive for *Pseudomonas* by Day 7. Despite the fact that the serum zinc content of burned rats remained within the normal range for the first 2 days postburn, there was a 50% increase in liver zinc concentration on Day 1 in these animals and the liver zinc content remained significantly elevated through Day 7. Burned-infected rats displayed a similar degree of zinc sequestration by the liver as burned rats on Day 1, but continued to accumulate zinc within the liver up to 120% above control values by Day 7 (Fig. 1).

Though all groups were initially closely matched for weight, burned rats did not gain as much weight as controls, even with food available, and burned-infected rats lost weight during the week of the study. There was no significant difference in total liver weight among these groups (Table 1). Using these data one can estimate the amount of zinc lost from the plasma and gained by the liver. It is clear that in burned and burned-infected rats the liver has accumu-

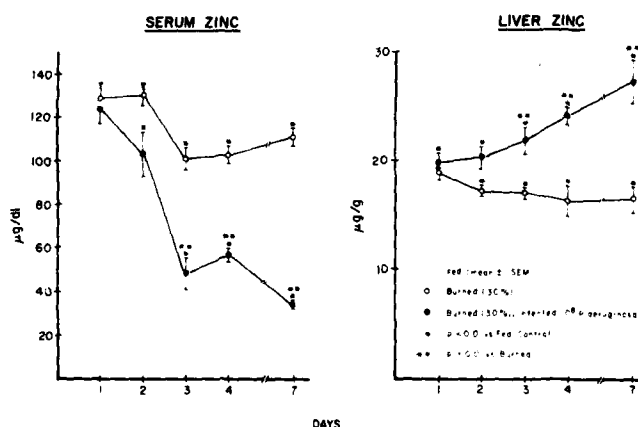


FIG. 1. Alterations in serum and liver concentrations in burned and burned-infected rats provided with food *ad libitum*.

TABLE I. BODY AND LIVER WEIGHT, PLASMA AND LIVER ZINC CONCENTRATION 1 WEEK POSTBURN

	Weight (g) <sup>a</sup>			Zinc ( $\mu$ g)		
	Body		Liver	Plasma	Liver	Liver gain-plasma loss ( $\mu$ g)
	Initial	Final	Total	Total ( $\Delta$ )	Total ( $\Delta$ )	
Control	188 $\pm$ 2	264 $\pm$ 3	12.63 $\pm$ 0.37	13	154	
Burned	187 $\pm$ 2	213 $\pm$ 7	11.22 $\pm$ 0.43	8 (-5)	184 (+30)	+ 25
Burned-Infected	190 $\pm$ 2	155 $\pm$ 4	10.95 $\pm$ 0.59	2 (-11)	330 (+176)	+ 165

<sup>a</sup> Mean =  $\pm$  SEM.

lated zinc in great excess of that lost by the serum (Table I).

To obviate differences in food intake caused by burning and/or infection, rats were fasted following burning and/or seeding with microorganisms. Fasting of itself resulted in a decrease in serum zinc on Day 2, with an additional decrement on Day 5. Burned rats incurred a 33% greater fall in plasma zinc concentration as compared with fasted rats on Day 1, but little change thereafter. Burned-infected rats not only had a one-third decline in serum zinc concentration on Day 1, but the serum zinc concentration continued to plummet in a linear fashion to a low 18  $\mu$ g/dl by Day 5 (Fig. 2).

Despite the gradual diminution of serum zinc in fasting rats, there was no accumulation of zinc within the liver. In contrast, burned rats more than doubled their hepatic zinc content on Day 1 and retained significant, though somewhat diminished, amounts of zinc within the liver through Day 6. Burned-infected rats also more than doubled their hepatic zinc content on Day 1, but continued to accumulate additional zinc through Day 5 (Fig. 2). Compared to fasting animals, burned-fasted rats accumulated approximately 50  $\mu$ g more zinc in their livers than they lost from plasma, while burned-infected-fasted rats accumulated about 280  $\mu$ g more zinc.

