# COPPER STATUS IN MULTIPLE TRAUMA PATIENTS: MEASUREMENT OF COPPER BALANCE, SERUM COPPER AND CERULOPLASMIN

by

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Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Human Nutrition and Foods

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October, 1990

Blacksburg, Virginia

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(ABSTRACT)

Changes in copper metabolism have been reported in both thermal injury and skeletal trauma; data regarding copper status in multiple trauma patients (MTP) are nonexistent. Hypercatabolism following multiple trauma may increase copper utilization, deplete copper stores and compromise cuproenzyme synthesis and function. The purpose of this study was to provide information on copper status in MTP and determine whether age, injury severity, clinical outcome or nutritional intake influenced copper status. Twenty-four hour copper losses, serum copper and ceruloplasmin were measured in 11 MTP with Injury Severity Scores (ISS) >12 at 24-48 hours post admission. Collections of biological fluids (urine, nasogastric, chest tube, drains, stools) were analyzed for copper using atomic absorption spectrophotometry (AAS) and quantified over 5 days. Serial serum copper and ceruloplasmin were determined on days 1,3,5,10,15 and patient discharge by ASS and rate nephelometry immunoprecipition, respectively. Eight patients received parenteral nutrition (PN). Three received intravenous glucose/electrolyte infusions (IV). Urine (n=11) and nasogastric losses (n=8) were statistically greater than normal ($p<.001$). The mean $\pm$ SEM cumulative copper losses of urine, chest tube drainage, nasogastric secretions and other drains were 790 $\pm$ 116 (n=11), 833 $\pm$

130 (n=7), 261 ± 46 (n=8), and 150 ± 58 µg/5 d (n=8), respectively. Urinary losses represented 10 to 12 times the normal copper excretion. Serum copper on day 1 and ceruloplasmin day 3 were significantly higher than normal ($p<.025$). Cumulative copper balance in the IV group was -2266 µg and -440 µg in the PN group. No relationship was found between copper loss and ISS. Patients in their twenties demonstrated the greatest urinary copper loss. The physiological and biochemical effects of extensive copper loss in the MTP require further evaluation. These patients may have a predisposition to copper deficiency due to excessive copper losses and may require increased copper supplementation.

ACKNOWLEDGEMENTS

Many individuals have provided steadfast guidance and support throughout the course of this study. Special consideration must be given to the patients and their families who willingly participated as subjects even after a life threatening experience. The author is indebted to Dr. Forrest Thye, Major Professor, for his suggestions, criticism, faith and sensitivity throughout the duration of this graduate program. Equally generous of her time, which often she did not have, her support and advice is Dr. Carol Gilbert, Director of Trauma at Roanoke Memorial Hospitals and a member of the graduate committee. She provided the avenue to conduct the research and served as a liaison between other hospital staff. Appreciation is also extended to the following individuals:

Dr. Ryland Webb, Dr. S.J. Ritchey, and Dr. Frank Gwazdauskas, members of the graduate committee, for their time, advice, humor and intellectual openness;

Carol Braunschweig for her long distance correspondence, encouragement, and faithful friendship;

Dr. Scott Malbon, for his contributions to the statistical analysis;

Jerry Houghton, Director of Laboratory Services at Roanoke Memorial Hospitals and his staff, for their interest, cooperation and assistance;

Leah Lineberry, Head Nurse, Neurological Intensive Care Unit at Roanoke Memorial Hospitals and her nursing staff for their competence, generosity of spirit and honesty when handling study patients' samples;

American Medical Laboratories for their timely analysis of samples;

Kathy Kieger, for her all around helpfulness and encouragement;

Surgical and Medical House Staff at Roanoke Memorial Hospitals, for their cooperation;

Last and most significantly, the author extends her love and gratitude to her husband, Joe. His patience, prodding, all-around helpfulness, words of encouragement, love, seriousness, as well as irrepressible humor, helped the author maintain a sense of direction and clarity. All of this, combined with the bright faces and wonderful personalities of her children, Jordan David and Eleanor Kathleen, has enabled the completion of this work.

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# CHAPTER I

## INTRODUCTION

Trauma is the major source of death in the United States among people younger than 40 years of age. In patients who survive the initial injury, survival relates directly to the body's ability to support itself during the recovery phase (1). Successful medical treatment depends on the ability to prevent sepsis and multiple organ failure and to provide adequate nutrition.

Multiple trauma patients (MTP) are a unique population. Victims are generally healthy and well nourished prior to injury; most have normal or marginal reserves of macro and micronutrients before the insult. Regardless of prior nutritional status, specialized nutrition support and micronutrient therapy is necessary due to the dynamic hypermetabolic or hypercatabolic conditions that follow injury. Guidelines for nutritional support are derived from the processes of the metabolic response to injury (1). Macronutrient and vitamin requirements are better defined for acute trauma than are trace element needs. The optimal trace element therapy for patients with serious injury remains an enigma because the experimental and clinical data are incomplete, and the degree of incompleteness varies considerably for the various minerals. Increased zinc and copper losses have been reported following skeletal trauma and thermal injury (2,3). Conflicting or minimal reports exist concerning copper losses in multiple trauma (2). As a result, trace element requirements are poorly defined in traumatic conditions.

The micronutrient requirement least understood after acute injury is copper, primarily since deficiency has been rare or unreported. Copper is an essential nutrient necessary for the functions of the cuproenzymes which include erythropoiesis, leukopoiesis, catecholamine metabolism, oxidative phosphorylation, thermal requlation, and immunity, among others (4,5). Until the advent of parenteral nutrition (PN), overt signs and symptoms attributable to copper depletion were nonexistent. Copper deficiency has been reported both in adults and children on PN (6,7,8). Recent literature has provided scattered case reports of copper deficiency (9). Most deficiencies documented have been limited to patients with primary disease of the gastrointestinal tract where malabsorption of copper was implicated (6,10,11). Parenteral solutions without adequate copper supplementation can result in negative copper balance and progressive hypocupremia (2,9,12,13).

The clinical status of MTP often prevents immediate nutrition support during the first 48 to 72 hours post injury, thus limiting copper intake. Trauma patients often require PN during the acute phase of injury, yet without data on copper losses appropriate copper replacement becomes indiscriminate. The response of serum copper, ceruloplasmin and urinary copper excretion after acute injury has not been studied. Among the consequences that would affect MTP are impaired utilization of iron for hemoglobin synthesis which alters oxygen delivery, osteoporosis, poor wound healing secondary to defective connective tissue synthesis, and impaired immune function (4,14,15).

Major trauma induces profound physiologic and metabolic changes.

The hormonal and biochemical alterations following injury have been well described (16-20). The greater the severity of injury the more pronounced the changes. Multiple trauma (MT) is associated with increased substrate catabolism which results in increased energy expenditure, negative nitrogen balance and electrolyte losses. Oxygen consumption and metabolic rate increase following injury which results in the outpouring of catecholamines (18). Furthermore, significant blood loss is associated with trauma. Since copper is required for the synthesis of both catecholamines and hemoglobin, it plays an essential role in the acute phase of injury. Consequently, it can be theorized there is increased utilization of copper which could result in an increased copper requirement. If combined with impaired or inadequate intakes and any urinary copper or miscellaneous losses, a negative copper balance could result. Although overt copper deficiency symptoms have not been reported post injury, the degree of copper depletion needed for immune dysfunction is less that that required for induction of the clinical signs of deficiency (21). Marginal or subclinical copper deficiency may be more common than currently recognized, particularly in the MTP.

Guidelines for essential trace element preparations for parenteral use have been published by the American Medical Association (AMA), Department of Foods and Nutrition (22). The suggested daily intravenous (IV) intake of copper in the stable adult is 0.5 to 1.5 mg/day. Additional intake in acute catabolic states is not provided as it is for other elements like zinc, due to lack of supportive data. Most patients receiving PN are supplemented with 1 mg copper every day

or every other day, regardless of clinical status (22). Reports of hypercupriuria in thermal injury, and increased losses of copper via high output stomas or fistulas, combined with inadequate energy intake, suggests additional copper supplementation during acute catabolic states such as MT. Unfortunately, little data are available to recommend IV replacement levels of copper in parenterally supported trauma patients.

Complete assessment of copper status and potential replacement therapy in trauma patients must rely on balance investigations (23,24). Balance studies have proven effective with other nutrients like nitrogen and zinc in defining the nutritional needs of MTP (24). This technique remains the most valuable functional index available for the assessment of copper status in the acutely traumatized patient (25). However, reports of balance studies for copper in the trauma patient are basically nonexistent (23).

Standard laboratory indices of copper status are serum or plasma copper, ceruloplasmin, and urinary copper (4). Suppressed serum or plasma values alone do not always reflect a deficiency. As suggested previously, balance studies are more sensitive indicators than serum concentrations; although, serial serum determinations can be useful when compared with copper balance.

Limited research is available regarding copper status in MTP. The possibility exists that the hypercatabolism which follows acute trauma may deplete copper stores and compromise the cuproenzymes synthesis and hence their functions in the MTP. Therefore, the intent of this research is to: 1) quantify copper losses in biological fluids; 2)

calculate copper balance; 3) measure serum copper and ceruloplasmin to determine deviations from normal; 4) determine the relationship between these serum values and copper balance; 5) determine what relationship exists between nitrogen and copper excretion; 6) record hematological parameters to determine the relationship between copper balance; and 7) examine what effect injury severity, age, clinical outcome, and nutritional intake have on 1, 2, and 3 above.

# CHAPTER II

# REVIEW OF LITERATURE

## Physiologic Functions of Copper

Copper is an essential trace element and the third most abundant metal in the human body. As a nutrient, copper has numerous physiological functions in man. These include erythropoiesis, leukopoiesis, skeletal mineralization, connective tissue synthesis, myelin formation, melanin pigment synthesis, catecholamine metabolism, oxidative phosphorylation, thermal regulation, antioxidant protection, cholesterol metabolism, immune function, cardiac function, glucose regulation, anti-inflammatory activity, and possibly coagulation (4,26-28). These metabolic roles are derived primarily from the known functions of the cuproenzymes, and from clinical manifestations of experimental, acquired, or hereditary copper deficiency states in animals and man.

## Biochemical Functions of the Cuproenzymes

The physiologically important cuproenzymes in humans include cytochrome c oxidase, superoxide dismutase, ferroxidase (ceruloplasmin), lysyl oxidase, and dopamine β hydroxylase. Most cuproenzymes are functionally oxidases or hydroxylases. Thus, the established role of copper proteins is intimately related to oxygen (29). A deficiency of copper may pose a significant aberration in the activities of these enzymes.

Cytochrome c oxidase is the terminal enzyme in the electron

transport chain of mitochondria, hence it is key to respiration and oxidative phosphorylation. Gallagher et al (30) reported a 10-fold decrease in the concentration of cytochrome c oxidase in liver tissue of copper deficient rats. Prohaska et al (31) found similar reductions in cytochrome c oxidase concentrations in mouse livers exposed to a copper deficient diet. Whether this limited respiration was unclear.

Superoxide dismutase (SOD) appears to have a role in protecting the cell from the damaging effects of the superoxide radical. Found in all living cells that utilize oxygen, including neutrophils, it catalyzes the dismutation of the superoxide anion, $O_2$-, as follows: $2O_2- + 2H+ \longrightarrow H_2O_2 + O_2$ (Haber Weiss reaction) (5). Superoxide dismutase acts as an free radical scavenger and thus represents a mechanism for oxygen detoxification. In addition, it protects against immune complex-induced vascular injury (32,33).

After tissue trauma, activated neutrophils and other phagocytes are uniquely endowed with the capacity to manufacture large quanitities of powerful oxidizing agents when they encounter opsonized bacteria, necrotic tissue or other appropriate stimuli (34). The neutrophils engulf debris from the damaged tissues, forming phagosomes. The lysosome fuses with the phagocytic vacuole which discharges lysosomal enzymes and toxic oxygen metabolites to destroy the engulfed particle. This metabolic process is associated with an abrupt increase in phagocyte oxygen consumption and is called the respiratory burst. During the respiratory burst, oxygen consumption of the cell can multiply dramatically which leads to the emptying of prostaglandin $E_2$,

leukotrienes, lysosomal enzymes, and oxygen radicals into the phagosome. The neutrophil remains protected since the cytosol contains the free radical scavenger, SOD, which inactivates the oxygen metabolites.

The activation of neutrophil oxidative metabolism to produce superoxide is an essential component of killing bacteria and control of infection after tissue trauma. Lanser et al (35) demonstrated a suppression in neutrophil oxidative metabolism in serum samples from 18 patients with multiple trauma. This suggested that host defense following injury could be compromised and perhaps predispose the patient to the onset of sepsis. The exact mechanism of action of the suppressor remains unknown but may involve SOD.

Some of the enzymes and free radicals are also extruded from the cell, and damage nearby endothelium (32). These toxic neutrophil products can act on the vascular smooth muscle and disrupt the endothelium to produce extensive tissue injury (36). Oxygen products such as the superoxide anion produced by neutrophils, have been implicated in lung injury after trauma for years (37). The protective effects of SOD appear related to a diminished influx of neutrophil products into the tissue (33,35). Tate and Respine (37) demonstrated that the administration of SOD as a free radical scavenger reduced the effects of vasoactive mediators in lung tissue of animals. Other studies are presently being conducted to evaluate the utility of free radical scavenger therapy after trauma.

Ferroxidase or ceruloplasmin is a cuproprotein found in blood plasma and is essential in promoting hematopoiesis. It is involved

with iron mobilization and incorporation into the oxygen carrying protein, hemoglobin (38-40). Osaki et al (41) proposed the following scheme for the role of ceruloplasmin in the mobilization of plasma iron. Iron (Fe) is released from ferritin-Fe(III) stored in liver cells by a ferritin reductase step involving reduced flavin mononucleotide and NADH (42). This ferrous form (Fe II) is presented to the surface of mucosal cells, parenchymal cells, and cells of the reticuloendothelial system. Ceruloplasmin oxidizes the ferrous form to ferric (FeIII), in which form iron combines with apotransferrin to form Fe(III)2 transferrin in the plasma. In this form, iron is distributed to all cells, predominately the reticulocytes of the bone marrow where it is involved in heme/hemoglobin synthesis.

Ceruloplasmin also serves as an acute phase reactant released from the liver in response to inflammatory injury secondary to trauma (43-45). Increases in ceruloplasmin synthesis and hence concentration occur during nonseptic trauma or inflammation and appear mediated by a leukocyte endogenous mediator (LEM) (44,45). Sganga et al (45) studied the temporal pattern of ceruloplasmin in 26 trauma patients immediately post injury. They found an early rise (< 5 d) in serum ceruloplasmin in both non-septic and septic patients with a late (> 5 d) decrease only in non-septic patients. Ceruloplasmin levels of septic patients levels continued to be elevated throughout the study's 16 d duration. They concluded that increases of acute phase proteins in acutely injured patients served as a predictor of sepsis and measured the adequacy of the host response to trauma. Kushner et al (44) studied 19 patients with myocardial infarctions and reported that

ceruloplasmin concentrations increased by as much as 50%. This elevation in serum cerloplasmin usually occurred after 8 h and peaked in 2 or 3 d. Patients with severe infarctions achieved the higher ceruloplasmin level for a longer period than patients with mild infarctions. They concluded that the duration and magnitude of the rise in ceruloplasmin are correlated to the extent of injury. The purpose of such elevations of ceruloplasmin is not certain. It may play a role in inactivation of leukocyte mediated proteases, superoxide responses involved in bacterial phagocytosis and killing, and in digestion of dead tissue (45,47).

Lysyl oxidase plays an important role in connective tissue integrity important in wound healing after trauma (5,48). It is an extracellular amine oxidase enzyme which catalyzes the oxidation of specific lysyl and hydroxylysyl residues on the peptide chains of elastin and collagen. Copper deficiency can result in impaired elastin and collagen synthesis (4). The trauma patient with cutaneous wounds needs adequate copper to support synthesis of new connective tissue.

Lastly, the copper containing hydroxylase, dopamine β hydroxylase, is a key enzyme in the synthesis of the catecholamine's, epinephrine and norepinephrine (26). Catecholamine production dramatically increased after injury and correlated directly to the severity of injury in 36 traumatized patients (17). Davies et al (49) demonstrated that the more severe the injury the greater the catecholamine release. It is unclear whether the activity of the cuproenzyme, dopamine B hydroxylase is depressed in copper deficiency. However, Prohaska and Wells (50) reported a lower level of dopamine and norepinephrine in

the brains of copper deficient rats. These results demonstrated that copper deficiency decreased brain catacholamine production which could alter the function of the central nervous system. Whether this would impact an individual's ability to respond to multiple trauma is unclear.

## Copper Metabolism

### Copper Content in the Body

The average 70 kilogram adult prior to injury contains approximately 75 mg copper (51). The estimated distribution of copper among body organs is as follows: skeletal muscle (24.7%), skin (15.3%), bone marrow (14.8%), skeleton (19.0%), liver (8.0-15.0%), and brain (8.0%). Cartwright and Wintrobe (51) performed copper analyses of the liver, brain, heart, kidney, and muscle in six normal male cadavers between the ages of 25 and 40 years. They reported the highest concentrations in the brain (6.3 µg/g) and liver (5.1 µg/g). Lesser concentrations of copper were found in the heart (3.0 µg/g), kidney (2.0 µg/g), and skeletal muscle (0.9 µg/g).