Thermal injury, with or without infection superimposed, induced only slight, generally nonsignificant increases in kidney zinc concentration as compared to fasting. Fasting itself resulted in a 15-30% increase

in kidney zinc concentration which was statistically significant from the second day on (Fig. 2).

Since about 50-70% of zinc in serum is bound to albumin, serum albumin was measured by using bromocresol green dye. Serum albumin was not depressed by fasting; burned rats had a somewhat lower serum albumin concentration than fasted rats on Days 2, 3, and 4. In contrast, burned-infected rats had significantly depressed serum albumin concentration on Day 1 which further decreased until by Day 5 their albumin concentration was little more than 2 g/dl (Fig. 2).

*Discussion.* The present data clearly indicate that nonfatal thermal injury in rats not only induces hypozincemia, but also results in an accumulation of zinc within the liver. The difference between the present data and that of Oh *et al.* (4) cannot be resolved on the basis of diet, since both fasted and fed rats displayed hepatic zinc sequestration. Nor is the dissimilitude merely a matter of zinc accumulating in the liver in some form other than metallothionein, since Oh *et al.* (4) measured both metallothionein and whole tissue zinc concentration and neither increased as a result of the burn. Resolution of the disparity in findings may have to do with the size of the lesion; in Oh's study the burned area was 2 cm in diameter, the size of a nickel (about 1% total body surface), while in the present study, the whole back was scalded (30% total body surface). Conceivably, one could have comparable depressions in serum zinc concentration, yet markedly dissimilar degrees of sequestra-

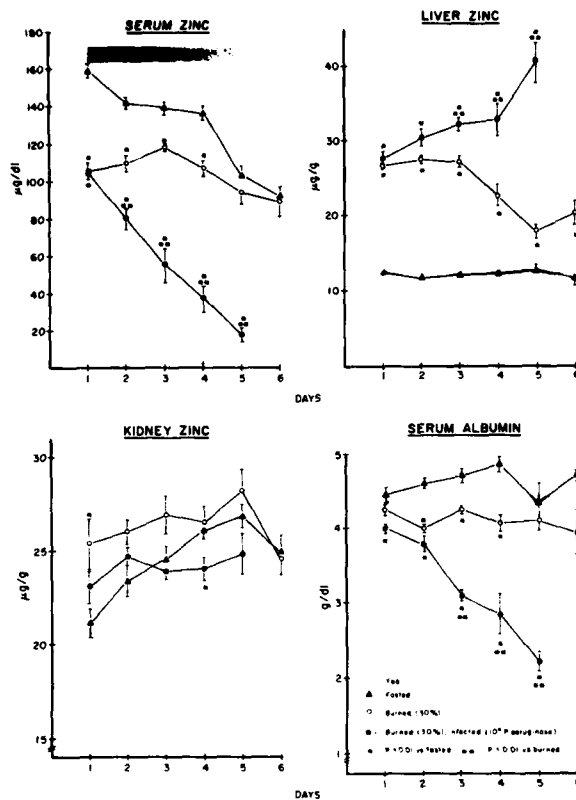


FIG. 2. The effect of fasting on alterations in serum, liver, and kidney concentrations of zinc and serum albumin in burned and burned-infected rats.

tion of zinc by the liver; for example, fasted rats have the same serum zinc concentration as burned-fasted rats on Days 5 and 6, yet significantly different hepatic zinc contents. The present data also indicate that the converse is at times possible, i.e., dissimilar serum zinc concentrations and yet comparable accumulations of zinc within the liver, as was observed on Day 2 when the rats were allowed food *ad libitum* (Fig. 1) and in the fed versus fasted rats (Fig. 2).

It is clear that the amount of zinc accumulated by the liver is far in excess of that lost by the serum. It appears that hepatic sequestration reflects the degree of injury and/or severity of infection. That fasted rats sequester more zinc than fed animals is puzzling, yet may indicate that zinc is of such import either in protecting against dis-

ease and/or injury, or in repairing the damage thereof (15), that the body goes to great lengths to store it.