### Copper Absorption

The maximum site of copper absorption is in the proximal portion of the gastrointestinal tract, that is, the stomach and upper duodenum (52,53). After oral administration of $^{64}$copper to humans, Van Berge Henegouwen et al (54) found the radioisotope appeared quickly in the blood. This suggested that absorption of copper occurred in the stomach and upper small intestine. Furthermore, Marceau et al (55)

reported that the acidic environment of the stomach promoted copper solubility and enhanced transport across the gastric mucosa. Approximately 25 to 60% of ingested copper is absorbed by the functioning stomach and small intestine (4,28,56). Evidence also suggested that copper absorption may be reduced in the elderly (57-59). Copper absorption in 5 elderly men determined by $^{65}$copper averaged 25.8 ± 3.1% (58) which is lower than the 57 ± 18% absorption reported by King et al (60) in young women.

## Mechanism of Copper Absorption

Gitlin et al (61) demonstrated that copper absorption in mice does not occur exclusively from simple diffusion. Crampton et al (53) reported two mechanisms for copper absorption in the mucosa of isolated hamster intestine. They demonstrated that while the major route for cellular uptake was carrier mediated diffusion, the minor route was energy dependent. Other investigators demonstrated that L amino acids whose transport across the intestinal mucosa is energy dependent, facilitated the intestinal absorption of copper (62,63). They concluded that a small fraction of copper was actively transported as a copper-amino acid complex.

The major mechanism involved in copper absorption is protein mediated diffusion. Identification of this copper binding protein revealed a metallothionein rich in sulfhydryl groups that bound copper through the formation of mercaptide bonds within the absorptive cell of the rat (64) and human (65) intestinal mucosa.

Copper Transport

Following release from the intestinal mucosa, copper is transported through the portal blood bound to albumin (6-7%) and amino acids (1%). Bearn and Kunkel (66) reported that after oral administration of $^{64}$copper, the isotope was mainly associated with albumin in the plasma. Neumann and Sass-Kortsak (63) first introduced evidence of a smaller copper fraction loosely bound to amino acids. They demonstrated that the amino acid bound fraction of plasma copper was in equilibrium with the albumin bound copper. Furthermore, these and other data (67,68) indicated that the predominant amino acid in these complexes was histidine. These authors suggested that the existence of the amino acid-copper complexes is probably important in copper transport in the blood. Amino acid-copper complexes are capable of actively diffusing acrosss the cell membranes such as those of erythrocytes, whereas albumin bound copper preferentially releases its copper to receptor proteins of the plasma membrane of hepatocytes and other cells. These copper complexes represent the direct reacting or labile copper pool in serum.

In the plasma (or serum), approximately 93% of copper is tightly bound to ceruloplasmin, the indirect reacting copper (69,70). Copper secreted from the hepatocytes is primarily in the form of ceruloplasmin. Apoceruloplasmin is synthesized in the liver (71), and later combines with 6 copper atoms to form holoceruloplasmin. Owen (72) observed that after infusion of radioactive copper ($^{64}$copper), the isotope only accumulated in extrahepatic organs after the appearance of ceruloplasmin-$^{64}$copper. Other investigators confirmed these results

using ceruloplasmin-$^{67}$copper (73,74). These authors suggested that since ceruloplasmin does not dissociate readily, the exchange of copper between ceruloplasmin and the extrahepatic tissues involved a degradation mechanism either within the cell or on the cellular membrane. Later work by Marceau and Aspin (75) confirmed that the peripheral delivery of copper to tissues involved the intercellular consumption of the ceruloplasmin molecule.

Copper Excretion

The major route of copper excretion is in the bile. Van Berge Henegouwen et al (54) determined a mean bile copper output of 1.7 mg/d using duodenal perfusion techniques. Cartwright and Wintrobe (51) estimated that biliary copper losses were between 0.5 - 1.3 mg/d. Lewis (76) examined the enterhepatic circulation of copper and demonstrated that reaborption is minimal due to the molecular binding of copper in bile.

Dreizen et al (77) first reported a mean copper concentration of 25.6 µg/100 ml in whole saliva of 14 normal humans. Later, DeJorge et al (78) determined a mean copper value of 31.7 µg/100 ml in 40 normal, fasting subjects, while Gollan et al (79) provided a mean value of 29 µg/100 ml. Based on these studies a daily saliva production of 1.5 liters would result in salivary copper secretion of 0.38 - .47 mg/d.

Gollan et al (79) reported that copper content in gastric secretions averaged 39 $\pm$ 20 µg/100 ml. Given this finding, a daily gastric secretion of 3 liters would calculate to approximately 1 mg/d of copper in gastric mucosa secretions. In addition, MacDonald et al

(80) examined the contribution of copper released into the feces via the dehiscence of epithelial cells of the intestinal mucosa which contain considerable copper bound to metallothionein. They observed that the absorptive cells lining intestinal villi are replaced every 5 to 6 d in man. They concluded that the copper contribution to the intestinal contents may represent 17% of the daily fecal excretion.

Fecal copper excretion represents unabsorbed dietary copper plus copper excreted via the biliary tract, salivary glands and gastric and intestinal mucosa, minus copper which is reabsorbed by the gastrointestinal tract. Other considerations are the body surface losses of copper. Klevay et al (81) estimated the surface loss of copper in sweat, desquamated skin, and hair to be .25 mg/d. This was consistent with the mean of .34 mg/d found by Jacob et al (82). Consolazio et al (83) reported the highest copper loss of 1.6 mg/d in adults who had sweated for 7.5 h at 37.8$^{o}$ C.

Urinary copper represents a minor route of excretion. Daily urinary copper obtained by Cartwright and Wintrobe (51) averaged 15 µg/d (range = 5 to 25 µg/day) in healthy subjects. They suggested that the copper excreted by the kidney represented the dissociation of copper from primarily copper-amino acid complexes as well as some copper-albumin complexes. Since most circulating copper binds to ceruloplasmin which has a high molecular weight (124 - 134,000), very little copper permeates the glomerular capillaries and hence urinary copper excretion is negligible under normal conditions.

## Copper Homeostasis

Copper homeostasis is regulated through a variety of interrelated mechanisms which may be influenced by endogenous secretions or copper intake. Bile has a negative influence on the reabsorption of secreted copper (84), while glucocorticoids promoted biliary copper secretion (85). Benson (85) concluded that hormonal status altered copper absorption by influencing the excretion mechanism for copper.

Turnlund et al (86,87) showed that copper absorption was dependent on the level of dietary copper. Absorption averaged 36.3 $\pm$ 1.3% with adequate copper intake (1.68 mg/d), 55.6 $\pm$ 0.9% with low copper intake (0.785 mg/d), and 12.4 $\pm$ 0.9% with high copper intake (7.53 mg/d) in 11 young men (86). These data demonstrated that the percent copper absorbed decreased as copper intake increased. They further concluded that absorption adapts more rapidly to a low copper diet than a high copper diet. In addition, retention averaged 0.17 mg/d with adequate copper intake, and -0.316 mg/d with low copper intake. During the first 6 d of high copper intake, retention averaged 3.9 mg/d, but average retention decreased linearly over the remaining period to a negative retention during the final 6 d. These results suggested that absorption is an important point of control when dietary copper is low which may protect against copper depletion. Copper absorption increased with low copper intakes and endogenous copper losses decreased. In contrast, when diets contained high amounts of copper, both reduced absorption and increased endogenous losses prevented excess accumulation.

Interactions

Copper-Zinc: The competitive interaction between zinc and copper has been documented in both experimental animals (88,89), and man (90-93). Individuals who received 1.5 to 7 times the recommended dietary allowance (RDA) of 15 mg/d zinc reported alterations in copper status measurements (91,93,94). Prasad et al (94) reported the diagnosis of copper deficiency in a sickle cell patient treated with 10 times the RDA for zinc (150 mg/d) over 2 years. The occurrence of hypocupremia, hypoceruloplasminemia, and the associated microcytosis and neutropenia prompted further evaluation of 13 other sickle cell patients. The mean ceruloplasmin levels in these 13 patients before zinc therapy was 55.7 mg/100 ml, but after zinc administration (4 to 24 wk), the mean level decreased to 24.3 mg/100 ml. After copper administration, microcytosis and neutropenia were corrected, and plasma copper and ceruloplasmin returned to normal. Since increased plasma copper levels are typical in sickle cell patients (95), the data suggested that oral zinc of 150 mg/d depleted the body of its copper stores.

Fischer et al (91) indicated that moderately high intakes of zinc (50 mg/d) over a 6 wk period in 26 healthy male adults resulted in decreased erythrocyte SOD (ESOD) activity by wk 4. Erythrocyte SOD activity decreased in a linear fashion over time which may be related to erythrocyte turnover. Plasma copper levels remained normal throughout the study. They concluded that the measurement of ESOD activity was a more sensitive indicator of copper status than were plasma copper and ceruloplasmin levels. Similar reductions in the activity of ESOD were reported after 50 mg/d zinc supplementation in

healthy adult females during a 10 wk study (93). In this study, ESOD activity measured 85% of pretreatment level after 6 wk. Activity further decreased to 53% of pretreatment level by wk 10. Again, no changes occurred in ceruloplasmin with this level of zinc supplementation. If these declines in ESOD activity are related to erythrocyte turnover, they would surface only after turnover of sufficient erythrocytes. Andrewartha and Caple (96) found that in sheep, copper was added to the ESOD apoenzyme only at the time of erythropoiesis. If the same situation applies to humans, and if copper was not lost from the enzyme during the approximate 120 d life span of the erythrocyte, then subjects in these studies (91,93) probably experienced impaired copper status as a result of zinc intake before impairment was actually detected, due to the long half life of the erythrocyte. Subsequently, only newly synthesized erythrocytes would reflect the decline in copper status.

Patterson et al (97) reported a case study of a 57 year old man with the diagnosis of sideroblastic anemia. The patient history revealed a daily zinc intake of 450 mg over a period of 2 years as treatment for prostate problems. Laboratory studies showed a hemoglobin level of 5.1 g/100 ml and a hematocrit of 15.6% on hospital admission day 1. Examination of bone marrow showed increased hemosiderin and 21% ringed sideroblasts. The patient received 3 units of packed red blood cells. At day 8 in the hospital, serum copper and ceruloplasmin levels were depressed to 4 µg/100 ml and 12 mg/100 ml, respectively (normal:70-90 µg/100 ml and 20-35 mg/100 ml, respectively). In addition, hemoglobin and hematocrit remained low.

Serum zinc levels were 320 µg/100 ml (normal:50-160 µg/100 ml). Zinc supplements were discontinued and by day 34, serum zinc levels had decreased to 160 µg/100 ml, but little change had occurred in serum copper, serum ceruloplasmin or hemoglobin levels. On day 83, the patients hemoglobin, bone marrow morphology, serum zinc, serum copper and ceruloplasmin levels were normal. This patient never received any supplemental copper. Since copper is critical for iron incorporation into heme, they concluded this patient's anemia was related to copper deficiency induced by megadoses of zinc. This conclusion was based on the eventual normalization of laboratory values after cessation of zinc therapy.

Another study in young men failed to demonstrate any effect on copper absorption at 5.5 mg/d or slightly above the RDA, 16.5 mg/d zinc intake (56). Taper et al (98) indicated zinc intakes between 8-24 mg/d posed no risk to copper utilization in adult females. These results implied that zinc intake must exceed 25 mg/d before there would be any impairment of copper status. However, adolescent females (n=11) fed even less zinc, either 14.52 or 14.84 mg/d exhibited an increased fecal copper excretion (92). In contrast, elderly adults (age 56-83 y) who consumed 7.8 or 23.26 mg of zinc daily and 2.33 mg/d of copper reported a reduced copper retention on the higher zinc intake (99). These data suggested that the higher zinc to copper ratio was a contributing factor in reduced copper utilization. The exact level of dietary zinc required to alter copper absorption or utilization remains unclear.

Van Campen and Scaife (100) demonstrated that zinc interacted with

copper at a site in or on the intestinal mucosa. Piscator (101) first developed the concept that metallothionein was an inducible protein and it binds both copper and zinc under physiological conditions. Hartman and Weser (102) showed that at physiological levels, zinc bound to metallothionein could be displaced by copper because of the higher binding strength of copper to thioneine sulfur. High levels of zinc have been shown to induce the synthesis of metallothionein in rats (103) and in humans (104). Sandstead (105) theorized that high intakes of zinc interfered with the metabolism of copper and that the mechanism involved zinc induction of metallothionein in the gut. These data indicated that metallothionein served as a regulator of zinc and copper absorption, and that a reciprocal relationship between zinc and copper existed, in part by competing for similar binding sites. Thus, it was proposed that displacement of zinc by copper imprisoned the copper in association with higher levels of metallothionein, and prevented the copper from being released and entering the circulation (104,105).

Another possibility is that high zinc intake could enhance the excretion of copper in bile (106). High intake of zinc induces metallothionein synthesis in liver, which may facilitate copper excretion through the exchange of copper for zinc on binding sites of liver metallothionein (102). The subsequent uptake of metallothionein-copper complexes by lysosomes would result in excretion of copper into the bile canaliculi (106,107).

Copper and Amino Acids: Changes in plasma amino acid concentrations in infants receiving PN suggested infusion of parenteral amino acids increased the amino acid bound fraction of plasma copper

(108). This caused increased urinary excretion of amino acid-copper complexes, and hypercupriuria. Furthermore, they demonstrated a positive correlation between urinary copper excretion and nitrogen excretion in infants receiving free amino acid solutions. Urinary copper losses ranged from <1 to 29 µg/d.

The interactions between copper and ligands in biological fluids such as blood plasma is complex. Berthon (67) analyzed how the infusion of PN modified normal copper distribution. Data suggested a competitive complexation between copper and the amino acids, particularly histidine, in the PN solution which resulted in a modest increase in urinary excretion of copper.

Copper and Antacids: Van Kalmthout et al (109) reported a case study of copper deficiency due to excessive antacid therapy. This patient consumed a normal diet supplemented with 7.5 g/d of antacid tablets (Trisibam$^{R}$, a gel of oxides of bismuth, aluminium, silica, magnesium, and sodium), and fiberoptic gastroscopy disclosed decreased gastric emptying. A low serum copper level of 1.20 µmol/l (N=12.0-25.0 µmol/l) and an extremely low ceruloplasmin level of 2 mg/100 ml (N=15-60 mg/100 ml) confirmed copper deficiency. This prompted an in vitro experiment to establish whether Trisibam antacid tablets lowered copper concentrations. Copper levels progressively decreased 20% per each additional tablet. They concluded that alkali oxides in the form of antacids caused the cupric salts to precipitate at alkaline pH, which removed soluble copper from the gastric juice. The bioavailability of copper was markedly reduced by the high levels of antacids. Another study found similar results in an infant with renal tubular acidosis

treated with alkali therapy (110). They concluded that the change in pH and osmolarity in the stomach and small intestine inhibited copper absorption.

Aggressive antacid therapy (aluminum hydroxide with magnesium hydroxide or aluminum hydroxide with magnesium hydroxide and simethicone) for stress ulcer prophylaxis is common in acutely injured patients. Doses range among 30 to 60 ml per 2 hours or approximately 3600 to 7200 mg/d of antacid. What impact these doses have on copper absorption is unknown.

Assessment of Copper Status

Measurements of plasma or serum copper levels and ceruloplasmin have been used most widely to monitor copper nutriture (111). The normal circulating levels of ceruloplasmin and serum copper are 22-70 mg/100 ml and 70-165 µg/100 ml, respectively (51,112). The SI reference values for serum ceruloplasmin is 220-700 mg/L and 11-26 µmol/l for serum copper. Clinical evaluation of copper status has also included urinary copper excretion (normal=5-25 µg/d). Proper interpretation is challenging since hypocupremia is not always synonymous with a copper deficiency (12), and because of the alterations in copper metabolism noted during an inflammatory response. Serum measurements often do not accurately reflect tissue stores or compartmental shifts from the vascular circulation to other extracellular compartments which occur following injury. Determination of serum copper and ceruloplasmin alone are insufficient for assessing copper nutriture in multiple trauma patients.

When accurately performed, balance studies provide a readily available assessment of copper (23,25). Shulman (113) reported borderline low normal serum copper levels ($\bar{x}$ =11.5 ± 4.7 μmol/l; normal range = 10.2-22.8 μmol/l), and baseline negative 24 h copper balance ($\bar{x}$= -0.3±0.7 μmol/$kg^{-1}/d^{-1}$) in 7 postsurgical infants. The high copper content found in the excessive gastrointestinal drainage (118.4 ± 53.8 μmol/l) after surgery may have contributed in part to the negative balance in this study. It was concluded that normal serum copper values did not always infer positive balance. In addition, Shike et al (11) reported that plasma copper levels in 28 patients with gastrointestinal disease maintained on TPN did not reflect the balance state. Patients with negative copper balances measured normal plasma copper values. These studies validated the need for balance studies in addition to serial serum copper determinations. Thus, the proper assessment of copper nutriture after acute injury needs to include 24 h balance studies.

Copper balance is defined as total intake minus total output for a given 24 h period. Classically, output includes urine and stool, however, unmeasured losses include the integument (body surface losses of copper in sweat, desquamated skin, and hair), respiration, blood draws, sputum, and saliva. In addition, possible unmeasured losses in trauma patients include chest tube, fistulas, surgical tube drainage, and nasogastric drainage. The difference between these measured values is used to estimate how much of the assayed substance is retained or lost. However, this simple subtraction method is limited since it is unable to account for the calculation of true intestinal absorption

which would include the endogenous contribution to fecal material. Another limitation inherent in the balance technique is that intake is often overestimated while output is underestimated which causes false positive balances.

Technical accuracy of metabolic balance data depends upon several factors. These include the ability to obtain precise weight and volume measurements of foodstuff and body excreta, to adequately homogenize aliquot samples, and avoid other metal contamination (24). However, through meticulous care and attention to detail these factors can be avoided or minimized during sample collection and analysis.

The balance technique is still considered a meaningful tool for assessing the nutritional and metabolic response to nutritional interventions or to metabolic or physiological alterations (24). Metabolic balance studies have proven effective in determining copper requirements in both healthy and sick individuals (2,56,57). In addition, balance techniques are particularly useful when small changes in output or balance are expected, or when the short-term responses to nutritional or metabolic events are under evaluation such as in trauma (24).

## Copper Deficiency

Copper deficiency in the normal population is rare (111,114). Copper deficiency has been reported due to excessive antacid therapy; zinc supplementation as previously mentioned, and with the administration of PN. The most consistent laboratory findings found in human copper deficiency, in addition to hypocupremia and

hypoceruloplasminemia, have been neutropenia, anemia and leukopenia. Cordano et al (115) and Sriram et al (116) found neutropenia to be the earliest and most consistent sign of copper deficiency, as well as, the most sensitive. The exact mechanism is unknown, though a deficiency of two white blood cell enzymes, cytochrome c oxidase and superoxide dismutase have been implicated (4,29).

The anemia of copper deficiency has been best studied in swine (38,117). Impaired intestinal absorption of copper, defective iron release from reticuloendothelial cells to plasma, excessive hepatic accumulation in parenchymal cells, and impaired conversion of bone marrow iron to heme within developing normoblasts contributed to defective hematopoiesis in these animals. Copper levels < 20 µg/100 ml impaired hematopoiesis in these animals and humans (8,39,117,118).

The anemia found in human copper deficiency is characterized as microcytic, hypochromic, yet this varies in degree. Vilter et al (8) described anemia in a patient with copper deficiency. Low serum iron level, and low plasma iron pool and turnover rate were reported in this patient, yet bone marrow iron stores were normal. This was consistent with a defect in iron release from tissue stores previously noted in swine. In addition, ferrrokinetic studies indicated that erythrocyte production rate was slower than normal as a result of underutilization of iron. The slight elevation of free erythrocyte protoporphyrin cited may reflect a defect in the incorporation of iron into heme. As in the copper deficient swine, iron was probably not being converted efficiently by the normoblasts for heme formation. Several investigators concluded that the anemia of copper deficiency results

from a reduced rate of red cell synthesis, shortened erythrocyte survival time, and impaired transferrin formation from ferrous iron caused by inadequate ceruloplasmin (14,39,117).

Other hematological parameters often, but not exclusively associated with the anemia of copper deficiency include the following: decreased hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (MCHC). MCV, the ratio of hematocrit (packed cell volume) to the red blood cell (RBC) count, expresses the average size of cells and indicates whether the most red cells are undersized (microcytic) or oversized (macrocytic), or normal (normocytic). MCHC, the ratio of hemoglobin weight to hematocrit, defines the volume of hemoglobin in an average red cell and helps distinguish normally colored (normochromic) red cells from paler (hypochromic) red cells. Therefore, the microcytic, hypochromic anemia found in copper deficiency corresponds with a low MCV and MCHC.

The biochemical mechanism responsible for leukopenia in copper deficiency has not been determined. However, histological evidence indicated that defective maturation of polymorphonuclear leukocytes, caused a reduction in the bone marrow granulocyte pool (119). Suboptimal leukocyte response to infection was reported in copper deficient patients (8). Thus, components of the immune system may be copper dependent as based on the data of Koller et al (21). They recorded antibody production, natural killer (NK)-cell cytotoxicity, synthesis of prostaglandin E2, and delayed hypersensitivity responses from rat pups fed an adequate (6 ppm), marginal (2 ppm), and copper

depleted (0 ppm) diet for 8 weeks. Liver copper and serum ceruloplasmin levels were measured which confirmed that immune measurements were from a copper deficient state in animals. Both of these indices were markedly decreased after 8 weeks on 0 ppm copper diet, although anemia did not develop. They theorized that at the end of the study period, depressed ceruloplasmin levels were of insufficient duration to produce anemia. Antibody response to the T-dependent antigen was severely suppressed in animals fed 0 ppm and 2 ppm copper, while delayed hypersensitivity and prostaglandin $E_2$ levels were unaffected. Natural killer-cell cytotoxic activity decreased significantly in rats fed the copper deficient diet. These NK cells are a nonphagocytic, nonadherent subpopulation of lymphoid cells referred to as granular lymphocytes (120). The natural cytotoxicity induced by these cells may aid in the control of viral and microbial infections, regulation of hematopoiesis, and graft-versus host disease. The results of this study demonstrated the impact of a marginally depleted or depleted copper diet on the immune function. Despite copper's role in erythropoiesis these animals showed no anemia, yet they exhibited a suppression in antibody production and NK cell activity. This suggested that the degree of copper depletion needed for immune dysfunction was less than that required for induction of the more accepted symptoms of deficiency. Furthermore, these changes in immune responses as a result of copper depletion or deficiency could increase susceptibility to viral and microbial infection. Other investigators supported this theory and demonstrated an increased susceptibility to infection in experimental animals (121,122).

The data of Failla et al (123) also supported the impact of a marginal copper deficiency. They reported a reduction in respiratory burst and the yeast killing ability of neutrophils in marginally copper deficient rats. Animals fed 2.7 ppm copper or less with 6.7 ppm serving as the control exhibited these reductions. They observed further decreases in tissue copper content and SOD activity when copper intake equalled or fell below 2.0 and 1.1 ppm. These alterations in neutrophil function and copper status surfaced after 1 week on the 0.6 ppm copper intake, but returned to normal in 1 week when fed the control intake of 6.7 ppm copper. They concluded that suboptimal copper intake quickly yet reversibly impaired neutrophil function.

With the advent of PN and use of parenteral solutions deficient in copper, deficiency has been reported both in adults and children (8,9,114,124). In 1972, Karpel and Peden (114) described copper deficiency in an infant with ileal atresia (pathological closure of the openning to the ileum) who had been maintained on TPN for 8.5 mo. Deficiency symptoms included normocytic, hypochromic anemia, as well as, neutropenia, hypocupremia and hypoceruloplasmin. The patient responded to oral copper therapy. Dunlap et al (14) reported similar findings in 2 older patients (45 y old and 12 y old) maintained on TPN without copper supplementation after extensive bowel surgery. After 3 months on home TPN the 45 year old patient (#1) returned to the hospital with the complaint of weakness. Significant laboratory findings included: hemoglobin, 9.4; hematocrit, 28; and 12% neutrophils (normal=46.6-76.8%) Erythrocyte morphology showed hypochromia with a dimorphous population. Bone marrow apiration showed

depressed erythropoiesis, increased iron stores, and definite sideroblastic changes (11% ringed siderblasts). Serum copper and ceruloplasmin levels were 11 ug/100 ml (normal=85-155 µg/100 ml) and 9 mg/100 ml (normal=10-40 mg/100 ml), respectively. These levels and the hematologic abnormalities returned to normal after this patient initially received 1 mg/d elemental copper that was later reduced to a maintainence dose of 0.4 mg/d. Patient #2 was not as severely hypocupremic and not anemic, but was neutropenic. Serum copper and ceruloplasmin levels were 32 µg/100 ml and 10 mg/100 ml, respectively. These levels, and the neutropenia corrected after 18 days of oral copper therapy (5 mg/d copper sulfate or 1.25 mg/d elemental copper). In patient 1 the hyperferremia and cytoplasmic sideroblastic granules disappeared with copper therapy which suggested that copper was associated in the incorporation of iron into hemoglobin.

Vilter et al (8) attributed copper deficiency in an infant to malabsorption as a consequence of systemic sclerosis and copper deficient TPN. The patient presented with leukopenia, neutropenia, and anemia but after IV administration of 1 mg/d copper sulfate, symptoms improved as quickly as 7 d. Sriram et al (116) found similar results in a 56 y old patient when TPN was initiated after a subtotal gastrectomy, massive bowel resection and partial colectomy. This patient developed neutropenia, leukopenia and severe hypocupremia (5 µg/100 ml;normal=70-140 µg/100 ml) approximately 1 1/2 years after long-term PN which confirmed copper deficiency. Within two weeks after the initiation of 2 mg/d of intravenous copper, the white blood count and neutrophil count returned to within normal limits. Serum copper

levels returned to normal after 12 weeks of copper therapy.

Fleming et al (118) conducted a prospective study to determine the onset of copper deficiency in 8 adults on copper deficient TPN for 3 to 13 weeks. Serum copper decreased in all patients after 1 week of TPN, and lasted 2 consecutive weeks in 5 of the 8 patients. Three of those 5 reported severe hypocupremia (< 30 µg/100 ml). The other 2 patients had decreases in serum copper detected at week 3 and 6. The mean rate of decline in serum copper during TPN was 10.8 µg/100 ml/wk. After the resumption of oral intake, the mean rate of increase in serum copper was 14 µg/100 ml/wk. More recently, Fujita et al (9) reported overt symptoms of copper deficiency in 3 adult cases which developed approximately 5.8 mo after the start of TPN. Leukopenia, neutropenia, low serum copper and ceruloplasmin were found in all 3 cases. Case #1 was an 18 y old male with a diagnosis of intestinal fistula. Admission laboratory findings were unimpressive. TPN was initiated and contained 85 to 113 µg/d of copper. Leukocyte and neutrophil count declined early (1.5 weeks), and by week 29 had decreased to 2200 and 440/mm3 (normal= 5000-9000; 2400-5490 $mm^3$), respectively. Plasma copper level gradually fell, and by week 27 had decreased to 3 µg/100 ml. A low ceruloplasmin level of 3.5 mg/100 ml was recorded at this time, as well as, a pronounced hypochromic anemia (red blood cell count: 259 x 104/mm3; hemoglobin,9.2 g/100 ml; and hematocrit,26.5%). A dosage between 0.3 and 1.8 mg/d of intravenous copper was administered at 29 weeks which resulted in the return to normal for plasma copper and ceruloplasmin values. Similar results were observed in the other 2 cases, however the onset of copper deficiency varied. Plasma copper

level decreased below 10 µg/100 ml in case 2 and 3 at week 9 and 34, respectively.

Other less common manifestations of copper deficiency include skeletal demineralization, arterial aneurysms, and blood coagulation. Bone changes of copper deficiency usually occur in neonates or young children (125-127). These included osteoporosis, metaphyseal changes, and micro-fractures. The cuproenzyme, lysyl oxidase, is essential for the cross-linking of collagen and elastin which are critical components in the formation and strength of arterial walls. According to Tilson (128), there seems to be a connection between vascular abnormalities and copper. Hepatic copper levels were decreased by 26% in 13 patients who died with abdominal aortic aneurysms. These data suggested that decreased hepatic copper stores were potential markers for the pathogensis of aortic aneurysms in man.

Cardiovascular pathology in animals deficient in copper revealed myocardial fibrosis (129), aortic rupture, intramural hemorrhages of the left ventricle, cardiac rupture, and myocardial infarction (130). Klevay et al (131) reported that experimental copper depletion significantly increased the total cholesterol value in a healthy 29 y old man from 202 to 234 mg/100 ml, yet hematologic indices remained normal. After copper repletion, total cholesterol fell to 198 mg/100 ml. These findings supported the author's previous hypothesis that hypercholesterolemia induced by copper deficiency could contribute to ischemic heart disease (132). Hypercholesterolemia was also found in rats fed increased zinc/copper ratios (10/0.25 or 20/0.5; control=10/2). Alterations in the activity of lecithin: cholesterol,

acyltransferase (LCAT), the enzyme responsible for free cholesterol clearance was suggested as a mechanism by which copper deficiency produced hypercholesterolemia (133). LCAT was diminished by 63.7% while plasma free cholesterol increased 74.2%. Klevay and Viestenz (134) also recorded electrocardiogram abnormalities in copper deficient animals; these included abnormal Q waves, large, wide R waves indicative of bundle branch block, and severe ST segment depression.

Little is known about the effects of copper deficiency on blood coagulation. Mann (27) recently found that Coagulation Factor V synthesized in the liver to accelerate conversion of prothrombin to thrombin, contained a constituent copper ion.

Multiple Trauma

Multiple trauma is a post-traumatic complex involving at least two injuries, each requiring hospitalization (135). These injuries could be one or more major fractures of long bones, pelvis, or vertebrae or injuries of the body cavities (head, thorax, or abdomen). The potential for multiple organ system involvement with disruption of the normal physiologic and metabolic functions of many organ systems is evident. The extent of injury is often graded according to the Injury Severity Score (ISS) for which the degree of injuries is assessed in each of 6 body zones and the score is calculated from the three most severely damaged zones; the range is from zero to 75 (136).

The Trauma Score is an index of injuries focusing upon the cardiovascular, respiratory, and neurological systems; each parameter is assigned a point value from 1 to 16 with the severity of injury

increasing as the numerical score decreases (137). Whereas, the Glasgow Coma Score, a component of the Trauma Score, is used to grade the degree of coma; the score ranges from 3 (least responsive) to 15 (normal response) (138).

Independent of the mechanism of injury, blunt or penetrating injuries activate an inflammatory response (139). This local response to injury serves three functions: (1) containment and removal of the injurious agent, if any, (2) removal of damaged tissue, and (3) repair, of affected tissue, with return of function to normal. In addition, a second process is stimulated, the acute-phase response, which results in increased hepatic synthesis of acute-phase proteins.

Metabolic Response to Injury

The metabolic response to trauma is characterized by an alteration in the body's neuroendocrine control mechanism (20). Such changes have a profound effect on the total body composition and vital organ function. The response to injury is essentially conditioned by hormonal and humoral mediators. Dead tissue, injured tissue, perfusion deficit, some resolving hematomas, and dividing and invading microorganisms activate mediator systems which regulate the metabolic manifestations of the response to stress.

Hormones play a major role in the metabolic response. Briefly, this complex response has 3 main components: (1) Catecholamine discharge inhibits insulin secretion and peripheral insulin action, and stimulates glucagon and ACTH production; (2) pituitary-adrenal and renal-adrenal stimuli increase corticosteriods, inhibit insulin

activity, and increase aldosterone, and sodium retention; and (3) posterior-pituitary stimulation produces water retention and antidiuresis (18). Most metabolic changes, which occur after the release of these hormones post acute injury, influence the use of nutrients as substrates.

The metabolic response to injury as originally described by Cuthbertson (16) consists of the initial ebb phase of variable duration and the subsequent flow phase. The ebb phase is characterized by low cardiac output and tissue perfusion, during which substrate utilization is depressed in most tissues in the body. Priority is given to resuscitation during this phase; nutritional support has a limited role until tissue perfusion is restored. Once this phase has passed, the flow phase follows. This hypermetabolic state is marked by high cardiac outflow and increased energy expenditure and nitrogen excretion. During this phase, insulin release is high but most of its metabolic effects are counteracted by the elevated levels of catecholamines, glucagon, and cortisol. The hormonal imbalance results in an increased mobilization of amino acids and free fatty acids from peripheral muscles and adipose tissue depots. A portion of these substrates is used for energy production, either directly, as glucose, or after being remodeled in the liver, as triglycerides. Another portion of amino acids participates in the synthesis of acute phase reactants in the liver. Provision of nutritional support during this hypermetabolic state in crucial.

The extent of these changes during both the ebb and flow phases is directly related to the severity of injury and to the patient's age,

sex and previous nutritional status as measured by serum albumin (140). Fellows et al (141) demonstrated that elderly patients who were nutritionally depleted prior to injury have a limited response and a higher incidence of morbidity and mortality. In addition there are data to support that nutritional depletion before trauma decreased the rate of catabolism after trauma (142,143). Other evidence suggested that both protein synthesis and catabolism increased in the trauma patient with sepsis (143,144). Other studies have shown the association between nutritional status and clinical outcome after injury (1,145). Nutritional support supplied in accordance with the metabolic milieu of the traumatized patient, greatly minimizes complications and achieves the goals of nutritional support.

Nutritional Support in Multiple Trauma

The desired effects of nutritional support in trauma patients are several: (1) to promote the end organ responses of nitrogen retention, (2) to preserve lean body mass, (3) to support hepatic protein synthesis and total body protein synthesis, and (4) to foster immune competence. The importance of nutritional support is clear, but the precise nature of fuel mixture to be used is still under investigation. Many injuries disrupt gastrointestinal continuity and preclude the immediate use of the gut. In these situations parenteral nutrition is clearly the choice. Parenteral support should be initiated after the stabilization of vital functions has been achieved post injury (146).