Normally some 40–50% of serum concentration of zinc is an integral part of the  $\alpha_2$ -macroglobulin (16–17), a protease inhibitor, and unless this protein decreases in concentration, the lowest serum zinc concentration one would expect to find if all the labile zinc, i.e., that bound to albumin and amino acids, were removed from circulation, is 60–75 µg/dl. Burned-infected rats, whether fed or fasted, have serum zinc concentrations in the agonal stage which are so low as to suggest that the  $\alpha_2$ -macroglobulin concentration has decreased in these animals.  $\alpha_2$ -Macroglobulin turnover has, in fact, been shown to be increased even in patients with small burns (10–15%

(18)). Moreover a decrease in  $\alpha_2$ -macroglobulin would not be unlikely considering that the infection was with a microorganism whose virulence may in part depend on elaboration of proteases (19) and  $\alpha_2$ -macroglobulin and  $\alpha_2$ -antitrypsin interact to eliminate released proteolytic enzymes (16).

Since liver accumulates more zinc than serum loses, the source of the zinc, especially in fasted animals, is in question. Muscle is a most likely source since marked muscle wasting occurs in both injury and infection (1). If the 40% weight loss that burned-infected rats experience within 5 days were primarily a loss of muscle of tissue and if one accepts an average muscle zinc content of 6–8  $\mu\text{g/g}$  (20), then sufficient zinc would be made available (480–640  $\mu\text{g}$ ) to account for liver sequestration as well as the excess urinary excretion which is observed following thermal trauma (21, 22). It is interesting that Davies and Fell found a highly significant correlation between the amounts of zinc and creatinine excreted (22) in light of the accumulation of zinc by the liver. If Davies and Fell's findings truly represent a constant stoichiometric release of zinc and nitrogen from peripheral tissue, then the accumulation of zinc by the liver may yield an estimate of nitrogen released from muscle which is available for recycling or reutilization, perhaps in the form of plasma proteins (15, 23). Assuming 7.25  $\mu\text{g}$  zinc and 33 mg nitrogen per gram of muscle tissue and the data in Table 1, burned rats would have 136 mg of nitrogen available for reutilization or enough to replace the total plasma content twice over while burned-infected rats would have 801 mg of nitrogen available for recycling or enough for almost a 12-fold replacement of circulating plasma proteins. If one assumes a value of 30 mg zinc and 33 mg nitrogen per gram of muscle (22) then the available nitrogen would be decreased by a factor of 4. Interestingly enough, these estimates are compatible with data on incorporation of radiolabel into total serum protein (23) and increases in acute phase globulin synthesis/turnover in burns and infection (2, 3, 23–26). Finally, these data provide additional circumstantial evidence

that zinc and nitrogen redistribution during injury and infection may indeed be coupled. In patients with gastrointestinal disease who are receiving total parenteral nutrition there does appear to be a link between the metabolism of protein and that of zinc (27).

**Summary.** Hypozincemia and hepatic sequestration of zinc occur in rats receiving a nonlethal scald injury as well as in rats with a lethal infection superimposed on the burn injury, somewhat in proportion to the severity of the injury. The amount of zinc accumulated by the liver is far in excess of that lost from serum and may be related to altered nitrogen distribution. Unlike liver, kidneys do not sequester zinc. The availability of food mutes, but does not obliterate either, the serum zinc depression or the hepatic accumulation of zinc.

Zinc redistribution during thermal injury with or without the complication of infection appears not to be related to alterations in serum albumin concentration.

The assistance of Harrel L. Walker and Ralph S. Harris in producing a reproducible scald injury. Major Albert T. McManus in providing *P. aeruginosa*, and Lt. Douglas Mills in statistical analyses is greatly appreciated.

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Received July 16, 1979. P.S.E.B.M. 1980, Vol. 163.

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