Cerra (1) devised a staging system based on the degree of metabolic stress from which nutritional guidelines are derived. The

degree of stress is measured according to urinary nitrogen loss, plasma lactate level, plasma glucose level, insulin resistence, oxygen consumption, and urinary 3-methylhistidine excretion. Clinical examples of each stage include starvation, stage 0; elective surgery, stage 1; polytrauma, 2; and sepsis, stage 3. Multiple trauma falls into the stage 2 category which correlates with an estimated caloric need of 40 kcal/kg/d; a nonprotein calorie:nitrogen ratio of 100:1; and 30 kcal/kg/d of non-protein sources (up to 50% fat as long as triglyceride clearance is intact). In addition, it is essential to provide adequate amounts of intracellular ions, vitamins, and trace elements during the acute catabolic phase. Unfortunately, the precise amounts are unknown, particularly regarding specific trace elements. Current practice is to provide a trace element formula daily or on alternate days. The AMA has published guidelines for essential trace element preparations for parenteral use (22). The suggested daily IV intake for zinc, copper, chromium, and manganese are given for the stable adult; additional intake in catabolic states is only given for zinc. This disparity clearly demonstrates that additional requirements for the other trace elements remain unknown.

Copper Administration in Parenteral Nutrition

A recommended dietary allowance for copper has yet to be established, but an Estimated Safe and Adequate Daily Dietary Intake (ESADDI) was given in 1989 (147). The recommended intake for copper in the healthy adult is 1.5 - 3 mg/day (147). The requirement assumes a 33% absorption rate. It is unclear how different diseases and injury

affect the absorption or losses and therefore the requirement for copper. In addition, adequate copper intake for the elderly population has not been determined. Several studies have found that the elderly consumed below the estimated safe and adequate dietary intake for copper (59,147).

The Expert Panel on Guidelines for Essential Trace Element Preparations for Parenteral Use published by AMA recommended 0.5 - 1.5 mg/d copper in the stable, non-hypermetabolic adult patient without additional allowances for acute catabolic states (22). This suggested dosage refers to total copper intake based on balance studies. The lower limit of this AMA copper intake (0.5-0.75 mg/d) resulted in acceptable balances in 8 critically ill patients, although 3 of these patients remained in slightly negative balances (13). These patients presented to the intensive care unit with one of the following diagnosis: bowel obstruction, pancreatitis, septicemia or peritonitis. Other investigators (8,10,14) have suggested intravenous (IV) copper intakes ranging from 1 mg to 1.6 mg/d for the correction or prevention of deficiency. Vilter et al (8) reported a dramatic response to the IV administration of 1 mg/d as copper sulfate to a copper deficient patient previously on TPN containing 0.1 mg/d copper. Leukopenia and neutropenia was reversed within 7 d, however there was no immediate change in the symptoms of anemia. Lowry et al (10) evaluated the response to IV copper supplementation by dividing the tumor-bearing patient population (n=24) into those with normal (99-136 µg/100 ml) or subnormal serum copper levels (<99 µg/100 ml). A good serum copper response was observed in 2 hypocupremic patients at a dosage of 39 to

71 µg/kg. Within the normal serum copper group, a daily parenteral copper intake of 60 to 65 µg/kg corresponded with unchanged or increased serum copper levels. While on TPN, another patient with copper deficiency, required an initial IV dose of 4 mg copper sulfate to restore serum copper, serum ceruloplasmin and hematological parameters to normal, and then a daily maintenance dose of 1.6 mg of IV copper sulfate (14). The amount of parenteral copper required to achieve copper balance in 28 adult patients with gastrointestinal disease maintained on TPN was 0.3 mg/day in stable patients, whereas requirements increased to 0.4 mg/d or 0.5 mg/d in patients with diarrhea or other excessive gastrointestinal losses (11). Inconsistency in dosage recommendations is clearly apparent in patients receiving PN. Furthermore, only two studies (11,118), documented the absence of copper as a contaminant in the TPN solutions. In 1978, Hauer and Kaminski (148) detected copper as a contaminant in the standard amino acid solutions FreAmine II (9-11 µg/L) and Aminosyn 5% (8 µg/L), as well as, in dextrose solutions (<10 µg/L) made by either McGaw or Cutter. In contrast, copper was not detected in the amino acid solutions Aminosyn 7% - 10% or Travasol 8.5% amino acid solutions, nor in dextrose solution made by Abbott (22). Hospital pharmacies have attempted to refine standardized TPN solutions to minimize any trace metal contamination since Hauer and Kaminski (148) first reported this information. Copper replacement in the MTP patient remains limited and unclear. Evidence is also lacking as to the effect of multiple trauma on copper needs in the elderly.

Copper Metabolism in Multiple Trauma

Changes in copper metabolism after acute injury have been documented, but reports are few and controversial (2,3). Copper balance in 10 male skeletal trauma patients between the ages of 18 and 54 y was determined over 5 - 6 days (2). Five patients received IV electrolyte/glucose infusions and 5 received blood or blood products in addition to IV electrolyte/glucose. Blood/blood product administration in this group of 5 patients received an average of 5 units whole blood. In addition, 2 patients each received either 1 unit packed red cells or 1 unit fresh frozen plasma and 2 other patients received 300 - 600 ml 25% albumin. Mean daily caloric intake (n=10) was < 500 kcal. Biological specimens analyzed for copper included 24 h urine, stool, emeses and drainages. Copper concentrations were determined by AAS. The mean daily copper in urine, stool and miscellaneous drains in the patients on electrolytes/glucose infusions measured 41 µg, 130 µg, and 18 µg, respectively. Skeletal trauma patients on both IV electrolytes/glucose and blood or blood products reported copper outputs of 35 µg, 4 µg, and 13 µg, respectively in urine, stool and miscellaneous drains. Patients who received only IV electrolytes/glucose infusions had greater mean copper losses than other patients who were given blood or blood products in addition to electrolytes/glucose infusions. However, this difference lacked statistical significance. Peak urinary loss occurred on metabolic balance day 3 for patients on IV electrolytes/glucose (98 ug) compared to day 5 or 6 for patients administered blood or blood products (57 ug). Mean copper balance was $-266 \pm 44$ µg, in patients given

electrolytes/glucose and +322 ± 109 µg in those given electrolytes/glucose and blood/blood products. One male patient (21 y old) who received the IV electrolytes/glucose infusions, had the largest negative mean copper balance (-487 µg). Another male patient (age 49), also received a total of 100 ml serum albumin. Consequently, the mean daily copper intake of 699 µg resulted in a mean balance of + 571 µg copper/d in this latter patient. They concluded that copper supplementation was still necessary since copper from blood products offered transitory relief, which over time would not sustain balance, and that the administration of blood products did not increase urinary copper output. Other investigators have shown that neither blood/blood product administration or small amounts of oral liquids were able to maintain serum or plasma copper concentrations in other patient populations on TPN (6,116,149).

Alterations in serum copper, ceruloplasmin and urinary copper excretion have been observed after thermal injury (3,150-152). Boosalis et al (3) determined serial serum copper, serum ceruloplasmin and 24 h urinary copper excretion in 23 thermally injured patients between the ages of 19 and 80 years. The second and third degree burns covered a mean total body surface area (TBSA) of 47% and were distributed as follows: 3 patients with < 20% TBSA burn; 7 with 21-40% TBSA burn; 7 with 41-60% TBSA burn; 6 with > 60% TBSA burn. Three patients with > 75% TBSA burn died. Nutritional support provided 2-3 g of protein/kg/d and 2-3 times basal caloric needs. All patients received either High Nitrogen Vivonex$^{R}$ and/or Ensure Plus$^{R}$. Five patients supplemented with Ensure Plus$^{R}$ received an average copper

intake of 1.45 mg/d. Vivonex$^{R}$ supplemented patients (n=18) received 1.25 mg/d of copper. Throughout their hospital course, 7 patients required TPN which was supplemented with 1.2 mg/d of copper. Serum copper, ceruloplasmin and urine samples were obtained within 48 hours of admission to the hospital and then every 2 days for the next 8 days, and weekly thereafter until discharge. Total concentrations of copper in serum was determined by flame AAS, whereas urine copper concentrations were analyzed by flameless AAS. The concentration of ceruloplasmin was performed using radial immunodiffusion. Depressed serum copper and ceruloplasmin paralleled one another and were related to severity of the burn. Serum ceruloplasmin extrapolated from a graph averaged 22 mg/100 ml (N=20-60 mg/100 ml). Patients sustaining >60% total body surface area (TBSA) burns had the lowest serum copper levels by wk 5, whereas serum ceruloplasmin was lowest at wk 4 postburn. Urinary copper excretion was within normal limits postburn days 1 to 4, but by day 7 excretion was elevated twice normal, and maximum between day 14 - 20 (2-3 times normal). The greater the percent TBSA burn the greater the urinary copper losses. Urinary copper excretion began to approach normal limits 53 - 59 days post burn. Two other studies in thermal injury reported similar elevations in urinary copper excretion (150,151).

Summarization of Literature

Multiple trauma induces a host of metabolic and physiologic responses that have profound impact on nutritional status. Copper metabolism is influenced by the body's response to trauma though the

mechanism is unclear. Micronutrient therapy in the multiple trauma patient involves an understanding of these metabolic alterations produced by trauma, and the practical application of that knowledge.

Limited research is available for adequately assessing the copper status of acutely traumatized patients. Reports of copper status in thermal injury and skeletal trauma have clearly demonstrated alterations in copper metabolism, though specific questions still remain regarding the duration of these aberrations and potential consequences. Data on copper status in multiple trauma are nonexistent. Therefore, this study evaluated copper nutriture in MTP during the acute phase of injury. Information on copper losses in available biological fluids (urine, wound drainage, chest tube drainage, nasogastric secretions, sputum, miscellaneous drains, and stool), and hence copper balance in multiple trauma patients during the first 5 days post injury was collected and analyzed for copper. In addition, serial serum copper and ceruloplasmin concentrations also were assayed. The influence of age, injury severity, clinical outcome, and nutritional intake on copper nutriture was examined.

# CHAPTER III

## COPPER STATUS IN MULTIPLE TRAUMA PATIENTS: MEASUREMENT OF COPPER BALANCE, AND SERUM COPPER AND CERULOPLASMIN


### ABSTRACT

Changes in copper metabolism have been reported in both thermal injury and skeletal trauma; data regarding copper status in multiple trauma patients (MTP) are nonexistent. The purpose of this study was to determine copper status in multiple trauma patients (MTP) and determine whether age, injury severity, clinical outcome, or nutritional intake influences the results. Twenty-four h copper losses and serum copper and ceruloplasmin were measured in 11 MTP with Injury Severity Scores (ISS) >12 at 24-48 h post admission. Collections of biological fluids (urine, nasogastric, chest tube, drains, stools) were analyzed for copper using atomic absorption spectrophotometry (AAS) and quantified over 5 days. Serial serum copper and ceruloplasmin were determined on days 1, 3, 5, 10, 15 and the day of discharge by ASS and rate nephelometry immunoprecipitation, respectively. Eight patients received parenteral nutrition (PN). Three received intravenous glucose/electrolyte infusions (IV). Urine ($n=11$) and nasogastric ($n=8$) copper losses were statistically higher than normal ($p<.001$). The mean $\pm$ SEM cumulative copper losses of urine, chest tube drainage, nasogastric secretions and other drains were 790 $\pm$ 116 ($n=11$), 833 $\pm$ 130 ($n=7$), 261 $\pm$ 46 ($n=8$), and 150 $\pm$ 58

(n=8) μg/5 d, respectively. Urinary losses represented 10 to 12 times the normal copper excretion. Serum copper on day 1 and ceruloplasmin day 3 were significantly higher than normal ($p<.025$). Cumulative copper balance in the IV group was -2266 μg/5 d, (0 copper intake), and -440 μg in the PN group ($\bar{x}$=204 μg/d copper intake). A significant difference was found in copper balance between the IV and PN groups on study days 3 and 5 ($p<.05$). No relationship was found between copper loss and ISS. A negative correlation existed between urinary copper excretion and age ($p<.05$). Patients in their twenties demonstrated the greatest urinary copper loss; patients > 50 y old demonstrated the least. There was no statistical relationship between clinical outcome or copper balance. Classical symptoms of copper deficiency were not observed; hemoglobin levels were below normal at discharge for all patients. The status of copper in MTP is altered. These patients are more susceptible to copper deficiency, and thus, may require increased copper supplementation to avoid either a marginal or classical deficiency. The physiological and biochemical effects of extensive copper loss in the MTP requires further evaluation.

## INTRODUCTION

The hypercatabolic response to injury as first described by Cuthbertson (1) is marked by an increased energy expenditure and nitrogen excretion. Knowledge concerning the mobilization, utilization and excretion of specific nutrients such as copper during varying degrees of trauma is incomplete. Data regarding copper status in multiple trauma patients (MTP) are nonexistent, however, alterations in copper metabolism have been reported primarily in patients with gastrointestinal disorders (2,3). Increased urinary copper excretion has been reported in both thermal injury and skeletal trauma (4,5). Data on additional losses via other routes (nasogastric, chest tube drainage, fistula drainage) are lacking in this hypercatabolic state. During an inflammatory response or after acute trauma serum copper and ceruloplasmin values rise (6,7). Kushner et al (8) described elevations in ceruloplasmin after acute injury which reflect an acute phase protein response. In contrast, Boosalis et al (5) reported a depression of both serum copper and ceruloplasmin in burn patients.

Copper is considered an essential nutrient in animals with an estimated safe allowance of 1.5 to 3 mg/day in humans (9). The American Medical Associations (AMA) guideline for IV copper administration recommend 0.5 to 1.5 mg/day in the stable patient, independent of clinical status or age (10).

This mineral mainly functions as a component of the cuproenzymes. Ceruloplasmin or ferroxidase mobilizes iron for incorporation into hemoglobin (11,12) and serves as an acute phase protein post acute trauma (8). Superoxide dismutase acts as a oxygen radical scavenger,

whereas lysyl oxidase aids in the crosslinking of collagen (11). Catecholamine synthesis, which is elevated after injury, requires the cuproenzyme dopamine β hydroxylase (13,14). In addition, cytochrome c oxidase, the terminal enzyme in the electron transport chain is key in respiration and oxidative phosphorylation. Copper deficiency may disrupt these enzymatic reactions resulting in impaired hemoglobin synthesis, wound healing, immune function, hormone synthesis, and oxygen detoxification.

Since the advent of parenteral nutrition (PN), copper deficiency continues to surface in a variety of patient populations (15). The classic overt symptoms of copper deficiency include microcytic, hypochromic anemia, neutropenia, and skeletal demineralization (16). Less obvious, but yet important manifestations of a marginal deficiency may involve the immune system and coagulation (17-19). Whether the MTP incurs the consequences of a marginal copper deficiency is unknown.

The response to trauma reveals hypercatabolism and may increase copper utilization depleting copper stores, thus compromising cuproenzyme synthesis and function. The purpose of this study was to provide information on copper balance, serum copper, and ceruloplasmin in MTP. The relationship between copper status and age, injury severity, clinical outcome, and nutritional intake also was examined.

## MATERIALS AND METHODS

### Study Design

Eleven adult patients with multiple trauma, 21 - 76 y of age ($\bar{x}$ = 45 y) were studied from May 1987 through July 1988 at the Level I

Trauma Center at Roanoke Memorial Hospital (RMH). Criteria for eligibility in the study included: Admission to the intensive care unit (ICU); multiple trauma with ISS > 12, Glasglow Coma Score > 4, Trauma Score > 4 on admission; 18 y of age or older; male or female; healthy prior to injury; and nutrition administered via parenteral support. Patients received standard trauma care upon admission to the ICU. The Institutional Review Committee on Human Research at RMH approved the study. Informed consent was obtained prior to participation in the study (Appendix A). Participants could withdraw from the study at any time.

In-service workshops were given to explain the study to all ICU nursing staff. Information sheets (Appendix B) were placed at each participants bedside as a reminder what needed to be collected and what laboratory tests were to be ordered by the medical resident using the assigned computer entries, Trauma Research I, II, and III.

Nutrition Support

Standard nutritional support consisted of 5, 8.5 or 10% Aminosyn (crystaline amino acids) mixed with 25% dextrose (Abbott) plus 10 or 20% lipid emulsion. Protein requirements were determined using 1 - 1.5 g protein/kg body weight. Physicians ordered PN with 1 mg copper administration on alternate days. All patients received antacid therapy (Maalox: aluminum hydroxide with magnesium hydroxide or Mylanta: aluminum hydroxide with magnesium hydroxide and simethicone) at a rate of 30 - 60 ml/h. Daily records were kept on the administration of other intravenous infusions including blood/blood

products and medications. Parenteral solutions were devoid of any metal contaminates.

24 Hour Collections

Daily 24 hour collections of available biological specimens (urine, stool, nasogastric secretions, chest tube drainage, and miscellaneous drains) were measured and analyzed for copper and nitrogen (urine) over 5 days starting within 24 hours of admission to the ICU. Urine from foley catheter bags was emptied into gallon plastic containers stored at the patient's bedside. Collection periods were 6 AM to 6 AM or 2 PM to 2 PM. Chest tube drainage was collected and volume recorded when the tube was changed.

Twenty-four hour urine volumes were recorded daily, and 100 ml aliquots preserved with 30 ml of 6N HCL in acid-washed trace element free containers. Urine samples were prepared by mixing 10 ml urine with 0.5 ml concentrated sulfuric acid. All instruments for transfering and mixing were made of polycarbonate plastic which were free of copper contaminants. Nasogastric, and drainage volumes were recorded and treated for digestion in a 1:3 ratio of 2N sulfuric acid for a minimum of 24 hours and periodically mixed by vortex to facilitate digestion. Five to 50 ml aliquots were transferred into acid washed, trace element free vials. Total volume of chest tube drainage was recorded and a 10 ml aliquot taken. Samples were diluted 1:5 with deionized water. Total concentration of copper in urine, nasogastric secretions, miscellaneous drains and chest tube drainage was determined by flame atomic absorption spectrophotometry (AAS)

(Appendix C). Urinary urea nitrogen was measured by the microkjeldahl technique. Stool quantity was insufficient for analysis.

Serum Copper and Ceruloplasmin

Serum copper and ceruloplasmin were measured on day 1, 3, 5, 10, 15, and within a day of discharge. A hospital staff Medical Techician obtained the blood by venipuncture. Care was taken to prevent hemolysis and external metal contamination. Plastic, metal-free syringes and/or tubes and containers were used to collect and store all specimens.

A red/black top vacutainer, a serum separator tube, was used to collect blood for serum copper and ceruloplasmin analysis. After blood collection, each tube was gently inverted 5 times to mix clot activator with blood. The blood was allowed to clot a minimum of 30 minutes but no longer than 1 h. The tube was centrifuged for 15 minutes at 1000 - 1300 G's and sent to AML. Specimen requirement for serum copper was 3 ml serum. Serum samples were diluted 1:5 with deionized water and assayed for copper using AAS. A 1 ml serum sample was collected for measurement of ceruloplasmin by nephelometry immunoprecipitation (Appendix D). All samples were analyzed in American Medical Laboratories (AML).

The following hematological parameters also were recorded when available: hemoglobin, hematocrit, red cell indices, reticulocyte count and white blood count with differential.

Copper and Nitrogen Balance

Metabolic balance for copper was determined by calculating the difference between copper intake and output. Nitrogen balance was calculated according to Blackburn et al (20) methodology [nitrogen intake (g) - (urinary urea nitrogen (g) + 3 g non-urea nitrogen)].

Statistical Methods

All data were analyzed using Systat (21). Mean values and standard error of the mean (SEM) were calculated for all parameters. The one-tailed "t" test (22) was used to determine the statistical significance between normal copper values and study samples. Differences in copper balance between the IV group and PN were summarized by the 2-tailed "t" test. A one way analysis of variance (ANOVA) was used to test significance between different variables when grouped by urinary copper losses. Pearson product moment correlation coefficients also were used to determine if relationships existed between variables (23). Differences were considered significant at $p<0.05$.

## RESULTS

Eleven multiple trauma patients with ISS ranging from 22 to 75 volunteered for the study. Four patients were male; seven were female. The average length of stay in the hospital and intensive care unit was 34 d and 18 d, respectively. A summary of each patient's injuries is found in Appendix E. Individual data for each parameter measured in this study are listed in Appendix F. Table 1 lists the age, sex, race, mechanism of injury, ISS, chief diagnosis on admission, outcome of each patient and whether PN was administered.

TABLE 1
MULTIPLE TRAUMA PATIENT PROFILE

| Pt# | Age | Sex | Race* | Mechanism of Injury** | Injury Severity Score*** | Chief Diagnosis | Outcome/ PN**** |
|---|---|---|---|---|---|---|---|
| 1 | 22 | M | C | GSW | 34 | Colon Laceration | S/no |
| 2 | 51 | F | C | MVA | 22 | Closed Head Injury (CHI) | S/yes |
| 3 | 21 | M | C | MCA | 26 | Pancreatic Transection,Left Adrenal & Kidney Laceration | S/yes |
| 4 | 29 | F | C | MVA | 30 | CHI | S/yes |
| 5 | 22 | F | C | MVA | 59 | Liver Laceration | S/no |
| 6 | 39 | M | C | GSW | 27 | Head Laceration | S/yes |
| 7 | 66 | F | C | MVA | 75 | Transected Aorta | S/no |
| 8 | 27 | M | B | MVA | 75 | Transected Aorta | S/yes |
| 9 | 72 | F | C | MVA | 41 | Partial Transected Aorta | D/yes |
| 10 | 76 | F | C | MVA | 33 | Partial Transected Aorta | D/yes |
| 11 | 71 | F | B | FALL | 38 | Smoke Inhalation | D/yes |

* Race: C: Caucasian; B: Black.

** Mechanism of Injury: GSW: Gun Shot Woung; MVA: Motor Vechicle Accident; MCA: Motorcycle Accident.

*** Injury Severity Score

**** Outcome/PN
S: Survived
D: Died
PN: Parenteral Nutrition; received PN: yes or no

Copper Losses

Daily urinary copper levels were significantly elevated in all eleven patients ($p<.001$). Urinary copper output represented 10 to 12 times the normal copper excretion. Daily and cumulative mean urinary copper excretion were 158 ± 27 and 790 ± 116 µg, respectively, and on the average accounted for about half of the total copper loss over the 5 days studied. The peak mean daily loss occurred on study day 2. Daily variations between individual patients were small. Patient #5, age 22 with an ISS of 59 excreted the greatest cumulative urinary copper, 1677 µg/5 d. This patient's peak loss of 539 µg occurred on day 2.

The mean daily and cumulative copper content in nasogastric secretions, chest tube drainage, and other drains are listed in Table 2. Stool specimens were collected from 3 patients but amounts were inadequate for analysis. The remaining 8 patients had post traumatic intestinal ileus and stool collection was zero. Day 5 nasogastric (NG) copper content (53 µg) was greater than normal levels ($p<.025$, $n=8$). Mean cumulative NG losses were 261 µg. Nasogastric tubes were pulled preceding day 5 in 3 patients. Copper losses from chest tube drainage were similar to urine losses and averaged 167 ± 26 µg/d or 833 ± 130 µg for the 5 d ($n=7$). Other drains consisted of wound, jejunostomy and gastrostomy drainage. Copper output in miscellaneous drains ranged from 2.8 to 182 µg ($n=8$). Mean cumulative copper from drains was 150.3 ± 58 µg. Total cumulative copper losses from all sources measured over the 5 study days totaled 2034 µg.

Listed in Table 3 are patients sorted by cumulative urinary output

TABLE 2

MEAN DAILY & CUMULATIVE COPPER LOSSES

| | Copper Loss (µg) | | |
|---|---|---|---|
| Specimen | Daily | Cumulative | NORMAL(daily) |
| Urine (n=11) | 158±27* | 790±116 | 5-25 µg/d |
| Nasogastric Secretions (n=8) | 53±16 | 261±46 | 1 mg/d |
| Chest Tube Drainage (n=7) | 167±26 | 833±130 | NA** |
| Miscellaneous*** Drainage (n=8) | 37±17 | 150±58 | NA |
| Stool | - | - | 1 mg/d |
| TOTAL | 415 | 2034 | |

* ± SEM.
** Not applicable.
*** Jejunostomy, gastrostomy and wound drainage and sputum.

TABLE 3

CUMULATIVE URINARY COPPER LOSS

| Copper Loss Group | CMUR5* | Patient # | Age | ISS |
|---|---|---|---|---|
| >1000 µg | 1677 | 5 | 22 | 59 |
| | 1218 | 4 | 29 | 30 |
| | 1192 | 3 | 21 | 26 |
| MEAN | 1362 | - | 24 | 38 |
| 600 - 1000 µg | 792 | 1 | 22 | 34 |
| | 699 | 9 | 72 | 41 |
| | 646 | 11 | 71 | 38 |
| | 611 | 6 | 39 | 27 |
| MEAN | 687 | - | 51 | 35 |
| < 600 µg | 520 | 10 | 76 | 33 |
| | 493 | 7 | 66 | 75 |
| | 491 | 2 | 51 | 22 |
| | 347 | 8 | 27 | 75 |
| MEAN | 463 | - | 55 | 51 |

*CMUR5: Cumulative Urinary Copper Day 5

categories of > 1000 µg, 600 - 1000 µg and < 600 µg. There was a statistical difference between age in the >1000 µg group and the <600 ug group ($p<.05$) but not ISS. A significant negative correlation existed between urinary copper excretion and age ($r=.55; p<.05$, one tailed test). As age increased, urinary copper excretion decreased. The maximum copper excretion was observed in 3 patients in their twenties ($\bar{X}$ = 24 yr). These patients all suffered a severe liver laceration among other injuries. Patient #8 who lost the least cumulative urinary copper (347 µg) over the 5 days suffered a spinal cord injury and was age 27 with an ISS of 75.

Urinary Urea Nitrogen Output

Urinary urea nitrogen (UUN) excretion averaged 16 g/d over the 5 study days but no significant relationship was found between UUN excretion and urinary copper. All patients except #4 who received PN study days 1 through 5 showed a mean negative nitrogen balance at the end of the 5 study days. Nitrogen balance ranged between +2.4 g and -18.5 g; mean equaled -9.2 g. The mean protein equilvalent of these nitrogen losses equaled -58 g. The mean daily balance ($n=11$) on study day 1 was -14.3 g which improved to -5.0 g by day 5. The mean nitrogen balance for each individual patient is shown in Table 4 along with the cumulative nitrogen balance and the protein equilvalent of these nitrogen losses.

Nutritional Support

Three patients received infusions of 5% dextrose during the first

TABLE 4

NITROGEN BALANCE DATA AND PROTEIN EQUIVALENT

| | Nitrogen Balance | | Protein Equivalent* | |
|---|---|---|---|---|
| Patient # | Mean (g/d) | Cumulative (g/5d) | Mean (g/d) | Cumulative (g/5d) |
| 1 | -15.2 | -76.2 | -95 | -476 |
| 2 | -10.5 | -52.2 | -66 | -326 |
| 3 | - 7.3 | -36.7 | -46 | -229 |
| 4 | + 2.4 | +12.2 | +15 | + 76 |
| 5 | -18.5 | -92.6 | -116 | -579 |
| 6 | - 1.3 | - 6.7 | - 8 | - 42 |
| 7 | -14.0 | -69.7 | -88 | -436 |
| 8 | -12.0 | -59.3 | -75 | -371 |
| 9 | - 7.4 | -36.7 | -46 | -229 |
| 10 | - 7.4 | -37.2 | -46 | -233 |
| 11 | -10.0 | -49.5 | -63 | -309 |
| AVERAGES | - 9.2 | -46.0 | -58 | -287 |

* Protein Equivalent = 6.25 x grams of nitrogen

5 days of the study while 8 received PN solutions with 8.5 - 10% crystalline amino acids and 25 - 30% dextrose plus 10 or 20% lipid emulsions on study day 1 (n=1), 2 (n=1), and 3 (n=6). Protein needs were estimated at 1.0 to 1.5 g/kg body weight. The mean caloric intake in the PN and IV group was 2102 kcal/d and 401 kcal/d, respectively. Daily protein and caloric intakes are listed in Table 5. The IV groups daily infusions of 5% dextrose were all initiated on study day 1. Only one patient, #4, received PN on study days 1 - 5. Patient #11 began PN on study day 2 while the remaining patients began on study day 3. There was no statistically significant relationship between protein intake and urinary copper excretion or total copper losses. Mean cumulative copper balance in the IV group was -2266 µg (n=3) and -440 µg (n=8) in the PN group.

There was no difference found in urinary excretion of copper between the IV and PN group. Mean cumulative copper loss in the IV and PN groups was 2266 µg and 1458 µg, respectively. There was no significant difference found between total copper loss and these 2 patients groups. Patient (#8) had the lowest urinary copper value and received PN on study days 4 and 5. The values for urinary copper on study day 3, 4 and 5 were 138, 86 and 88 µg, respectively. In contrast, patient (#5) lost the most urinary copper (1677 µg/5 d) and only received IV glucose infusions.

Copper intakes varied among the patients given PN and were usually less than the 1 mg/alternate days ordered (Table 6). During the first 5 study days, IV copper intakes ranged from 137 to 1753 µg. Patients (n=6) first received an average of 495 µg copper sulfate on study day

TABLE 5
MEAN DAILY CALORIC and PROTEIN INTAKE

| Patient # | IV Dextrose (kcal) | Parenteral Nutrition Calories (kcal) | Protein (g/d) |
|---|---|---|---|
| 1 | 330 | - | - |
| 2 | - | 2212 | 84 |
| 3 | - | 2126 | 83 |
| 4 | - | 2392 | 107 |
| 5 | 364 | - | - |
| 6 | - | 3140 | 120 |
| 7 | 509 | - | - |
| 8 | - | 3140 | 120 |
| 9 | - | 1598 | 63 |
| 10 | - | 1108 | 68 |
| 11 | - | 1099 | 42 |

TABLE 6
COPPER INTAKES (µg)

| Patient # | Study Days 1 | 2 | 3 | 4 | 5 | TOTALS |
|---|---|---|---|---|---|---|
| 2 | 0 | 0 | 887 | 0 | 0 | 887 |
| 3 | 0 | 0 | 556 | 0 | 1000 | 1556 |
| 4 | 0 | 0 | 450 | 0 | 1000 | 1450 |
| 6 | 0 | 0 | 753 | 0 | 1000 | 1753 |
| 8 | 0 | 0 | 0 | 0 | 1000 | 1000 |
| 9 | 0 | 0 | 189 | 0 | 1000 | 1189 |
| 10 | 0 | 0 | 137 | 0 | 0 | 137 |
| 11 | 0 | 175 | 0 | 0 | 0 | 175 |
| | | | | | MEAN INTAKE | 204/d |

3. Four of those 6 patients later received 1000 µg on day 5. Over the 5 study days, 3 patients received only one dose of copper. These 3 patients received 175 µg, 137 µg and 1000 µg of copper on study days 2, 3, and 5, respectively. There was no significant relationship between copper intake and urinary excretion. Patient #4 received PN on study day 1 and received a total of 1450 µg copper over the 5 study days, yet had a negative cumulative copper balance. Mean copper intake in elderly patients (n=5) > 50 y of age was 278 µg/d, and 960 µg/d in patients < 39 y of age (n=6).

Copper Balance

The mean cumulative copper balance on study day 5 was -938 µg ± 315 (n=11). Daily and cumulative copper balances are given in Table 7. Mean copper balance on study days 1, 2, 3, and 4 was negative in all patients. Nine patients had negative cumulative copper balances by study day 5 ranging from -153 to -3524 µg. Six of those 9 subjects received PN by day 1 (patient #4), day 2 (patient #11), and day 3 (patients #3,6,8,10). Cumulative balances greater than -1000 µg were reported in 5 patients, although two of these patients (#10 and 11) received PN. The largest cumulative negative copper balance of -3524 µg was found in patient #5 who received only IV infusions. This patient's copper balance on study day 5 was -622 µg. Patient #6 received the greatest amount of copper over the 24 h collection period, 1753 µg, yet cumulative copper balance was -333 µg. The 2 patients (#2,9) with cumulative positive balances (+149 and +266 µg respectively) received PN. The daily copper balance on study day 5 was

TABLE 7

COPPER BALANCE
(µg/d)

| Study Day | Mean ± SEM Daily | Mean ± SEM Cumulative |
|---|---|---|
| 1 | - 318.0 ± 33 | - 318.0 ± 33 |
| 2 | - 322.0 ± 62 | - 639.3 ± 88 |
| 3 | - 96.0 ± 22 | - 735.2 ± 186 |
| 4 | - 359.0 ± 46 | -1094.0 ± 218 |
| 5 | + 156.3 ± 54 | - 938.0 ± 315 |

postitive in 5 patients (#3,4,6,8,9) who received PN supplemented with 1000 µg copper on day 5. There was a significant difference in cumulative copper balance between patients receiving IV infusions (n=3) and PN (n=8) on study days 3 and 5 ($p<.05$). Table 8 gives the data for copper balance between the PN and IV groups.

A significant correlation between cumulative nitrogen balance and cumulative copper balance was found ($r=0.82; p<.0001$). Mean daily and cumulative nitrogen balance were negative.

Serum Copper and Ceruloplasmin

The mean serum copper and ceruloplasmin concentrations are listed in Table 9. Mean serum ceruloplasmin levels and serum copper levels were within normal limits throughout the hospital course. Patient #9, age 72, had mildly depressed serum copper and ceruloplasmin throughout the course of the study.

Clinical Outcome and Injury Severity

The 3 patients who died were 71, 72, and 76 years of age with ISS of 38, 41, and 33, respectively. All received PN. There was no significant difference in copper loss between these patients and survivors, though mean cumulative loss was less, 1253.68 µg. Mean cumulative copper balance was -753.4 µg in non-survivors and -1007 µg in survivors. There was no stastistical relationship between clinical outcome and copper balance. Mean serum copper and ceruloplasmin values in the 3 non-survivors were less than survivors. Both serum copper and ceruloplasmin were moderately depressed throughout the study in the 72

TABLE 8

MEAN COPPER BALANCE BETWEEN PARENTERAL NUTRITION GROUP (PN) INTRAVEOUS GROUP PATIENTS (IV)
(μg/d ± SEM)

| Study Day | PN Group | IV Group |
|---|---|---|
| 1 | - 289 ± 30 | - 393 ± 75 |
| 2 | - 264 ± 23 | - 477 ± 192 |
| 3* | 69 ± 117 | - 563 ± 114 |
| 4 | - 314 ± 42 | - 478 ± 95 |
| 5* | 358 ± 157 | - 382 ± 103 |

* PN and IV Groups significantly different ($p<.05$).

TABLE 9

MEAN SERUM COPPER AND CERULOPLASMIN CONCENTRATIONS
(mean ± SEM)

| Study Day | Serum Copper (µg/100 ml) | Serum Ceruloplasmin (mg/100 ml) |
|---|---|---|
| 1 | 86.5 ± 8.3 | 41.2 + 4.4 |
| 3 | 107.5 + 9.7 | 45.6 + 5.2 |
| 5 | 116.5 + 9.3 | 51.6 + 4.5 |
| 10 | 136.3 + 10.4 | 62.9 + 5.7 |
| 15 | 149.7 + 15.0 | 63.6 + 8.6 |
| Discharge | 160.1 + 17.8 | 74.5 + 6.7 |

(Normal serum copper=70-165 µg/100 ml;normal serum ceruloplasmin=22-70 mg/100 ml).

year old (patient #9).

Injury severity scores ranged from 22 to 75. Patient's #7 and #8 had the highest ISS of 75 each and excreted the least urinary copper. Patient #5 with an ISS of 59 had the greatest cumulative copper loss. There was no significant relationship between ISS and copper losses or copper balance.

Blood and Blood Product Administration

All patients received blood and/or blood products during collection of study samples. Appendix G gives the estimated blood loss from surgery, when known, and blood replacement during the first 5 study days. Patient #5 received autotransfusions in excess of 35 units of blood/blood products due to an estimated blood loss of 15 liters during surgery on study day 2. This patient lost the most copper (3524 µg/5 d). There was however, no significant relationship between blood infusions and copper output.

Hematological Parameters and Serum Albumin

Appendix H summarizes individual values for hemoglobin, hematocrit, red cell indices, reticulocyte count, white blood count with differential, and serum albumin on study days 1, 2, 3, 4, 5, 10, 15, and the day of discharge when available. Serum albumin levels were depressed in all patients (<3.0 g/100 ml) during the 5 study days.

Patient #4 suffered from a "low grade" chronic anemia. Patient #3 reported decreased serum iron and transferrin, in addition to a low hemoglobin.

## DISCUSSION

The care of the MTP involves understanding the metabolic alterations produced by trauma which in turn influences nutrient needs. Although there were reports on copper status after injury, they were specific to thermal or skeletal injury (4,5). The present study provided several important observations regarding copper balance, serum copper and ceruloplasmin values in MTP.

The 24 h collections of biological fluids for the measurement of copper balance provided previously unrecorded copper losses. All 11 MTP had an elevation in urinary copper excretion during the 5 day study period. Daily urinary losses represented 10 - 12 times the normal which differed widely from other research groups. Boosalis et al (5) found urinary excretion of copper to be within normal limits in thermally injured patients day 3 postburn and twice the normal copper loss by day 7 postburn. Maximum urinary copper losses occurred at 2 wk postburn and were 2 to 3 times normal. Excretion returned to normal during wk 7 after injury. These urinary copper determinations were performed intermittently rather than daily. Another study demonstrated urinary copper loss at twice normal in 10 skeletal injury patients the first day after injury with peak loss at three times normal by day 3 (4). In the present study, peak urinary copper losses occurred a day earlier.

One possible mechanism for the increase in urinary copper may have been related to depressed serum albumin concentration. Nutritional status in trauma patients in part, relies on the evaluation of serum albumin (1). Approximately 6 - 7% of copper is normally bound to

albumin which functions as the immediate transport form of copper in plasma. If serum albumin levels fall, more copper would be available to bind amino acids in the plasma and subsequently greater amounts of amino acid-copper complexes would be filtered by the kidney resulting in greater urinary copper loss. Hypoalbuminemia (<3.0 g/100 ml) was present in all MTP during the 5 day study. Thus, the lack of sufficient levels of serum albumin to bind copper could have resulted in ultrafiltrable losses of amino acid-copper complexes. This might have explained some of the urinary copper loss, but probably not the 10 to 12 fold increase observed in these MTP. Lowry et al (10) suggested that the dissociation of the copper-albumin complex resulted in urinary copper losses at twice the normal magnitude in tumor bearing patients on TPN for 5 to 42 days. Shike et al (11) also reported that urinary copper excretion in patients receiving TPN was twice normal. Based upon the data from this present study, it was not possible to identify why MTP lost the magnitude of copper in their urine.

Other sources for copper loss were quantified in other biological fluids besides urine. The copper output in chest tube drainage, nasogastric fluid, and miscellaneous drainage demonstrated other routes for loss which usually go unrecorded. Total cumulative mean copper loss from all fluids of 2034 µg over 5 days may predispose MTP to a marginal copper deficiency especially when coupled with the sporatic copper supplementation which occurred in these MTP.

A significant correlation surfaced regarding age and urinary copper excretion; that is, urinary copper loss decreased as age increased. Average losses were 570 µg in patients older than 50 years

of age (n=5) and 973 µg in patients 39 and younger (n=6). The trend for less excretion in the elderly subjects was unaffected by nutritional intake. The greatest urinary copper losses were in individuals in their twenties. Bunker et al (24) have suggested that elderly patients may have reduced cellular uptake of copper. Also poor nutritional status prior to injury may reduce the rate of copper loss in the elderly subjects (25). The copper intake in elderly patients from the current study was less than younger patients. However, in this study patients age 60 and over were assessed as healthy before injury. What should not be ruled out are differences in body composition and copper stores between the elderly and younger population. Studies have supported the concept of decreased lean body mass and reduced cellular metabolism with aging (26,27). The decline in muscle mass could be associated with a decreased storage depot for copper since approximately 50% of the total body copper is located in the large muscle mass. If copper stores in the elderly were limited for this reason, less would be mobilized or lost from the tissues and less excreted. Some level of regulation of copper metabolism may also have occurred in patients in the present study which protected the elderly against copper depletion and the younger patients against copper toxicity. This would agree with the data of Turnlund et al (28) which demonstrated that the absorption of copper was dependent of copper intake and it adapted more rapidly to a low copper intake (protection against depletion) than to higher copper intakes (protection against accumulation of copper). In contrast, Bunker et al (24) found that housebound elderly people did not compensate for low

copper intakes with increased absorption. Although the 3 nonsurvivors in the present study were all elderly, no statistical difference in copper loss was found between them and survivors. Additional studies of elderly MTP are required to determine whether current IV copper recommendations are adequate in this age group.

The relationship between PN and its possible affect on increased urinary copper was inconsistent with previous work. Clinically stable patients receiving PN with copper supplementation have reported twice normal urinary copper loss rather than 10-12 times normal (2,3). Data revealed no difference in urinary copper losses between the PN (n=8) and IV (n=3) group in the present study, though mean cumulative copper excretion in the IV group was greater. Lack of a significant difference may have resulted from the disparity in patient number between the groups.

Four patients with copper supplementation >1000 µg per the 5 study days had similar copper excretion as those without copper supplementation (n=3). Actually, the patient who excreted the greatest quantity of copper never received any IV copper or PN. Urinary losses appeared to be unaffected by the amino acid concentration in PN patients when compared to urinary copper losses in IV patients. There data conflicted with observations by other investigators that suggested that amino acids in PN resulted in an elevated plasma amino acid concentration and caused the amino acid bound fraction of plasma copper to increase, which resulted in increased urinary excretion of amino acid-copper complexes and increased losses of copper in the urine (29,30).

The mean parenteral copper intake of 1018 µg over a 5 day period, with a mean of 204 µg/d, fell short of the AMA guidelines for trace element preparations for parenteral admininstration of 1 mg/d of copper TPN solutions (10). These AMA recommendations are for clinically stable not hypercatabolic patients.

Study results failed to demonstate a relation between injury severity and copper losses as hypothesized. Investigators reporting a relationship between injury severity and copper loss have been unique to thermal injury (5). The greater the percent body surface burned the greater the urinary copper excretion. A direct comparison with thermal injury and multiple trauma is difficult because of the heterogeneity in patients and their injuries. The present data regarding the effect of injury severity on copper loss or balance should be interpreted with caution since the sample size was small and injuries with ISS <12 were excluded. The question arises whether more patient observations encompassing a wider range of ISS (0 - 75) would have revealed a significant relationship between injury and copper output.

In the present study, the duration of these copper losses are unknown and may vary because of individual patient response to injury. The 24 hour measurements in this study were limited to 5 days which raises the question of how long these catabolic losses might have lasted. Elevated urinary copper excretion and chest tube losses pose the greatest concern and have the most potential to continue beyond 5 days. Although, Boosalis et al (5) observed normal urinary copper excretion initially, and twice normal at day 7 after thermal injury, by week 2 excretion of copper was 2.5 to 3 times normal. Cohen et al (31)

reported increased urinary copper in 2 of 14 patients at 2 months postburn. Although increases in urinary copper were observed in these other reports, urinary copper levels never exceeded 3 times normal in the present study. The magnitude of copper loss in these MTP must not be ignored since the cuproenzymes affect physiological functions necessary for hemoglobin synthesis, wound healing, hormone synthesis, immunity and blood clotting.

The mean daily copper balances (n=11) were positive on study day 5 (+156 µg) which reflected the 1000 µg of copper administered to 5 patients on day 5. However, mean cumulative copper balance by study day 5 was negative (-938 µg) which meant that copper losses exceeded copper supplementation. The difference found in copper balance between the IV group and the PN group indicated copper administration improved copper balance in MTP. Although copper balance in the PN group was positive on days 3 and 5, 6 of the 8 patients in this group were still in negative cumulative copper balance by study day 5. This change in copper balance demonstrated a dose related response since 6 of 8 patients in the PN group were in negative cumulative copper balance by study day 5. The AMA guidelines for IV copper therapy proposed that 0.5 - 1.5 mg/d of copper would maintain balance in stable patients, yet recommendations remain unclear for hypercatabolic states such as MT (10). Four patients who received adequate copper based on AMA guidelines still had negative cumulative copper balances on study day 5. The data from this study suggested that actual copper supplementation ranging from 0.14 to 1.0 mg/d was inadequate to maintain copper balance in the MTP. Another point to be made, which

complicates the situation further, is that the amount of copper ordered seldom was the actual amount administered. Hence, the potential for copper deficiency becomes even more obvious. These results question just how appropriate the current AMA copper recommendations are for hypermetabolic states like MTP.

A recent report reviewing the current knowledge about trace element metabolism indicated a copper intake of 0.3 mg/d was sufficient to achieve balance in the stable adult patient receiving PN (32). If this maintenance dose were administered daily to the MTP in this study, cumulative copper balance would have been less negative. However, if stool, sweat, ventilator or respiratory, and integumentary losses were also taken into account, balance would be more negative in most MTP. Fecal copper was not determined in this study due to insufficient samples, though the lack of sample volume does not preclude stool collecting in the bowel implicating another source of copper loss. It has recently been established that fecal copper decreases when dietary copper decreases, and increases when copper intake increases (33), thus the level of copper in the diet determines the rate or percent of absorption. This probably plays a role in the elimination of excess dietary copper and is a factor in the control of total body copper balance. The cause of the negative balances for copper are most likely attributed to the excessive copper losses from urinary and chest tube drainage. Other factors which probably influenced copper balance included copper loss in nasogastric secretion, altered copper utilization, recurring infection, changing clinical condition, and inadequate copper supplementation.

Urinary urea nitrogen excretion was not a reliable index of urinary copper output, however, a correlation did exist between the cumulative nitrogen balance and copper balance ($r=.82$, $p<.0001$). A positive copper balance was associated with increased nitrogen retention which was probably a result of the administration of PN with added copper. Patients who received PN in the present study demonstrated either positive or less negative cumulative copper and nitrogen balances than patients who received IV electrolytes and glucose. Wolman et al (34) reported a similar finding but between zinc and nitrogen balance in 24 patients who had gastrointestinal disease; a positive zinc balance was associated with a positive nitrogen balance. The clinical significance of the relationship between copper balance and nitrogen retention revealed that nitrogen balance could serve as an index of copper balance. In addition, these findings endorsed the early administration of PN supplemented with copper in MTP.

The usual 50% rise in the acute phase protein, ceruloplasmin post injury or stress was expected (8). Instead, the mean level of serum ceruloplasmin and serum copper were within normal limits throughout the hospital course. Since approximately 90% of copper is bound to ceruloplasmin, serum copper and ceruloplasmin values should and did track one another. Unlike previous studies, the present study failed to see any significant elevation in ceruloplasmin or serum copper following injury. The etiology was probably multifactorial. It is possible the rate of hepatic synthesis of ceruloplasmin was increased, but only matched the rate of catabolism, thus blunting the expected

rise in both ceruloplasmin or circulating serum copper levels. This theory was suggested in thermally injured patients, however, serum copper and ceruloplasmin values were decreased rather than being normal or elevated (5). If ceruloplasmin catabolism equaled synthesis, then circulating copper and ceruloplasmin levels would be expected to be normal. In the normal steady state conditions, a significant correlation existed between ceruloplasmin and serum copper (35). How this affects the MTP copper status remains uncertain especially since tissue stores can enable serum levels to be maintained for sustained periods before the onset of acute copper deficiency. The hyperexcretion of urinary copper or other sources of copper losses were not shown to reduce serum copper values in this study.

The lack of correlation between serum copper and copper balance was not an unexpected finding. All patients were in negative copper balance sometime during the 5 study days but they did not have low plasma copper levels. Shike et al (3) concluded that plasma copper values did not reflect the balance state in mildly malnourished patients. Others suggested that the development of hypocupremia under conditions of negative copper balance would be unpredictable and may manifest itself only after several months on TPN (14,36). Therefore, it could be suggested that normal serum copper levels in these MTP were maintained during the periods of negative copper balance by the continued release of copper from the liver. Serum copper would be declined once copper stores were depleted. Furthermore, based on the present data, the amount of copper required to achieve copper balance should not be determined by the levels of serum or plasma copper.

In the present study, the classical symptoms of copper deficiency were not observed. As observed by Vilter et al (37), deficiency symptoms of neutropenia, leukopenia and anemia occurred after serum copper values became significantly reduced (<20 µg/100 ml); the copper deficiency developed after 2.5 months of TPN. In another study, hypocupremia was not associated with clinical evidence of copper deficiency (38). These data related the onset of clinical symptoms to the severity and duration of hypocupremia. The lack of overt deficiency symptoms in these MTP does not discount the possibility that subtle defects in immunocompetence may have occurred as previouly reported (17,18). Another observation about this study was that all patients were discharged with below normal hemoglobin values (mean=10.6 g/100 ml). Whether copper played a physiologic role would be speculation.

The administration of blood and blood products which contain copper may also be a contributing factor temporarily maintaining serum copper levels (39). Controversy surrounds whether the amount of copper supplied by blood transfusions is a significant nutritional source (2,4,40). All MTP received blood transfusions during the first 5 days of this study for blood loss replacement or restoration of hemodynamic stability. The volume of replacement was not related to the severity of injury. No significant difference was found between patients receiving multiple transfusions and urinary copper output. Thus, the dumping of copper in the urine after blood and blood product administration was refuted. One patient however, who was autotransfused (reinfusion of patient's own blood) with approximately

35 units reported the greatest urinary copper excretion. The serum copper concentration in this patient remained within normal limits throughout the study. Askari et al (4) observed similar results in patients with skeletal injury. In a group of neonates, Lockitch (40) found no effect on serum copper levels after the transfusion of packed red blood cells, and brief yet significantly raised values after plasma transfusions. In addition, Lowry (2) found that blood products were inadequate towards the maintenance of serum copper and ceruloplasmin concentrations in most patients. The results of the present study suggested that the affect multiple transfusion may have had on urinary copper excretion or circulating copper levels was minimal. It was doubtful that the elevation of serum copper by transfusion would confer any clinical benefit.

The present study confirmed that MTP excreted excess copper during the catabolic phase of injury, and that the losses were more striking than previously reported (4,5). In addition, losses were independent of PN intake, clinical outcome, and injury severity but influenced by age during the 5 study days. Furthermore, the copper loss from chest tube drainage was marked enough to alter copper balance. Allowances for these and body surface losses could make the difference between a positive or negative balance. The cumulative negative copper balances reported, if protracted over an extended period, may lead to a gradual depletion of copper reserves, and consequently precipitate either a classical copper deficiency or a subclinical deficiency. Additional copper supplementation may be required, particularly for young MTP, but only after careful monitoring of the amount of copper actually received

from whatever the source.

Factors which influence copper status of MTP should be considered when providing replacement or maintenance doses. These include pre-existing nutritional status, age, accurate nutrient administration, and individual clinical differences. In addition to serum copper, ceruloplasmin, and copper balance, other static or functional indices are needed to assess the copper status of MTP. The determination of erythrocyte SOD activity or neutrophil function may prove more effective in the assessment copper status after MT. These parameters are more sensitive indicators of copper depletion and therefore, could detect marginal copper deficiency earlier than traditional parameters (41). The results of the present study justify the conclusion that greater attention be given to copper nutriture in MTP. Future studies should consider longer duration and larger patient numbers over a wider range of ISS.

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# CHAPTER IV

# DISCUSSION

Several concerns regarding copper status after multiple trauma remain speculative and complex, yet worthy of discussion. These include the effects of inflammatory processes on copper, the influence of copper on the immune system, copper bioavailability after zinc supplementation and aggressive antacid therapy, how the hormonal milieu accompanying trauma alters copper nutriture, and nutritional status prior to injury.

The inflammatory response post injury varies among patients but partially depends on the function of neutrophils. The cuproenzyme, superoxide dismutase found in neutrophils serves as an free radical scavenger. The stress response to trauma causes an increase in neutrophils which serve a central role as a cellular mediator of acute inflammation (139). Interleukin-1 released in response to tissue damage induces the release from bone marrow of preformed immature and mature neutrophils and activates these neutrophils which results in their elevation. Patients in the present study typified this neutrophil response throughout the first 5 days of the data collection. Neutropenia was never observed, yet the concentration of superoxide dismutase (SOD) was not assayed. Antioxidant protection could be jeopardized if inadequate copper nutriture was compromising SOD synthesis.

Many patients, after resuscitation from severe injuries, become immunosuppressed (153), and some die of septic complications. Koller

et al (21) supported the theory that immune dysfunction surfaces prior to the detection of any clinical sign in experimental animals. A decrease in IgG production and natural-killing cytotoxicity was reported and attributed to copper dependent enzymes. More recently, Failla et al (123) reported low copper content in neutrophils, decreased respiratory burst, and impaired yeast killing ability after feeding rats a suboptimal (2.7 ppm) intake of copper for as short as 1 week. Such results show that copper deficient animals are more susceptible to infections and septicemias. These various immune indices may reflect the consequences of marginal copper deficiency before clinical signs are detected. Several of the MTP patients had septic episodes during hospitalization, yet it would be speculation to conclude it was solely related to copper status.

Unlike previous studies, the marked rise in serum copper and ceruloplasmin post injury never occurred (44,45). An explanation might relate to the competitive nature between the binding of zinc and copper to the regulatory protein, metallothionein. Induction of metallothionein by the mediator, interleukin-1, and subsequently increased zinc from muscle catabolism occurs in response to stress (154). Metallothionein synthesis is induced and zinc bound to metallothionein can be displaced by copper since it has a higher binding constant to this protein than zinc. The metallothionein copper complexes are taken up by lysosomes and the copper is excreted into the bile canaliculi (102,106). This would prevent copper from being released into circulation, thus possibly explaining why the expected increases in serum copper and ceruloplasmin were not observed

in the present study.

The zinc-copper interaction may have clinical significance when zinc doses exceed copper. Hypocupremia induced by zinc therapy has been reported (94). Individual doses of zinc are often recommended during catabolic states in addition to the standard trace element formula. This could alter the zinc/copper ratio enough to disrupt circulating copper values. One patient (#9) in the present study, received additional parenteral zinc supplementation and had a serum copper value in the low range of normal. It was possible that the administration of extra intravenous zinc stimulated metallothionein synthesis, and consequently decreased the amount of copper released into circulation. Yadrick et al (93) demonstrated a decrease in erythrocyte SOD activity in adult females consuming 50 mg zinc/d during a 10 wk study. It was apparent that zinc supplementation posed a risk to copper status as early as 6 weeks into the study. For these previous reasons, further research of copper status in MTP should include the measurement of serum zinc concentrations and urinary zinc excretion.

Aggressive antacid therapy is standard procedure post trauma. A case report of antacid induced copper deficiency was reported after ingestion of approximately 7.5 g of antacid (109). All patients in this study received between 3.6 to 7.2 g/d of antacid which could potentially decrease copper's bioavailability once enteral or oral intake resumed. In a situation where copper losses could very well be significant and stores questionable, the risk of further compromising copper status must be addressed by clinicians.

Differences in utilization of IV administered copper compared to oral copper intake may alter copper bioavailability. Turnlund et al (86) concluded that the percent copper absorbed decreased as dietary copper increased. Whether such regulation applies to patients receiving IV copper is uncertain since absorption mechanisms via the gut are bypassed. Bush et al (155) compared the absorption between orally and IV administered radioactive copper but found little difference. It was assumed that the intravenouly delivered copper was handled in the same manner as radiocopper absorbed from the gastrointestinal tract. In contrast, Shike et al (11) reported that the amount of copper in parenteral nutrition solutions when given in excess was retained in the body rather than excreted. The explanation was attributed to differences between metabolism of intravenously and orally administered copper. Therefore, these contradictory findings would still make a direct comparison between burn patients who received enteral nutrition (3), and the MTP who either received IV glucose infusions or PN difficult. Under normal physiologic conditions approximately 25 to 60% of the copper is absorbed by the gut, whereas the percent of IV copper retained in the body is unknown.

Hormonal status of MTP could influence copper metabolism. Unfortunately, little is known about how the post injury secretion of catecholamines, the adrenal glucocorticoids and mineralocorticoids, adrenocorticotropic hormone (ACTH), growth hormone, antidiuretic hormone, the thyroid hormones and the glucoregulatory hormones affect the copper nutriture in humans. In neonatal rats, glucocorticoids stimulated copper secretion as ceruloplasmin by hepatocytes and

augmented biliary removal of hepatic copper via the mediator leukocyte endogenous mediater (LEM) (156). Multiple injections of ACTH in young rats produced conflicting results with elevations and depressions in both ceruloplasmin and plasma copper (52). Growth hormone counteracted the increased hepatic copper content found in hypophysectomized rats (52). Meyer et al (157) concluded that the administration of epinephrine to rats caused an increase in plasma copper and ceruloplasmin. Since catacholamine excretion increases in relation to injury severity, the correlation between urinary catecholamines and urinary copper after trauma deserves further study. Despite the known relationship between injury severity and hormonal response, no correlation was found between ISS and copper losses among the MTP.

Anemia, defined as chronically low hemoglobin levels, was reported in three patients throughout the study. One of these 3 patients (#9) reported borderline hypocupremia. Serum copper levels in this patient were 30 µg/100 ml, 70 µg/100 ml, and 65 µg/100 ml on study days 1, 3, and 5, respectively. The other 2 patients with anemia only had decreased hemoglobin values. Hemoglobin values at discharge were below the acceptable normal value in all 11 patients. The possibility exists that copper stores were eventually exhausted secondary to either increased utilization and/or loss. Once again, the question of whether MTP are more susceptible to a marginal copper deficiency becomes clinically apparent.

The nutritional status of patients preoperatively is a reliable predictor of clinical outcome (141). Elderly patients who are nutritionally depleted prior to injury have both a blunted stress

response and an increased incidence of morbidity and mortality (145). The 3 elderly patients in this study who died were presumably healthy prior to hospitalization. If previous nutritional status were compromised in these patients, copper stores could have been limited. This would place the elderly MTP at greater risk for a copper deficiency than younger patients though copper losses were significantly greater in the young patients. Furthermore, it is possible that the greater copper losses in young MTP reflects better copper nutriture and therefore better prognosis for survival. The greater copper loss may be good and even serve as a predictor of the ability to withstand the acute phase of trauma.

The results of this study regarding copper nutriture in the MTP should be interpreted with caution for the following reasons: (1) the study was of short duration and the copper intakes were variable so it is questionable whether true steady state was obtained in these critically ill patients; (2) the lack of correlation between injury severity and copper output suggested too small a sample size to demonstrate any differences; (3) patients were selected on the basis of requiring parenteral as opposed to enteral nutrition which could represent a bias for data interpretation; and (4) although overt copper deficiency symptoms were lacking, subtle changes in any particular patient's immunity as a result of a marginal copper deficiency may have impacted the overall patient status.

# CHAPTER V

## SUMMARY AND CONCLUSIONS

The MTP in this study were between the ages of 21 and 76 years of age with ISS ranging from 22 to 75. The average length of stay in the hospital was 34 days, with approximately 18 days spent in the intensive care unit. Three patients received only IV glucose infusions during the first 5 days of the study while 6 received PN by study day 3, 1 on day 2, and 1 on day 1.

Urinary copper output increased during the catabolic phase, but the losses were more striking than previously reported. Total cumulative copper losses were similar among all MTP with the greatest loss observed in urine. Daily urinary copper loss was statistically different from normal ($p<.001$) in all 11 patients. These losses represented 10-12 times normal copper excretion. Furthermore, urinary copper losses may have remained elevated had measurements extended beyond 5 days. Nasogastric secretions on study day 5 were also significantly greater than normal ($p<.025$, $n=8$). The average daily copper lost in chest tube drainage ($167\pm26$ µg;$n=7$) was similar in amount to the average urinary copper loss ($158\pm27$ µg). Data in the literature with which to compare our data on copper content in chest tube drainage are nonexistent. Miscellaneous drains produced on average, 37 µg/d ($n=8$). Stool quantities were insufficient for copper analysis. Cumulative copper losses totalled 2034 µg.

Urinary urea nitrogen excretion was not a reliable index of urinary copper output. However, a correlation did exist between the

cumulative nitrogen balance and copper balance ($r=0.82;p<.0001$).

A significant negative correlation was found between urinary copper excretion and age where urinary copper loss decreased as age increased ($r=.55;p<.05$). The greatest urinary copper output was observed in 3 patients in their twenties. Whether this relationship between age and copper loss reflected a causal effect remains unknown.

Cumulative copper balances in 9 patients were negative by study day 5, though 6 received PN. These cumulative negative balances if protracted over an extended period, could lead to the gradual depletion of copper reserves. A significant difference was observed in cumulative copper balance between patients receiving IV infusions and PN ($p<.05$). No significant difference was found between the PN or IV group in regard to urinary copper losses; however, mean cumulative copper excretion was greater in the IV group. Urinary copper excretion was independent of copper and amino acid intake.

Intravenous intake of copper was quite variable in patients receiving PN plus trace element supplementation. Discrepancies between amounts ordered and amounts actually administered were found. Average IV copper intake over the 5 days in the parenterally nourished group was 1018 µg. This calculated to an average of 204 µg/d which fell short of the AMA guidelines for IV trace element administration by approximately 800 µg/d.

No significant relationship was observed between ISS and copper loss or balance as was hypothesized. However, such a relationship might have existed if patient number had been increased, a broader range of ISS included, and the experimental collection period extended.

There was no statistical difference between clinical outcome and copper balance or loss. Although, the non-survivors (n=3) were in their seventies, they had lower mean cumulative copper losses and lower negative balances than the survivors.

The expected 50% rise in ceruloplasmin post injury never occurred. Mean serum ceruloplasmin and serum copper values were within the normal reference range.

The mean serum copper concentration remained unaffected after infusion of blood and/or blood products in all the MTP. No significant differences were found between patients receiving multiple blood or blood product transfusions or urinary copper excretion. The one patient (#9) who was autotransfused with approximately 35 units of blood and blood products did have the greatest 5 day urinary copper excretion.

In conclusion, the MTP in this study demonstrated excessive catabolic losses of urinary copper as well as previously unreported losses from chest tube, nasogastric, and miscellaneous drainage which were independent of injury severity, nutrition support, blood and blood products, or clinical outcome. In contrast, urinary excretion of copper was dependent on age. Serum copper and ceruloplasmin values were low for the degree of stress. Copper administration in most (n=8 of 11) patients was not sufficient to prevent negative copper balances.

Despite the lack of evidence of overt deficiency symptoms, such hypercatabolic copper losses and variable copper intakes places the MTP at risk for a marginal copper deficiency. Needed adjustments for the

excessive urinary copper and chest tube losses were apparent for the MTP. Furthermore, the data provided by the present study could be used for comparison to other MTP populations, and as baseline information for future studies on copper status in multiple trauma.

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APPENDIX A

COPPER STATUS IN MULTIPLE TRAUMA PATIENTS

1. I hereby volunteer and consent to participate in a research project being conducted by _____ through the facilities of Roanoke Memorial Hospital.

2. _____ has talked with me about the research and given me enough time to consider participation. Specifically, it was explained to me that I was chosen for this study because of the nature of my injury. I understand that the purpose of this study is to determine copper needs in trauma patients.

3. I understand that 5 blood samples will be taken during the total study (10 ml/sample) for analysis of serum copper and ceruloplasmin. A total of no more than 50 ml will be taken by a qualified medical tech. Urine and feces will also be collected for determination of copper.

4. No definite statement can be made to say that your participation will directly benefit you. There is no payment for participation in this study. There is no compensation for a physical or psychological injury which might be incurred as a result of this study. While medical care is available should an injury occur, the cost of such medical care is the responsibility of the patient. If you have any questions concerning your rights as a patient in this research study, you may contact Dr. Carol Gilbert or Susan F. Clark at 981-7441.

5. I understand that the researcher and the hospital will not identify me in any write-ups of this procedure and will keep records identifying me confidential to the extent provided by federal, state, and local law.

6. I understand that participation in this project is voluntary and that I may withdraw and discontinue participation at any time without penalty or loss of benefits to which I may otherwise be entitled.

7. I understand that the tests conducted in conjunction with this study will be free of charge.

8. I have the right to ask and have answered any questions concerning the procedures to be used in this study. Questions, if any, have been answered to my satisfaction. I have read and understand the above and have received a copy of this form. (ORAL CONSENT: I have been read and understand the above and have received a copy of this form).

_____ Date

_____ Subject or Legally Authorized Representative

APPENDIX A continued

I have explained and defined the research procedure in which the subject or the legally authorized representative has consented to participate.

_____ _____

Date Signature of Investigator

## APPENDIX B
## COPPER STUDY INFORMATION SHEET

Patients will be entered into the study within 48 h of admission if they meet the following criteria:

1. Previously healthy prior to injury;
2. Total parenteral nutrition should be expected;
3. Glasgow Coma Score >4 on admission,
   Trauma Score >4 on admission,
   Injury Severity Score: minimum of 12 or greater;
4. 18 years of age or older.

Informed consent will initially be obtained by either Susan Clark or Dr. Gilbert in triplicate (patient copy, medical record copy, study copy).

Instructions for ordering assays for copper study:

1. When writing order for 24 h sample collections please select a 6am - 6am or 2pm - 2pm collection period/patient. The 24 h collections are continuous for the first 5 ICU days so collection periods need to be the same per patient.
2. Patients with chest tubes: Order collection of chest tube drainage ONLY when chest tube is changed.
3. Computer entry for study's labs: ICU DAY 1, 3, 5 - Trauma Research I
   ICU DAY 2 & 4 - Trauma Research II
   DAY 10, 15 & DAY of DISCHARGE - Trauma Research III

<u>ASSAYS TO BE ORDERED BY MEDICAL RESIDENTS</u>

ICU DAY 1 (Trauma Research I)
- Serum Copper
- Ceruloplasmin
- 24 h Urine collection for copper & urea nitrogen
- 24 h Nasogastric collection for copper
- 24 h Chest tube drainage for copper
- 24 h Stool collection for copper
- 24 h Ileostomy or small bowel fluid for copper

ICU DAY 2 (Trauma Research II)
- 24 h Urine collection for copper & urea nitrogen
- 24 h Nasogastric collection for copper
- 24 h Chest tube drainage for copper
- 24 h Stool collection for copper
- 24 h Ileostomy or small bowel fluid for copper

ICU DAY 3 (Trauma Research I)
- Same as ICU DAY 1

ICU DAY 4 (Trauma Research II)
- Same as ICU DAY 2

ICU DAY 5 (Trauma Research I)
- Same as ICU DAY 1

DAY 10, 15, & DAY of DISCHARGE
- Serum copper & Ceruloplasmin

# APPENDIX C

## DETERMINATION OF COPPER IN URINE, NASOGASTRIC FLUID & DRAINS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

Specimen Requirement

100 ml aliquot 24 hour collection.

Reagents and Solutions

A. Reagents

1. Sulfuric acid, reagent grade, Fisher, A-305
2. Concentrated Copper Standard, 1000 ppm, Harleco, 7633

B. Solutions

1. Dilute Copper Standard - 10 µg/ml
   Using a volumetric pipet, transfer 1.0 ml of concentrated copper standard to a 100 ml volumetric flask and dilute with deionized water.

2. Working Copper Standards:
   a. 10 µg/100ml
      Pipet 1.0 ml of 100 µg/ml copper standard into a 100 ml volumetric flask and dilute with deionized water.
   b. 50 µg/100 ml
      Pipet 5.0 ml of 100 µg/ml copper standard into a 100 ml volumetric flask and dilute with deionized water.
   c. 100 µg/100 ml
      Pipet 10.0 ml of 100 µg/ml copper standard into a 100 ml volumetric flask and dilute with deionized water.

Sample Preparation

Nasogastric and drainage fluids treated for digestion in a 1:3 ratio of 2N sulfuric acid for a minimum of 24 hours and periodically mixed by vortex to facilitate digestion.

10.0 ml aliquots of urine unknown and 5 - 50 ml aliquots of nasogastric and drainage fluid, standards and distilled water blanks are pipetted into plastic disposable cups*; 0.5 ml of concentrated sulfuric acid is added to all cups and mixed with a wooden applicator stick.

4.0 ml of Ortho II Urine Control is pipetted into a cup and 0.2 ml of concentrated sulfuric acid is added and mixed (Multiply control reading by 10 for µg/L).

*Dispo-pipettes are used for these transfers. These pipettes are made of polycarbonate plastic and are thus free of copper contaminants and interfering substances.

APPENDIX C continued

Procedure

1. Operating parameters for copper

| | |
|---|---|
| Function: | ABS |
| Wavelength: | 325 |
| Range: | UV |
| Slit: | 4 |
| Light Source: | Copper Hollow cathode lamp |
| Flame Type: | Air acetylene flame, oxidizing (lean blue) |
| Air Rotometer: | 55 |
| Acetylene Rotometer: | 30 |
| Burner Head: | 4" single-slot |

2. Set the "Mode" function switch to "Conc", aspirate the 100 µg/100 mg standard and adjust the "conc" dial for 100% deflection on the read-out scale. "Damping" is on "1".

3. Aspirate an acidified water blank and set the "Absorbance/Concentration" meter to Zero by depressing the "Auto-Zero" button.

4. Switch "Damping" to "Int.2" and analyze the 10, 50, and 100 µg/100 ml standard along with the patient unknowns and controls.

5. If the unknowns are below 5% deflection, go back to step 2. Switch "damping" to "1". Aspirate the 50 µg/100 ml standard and adjust the "conc" dial for 100% deflection.

6. Repeat step 3.

7. Switch "Damping" to "Int.2" and analyze the 10 and 50 µg/100 ml standards along with the patient unknowns and controls.

8. Calculate unknowns per 24 hour total volume; the control is calculated per liter.

9. Repeat undetected results using standard additions method.

Quality Control

| Ortho II | Sample Control |
|---|---|
| Linearity | 0-300 µg/100 ml |
| Detection Limit | 1 µg/dl |

APPENDIX C continued

ATOMIC ABSORPTION SPECTROPHOTOMETRY: Serum Copper and Chest Tube Drainage

Specimen Requirement
3.0 ml serum

Reagents and Solutions
A. Reagents
1. Glycerol, Fisher, G-33.
2. Concentrated Copper Standard, 1000 ppm, Harleco, 7633.

B. Solutions
1. 5% glycerol. 50 ml of glycerol is diluted to 1000 ml with deionized water.
2. Dilute copper standard: 10 µg/100 ml. Dilute 1.0 ml of the concentrated standard to 100 ml with deionized water.
3. Working copper standards: 10 µg/100 ml and 50 µg/100 ml.
   a. 10 µg/100 ml
      Pipet 1.0 ml of dilute Copper standard into a 100 ml volumetric flask. Dilute to volume with 5% glycerol solution.
   b. 50 µg/100 ml
      Pipet 5.0 ml of dilute Copper standard into a 100 ml volumetric flask. Dilute to volume with 5% glycerol solution.
4. 5 µg/ml test solution = 5 ml of concentrated copper standard is diluted to 1 L with deionized water.

Sample Preparation

Unknown samples are diluted 1:5 with deionized water. American Medical Laboratories (AML) metal control is diluted 1:5 with deionized water.

Procedure

1. Standard conditions for copper

| | |
|---|---|
| Function: | ABS |
| Wavelength: | 325 nm |
| Range: | UV |
| Slit Settings | 4 |
| Light Source: | Copper hollow-cathode lamp |
| Flame Type: | Air-acetylene flame Oxidizing (lean, blue) |
| Burner Head: | 4" single-slot |
| Flame Settings: | Air rotometer: 55 |
| | Acetylene rotometer: 30 |

APPENDIX C continued

2. Set "Mode" function switch to "Conc". Aspirate the 100 µg/100 ml standard and adjust the "Conc" dial for 100% deflection on the read-out scale. Damping is on "1".

3. Aspirate a blank solution of 10% glycerol and depress the "Auto-Zero" button until read-out scale reads 0. Switch damping to "Int. 2".

4. Aspirate the 50 µg/100 ml and 100 µg/100 ml standards, the unknowns and control and record the "absorbance/conc." meter readings. Multiplication of the unknown and control meter readings by a factor of five will give the copper concentrate in each sample in µg/100 ml.

Quality Control
AML Metal Control
Linearity: up to 400 µg/100 ml.
Detection limits: 5 µg/100 ml.

# APPENDIX D
## QUANTITATION OF CERULOPLASMIN IN SERUM
## Nephelometric Immunoprecipitation

### Specimen

1. 1 ml of serum collected using a red/black top vacutainer, a serum separator tube.

2. Specimen is refrigerated and serum separated as soon as possible. Sera not assayed immediately are refrigerated at $2^{o}$ to $8^{o}$ C.

3. Specimens that are grossly hemolytic, hyperlipemic or turbid may give erroneous results.

### Reagents

1. Polymer Buffer (Beckman Instruments cat. no. 663600)

2. Diluent (Beckman cat. no. 663630) or prepared by Reagent Room: PBS, 0.2M, pH 7.0.
   a. Dissolve following in approximately 800 ml of deionized water:
      27.9 g $Na_2HPO_4$
      0.15 g $NaH_2PO_4$
      11.69 g NaCl
   b. Adjust the pH of the solution to 7.0 and then add 1.0 g of sodium azide. Adjust the volume to 1 liter in a volumetric flask.

3. Beckman Serum Calibrator II for calibration.

4. Antiserum - monospecific goat anti-human ceruloplasmin containing a trigger dye (Beckman Instruments).

5. Atlantic Antibodies #4 used as a control.

6. Fisher Abnormal Control.

7. Storage
   All reagents should be stored at $2^{o}$ to $8^{o}$ C.

### Procedure

1. Array Protein System Protocol from AML.
   a. A pipettor-dilutor set to pick up 100 µl and dispense it, plus 500 µl of saline diluent prepared a set of serial dilutions of each sample, the serum calibrator, and serum control.

APPENDIX D continued

b. Reaction tube is filled with 500 µl of polymeric buffer reaction medium and introduced into the cell compartment of the Beckman.

Immuno-Chemistry System instrument. The appropriate dilution of calibrator is picked up with a 35 µl air-piston pipet and introduced into reaction tube. Instrument displays the target value for the calibrator. Successive samples are similarly run, each employing a new reaction tube containing 500 µl of buffer into which 35 µl of the specified dilution sample and 35 µl of the antiserum are sequentially introduced. Instrument provides direct read-out in concentration units.

2. Measuring Range
   The Beckman ceruloplasmin test has been designed to detect serum concentrations within a range from 10 to 100 µg using a normal "C" (1:36) dilution.

Quality Control

With every run Atlantic Antibodies CA #4 and Fisher Abnormal Control is assayed. All controls must be within established limits before the assay is reported.

A check of the Reference Scatter is made and recorded in the Quality Control Book.

Normal Range

22 - 70 mg/100 ml

Limitations

1. Nonspecific interferences may occur between serum at the "A" (NEAT) dilution and the polymer-enhanced buffer leading to results that may not be accurate. For reporting, samples outside low at the "B" (1:6) dilution should be entered as less than 1.7 mg/dl.

2. Lipemic specimens may give falsely high results.

3. The presence of antibodies against the constituent being measured or complement components in immune complexes, or polymerization of proteins present in extra ordinarily high concentrations or the coexistence of monomeric, polymeric or aggregated forms of the protein in question, result in inaccurate quantitation.

# APPENDIX E

## SUMMARY OF INJURIES

| | |
|---|---|
| **Patient 1** | |
| ABD 61406.4 | Transection ileum with ischemia & multiple perforations |
| ABD 61206.5 | Perforation sigmoid colon |
| EXT 92803.3 | Open fracture (fx) left iliac wing |
| **Patient 2** | |
| HEAD 32305.4<br>92403.2 | Multiple facial fractures, circumzygomatic & mandible; |
| EXT 92201.2 | Open fx of right tibial shaft & fibula |
| 92403.3 | Closed fx of right os calcis |
| 92505.2 | Fx/dislocation of left ankle |
| 10303.1 | Closed fx of right acetabulum |
| **Patient 3** | |
| ABD 62009.5 | Transection pancreas |
| 61806.2 | Laceration right lobe liver |
| 61706.2 | Laceration left adrenal (transection) |
| 61707.3 | Laceration left kidney |
| EXT 10101.1 | Multiple abrasions |
| 61405.3 | Small bowel perforations |
| **Patient 4** | |
| ABD 61909.5 | Laceration spleen-avulsion hilum |
| 61806.2 | Laceration liver 25 cm superficial |
| HEAD 20608.2 | Closed head injury - combative on admit CT negative |
| FACE 31903.1 | Gingival laceration |
| EXTRM 82202.1 | Fx left finger #4 proximal phalanx |
| ABD 61702.2 | Right perinephric hematoma |
| | Aspiration pneumonia |
| | Hepatitis |
| | Acute Respiratory Distress Syndrome (ARDS) |
| **Patient 5** | |
| EXTM 92403.3 | Grade II open fx left tibia/fibula |
| 82402.2 | Fx right radius ulna |
| EXT 10303.2 | Laceration right calf >20 cm |
| 10602.1 | Degloving injury left heel |
| 10301.1 | Laceration left prepatellar bursa 4 cm |
| THOR 52530.5 | Flail chest requiring ventilation |
| ABD 61907.3 | Laceration spleen multiple superficial |
| 61809.5 | Laceration liver extensive including middle hepatic vein |

APPENDIX E continued

| | |
|---|---|
| Patient 6 | |
| ABD 61806.2 | Laceration liver 5 cm caudate lobe |
| THOR 52303.3 | Right hemothorax; left pneumo |
| EXTM 82503.3 | Open fx right elbow condylar |
| ABD 61405.3 | Laceration bowel ileum |
| THOR 52504.2 | Fx one rib right open |
| EXT 10301.1 | Laceration forehead-scalp < 10 cm |
| 10301.1 | Laceration left arm |
| Patient 7 | |
| THOR 50303.3 | Disruption innominate artery |
| THOR 50206.6 | Disruption descending aorta - complete |
| EXT/ PELVIS 92803.3 | Fx right acetabulum comminuted with pubic rami |
| THOR 52521.4 | Fx right ribs 4 - 8 with hemopneumothorax |
| EXT 10302.1 | Laceration nose |
| Patient 8 | |
| THOR 50206.6 | Traumatic aortic transection; Left pleural effusions (recurrent); |
| HEAD/NECK 70610.3 | Fx C5 cervical spine; Ischemic spinal cord injury with paraplegia; |
| ABD 61906.2 | Splenic laceration; |
| 61803.2 | Small liver peritoneal tear; Retroperitoneal hemorrhage involving cecum, ascending colon, duodenum and descending colon; |
| EXT 62102.2 | Fx right hand |
| HEAD 20608.2 | Closed head injury |
| Patient 9 | |
| EXT 10403.3 | Degloving injury abd wall |
| ABD 61406.4 | Avulsion small bowel with transection |
| FACE 32002.1 | Laceration tongue |
| EXTM 92601.3 | Fx right femur open grade III |
| EXTM 82502.2 | Fx right humerus closed neck |
| EXT 10704.1 | Burns 5% BSA 3rd degree |
| THOR 50204.4 | Partial laceration descending aorta |
| EXTM 82303.3 | Fx left forearm colles; displaced closed |
| FACE 32304.3 | Fx face Le Fort II |
| FACE 32203.2 | Fx jaw - symphyseal |
| HEAD 20503.3 | Closed head injury with brief LOC, subarachnoid blood |
| EXTM 92403.3 | Fx right medial malleolus (closed) (displaced) |
| THOR 52509.2 | Fx right ribs 5,6 |
| ABD 76708.2 | Fx L2 20% compression (Tension Pneumo) (Sepsis MSOF) |

APPENDIX E continued

Patient 10

| | |
|---|---|
| THOR 50205.5 | Aortic transection 270 circumference |
| 51401.2 | Pulmonary contusion (at surgery) |
| 51702.4 | Cardiac contusion (at surgery) |
| 51810.4 | Lacerated pericardium (at surgery) |
| EXT 10301.1 | Laceration head |
| THOR 52519.3 | Fx ribs left 4 |
| HEAD 20606.2 | Closed head injury LOC < 1 h |
| ABD 61803.2 | Subcapsular liver hematoma (CT) |
| | (Pulmonary embolism) |

Patient 11

| | |
|---|---|
| THOR 50106.5 | Inhalation injury |
| EITRM 92601.3 | Fx right femur |
| 82601.2 | Fx right clavicle |
| 82502.2 | Fx right humerus |
| THOR 52503.1 | Fx right 1st rib |
| 50713.3 | Right subclavian artery thrombosis |
| ABD 61903.2 | Sub capsular hematoma spleen |
| EXT 10202.1 | Hematoma scalp |

# APPENDIX F
## INDIVIUAL PATIENT DATA

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PART A: CLINICAL INFORMATION

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Patient number: 1 Hospital number: 4694469
Age: 22 (years) Sex: M Survived/Died: S
Mechanism of Injury: GSW* Blunt/Penetrating: P
Diagnosis: LAC COLON, SMALL BOWEL
Length of Hospital Stay: 23 days
Length of Intensive Care Unit Stay: 2 days
Total Parenteral Nutrition: N (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

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PART B: LABORATORY VALUES

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(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 70 | Day 1: | 42 |
| Day 2: | | Day 2: | |
| Day 3: | 110 | Day 3: | 59 |
| Day 4: | | Day 4: | |
| Day 5: | 120 | Day 5: | 49 |
| Day 10: | 195 | Day 10: | 90 |
| Day 15: | 185 | Day 15: | 25 |
| Discharge: | 160 | Discharge: | 75 |

SECTION II: SOURCES OF COPPER LOSS

Daily Losses measured in micrograms/24 h
Cumulative Losses are in micrograms and are in parentheses

| Day | Urine | Gastric | Drain(s) |
|---|---|---|---|
| 1 | 203.00 (203.00) | 5.80 (5.80) | 0.00 (0.00) |
| 2 | 134.00 (337.00) | 4.19 (9.99) | 0.00 (0.00) |
| 3 | 126.50 (463.50) | 364.00 (373.99) | 0.00 (0.00) |
| 4 | 135.00 (598.50) | 308.00 (681.99) | 0.00 (0.00) |
| 5 | 193.00 (791.50) | 0.00 (681.99) | 0.00 (0.00) |

Total Chest Tube Loss: (0.00)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 1 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | Nitrogen In | Nitrogen Balance |
|---|---|---|---|
| Day 1: | 19.50 (19.50) | 0.00 | -22.50 (-22.50) |
| Day 2: | 8.40 (27.90) | 0.00 | -11.40 (-33.90) |
| Day 3: | 6.80 (34.70) | 0.00 | - 9.80 (-43.70) |
| Day 4: | 13.10 (47.80) | 0.00 | -16.10 (-59.80) |
| Day 5: | 13.40 (61.20) | 0.00 | -16.40 (-76.20) |

SECTION IV: COPPER BALANCE
Daily Values measured in micrograms/24 h
Cumulative Values measured in micrograms and are in parentheses

| | Copper Out | Copper In | Copper Balance |
|---|---|---|---|
| Day 1: | 208.80 (208.80) | 0.00 (0.00) | -208.80 (-208.80) |
| Day 2: | 138.19 (346.99) | 0.00 (0.00) | -138.19 (-346.99) |
| Day 3: | 490.50 (837.49) | 0.00 (0.00) | -490.50 (-837.49) |
| Day 4: | 443.00 (1280.49) | 0.00 (0.00) | -443.00 (-1280.49) |
| Day 5: | 193.00 (1473.49) | 0.00 (0.00) | -193.00 (-1473.49) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 2 Hospital number: 4710349
Age: 51 (years) Sex: F Survived/Died: S
Mechanism of Injury: MVA* Blunt/Penetrating: B
Diagnosis: CHI,PULM CARDIAC CONTUSION, FX TIB FIB
Injury Severity Score: 26 Glasgow Coma Score: 10
Length of Hospital Stay: 51 days
Length of Intensive Care Unit Stay: 4 days
Total Parenteral Nutrition: Y (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 115 | Day 1: | 52 |
| Day 2: | | Day 2: | |
| Day 3: | | Day 3: | |
| Day 4: | | Day 4: | |
| Day 5: | 110 | Day 5: | 61 |
| Day 10: | 155 | Day 10: | 76 |
| Day 15: | 178 | Day 15: | 77 |
| Discharge: | 225 | Discharge: | 101 |

SECTION II: SOURCES OF COPPER LOSS
Daily Losses measured in micrograms/24 h
Cumulative Losses are in micrograms and are in parentheses

| Day | Urine | | Gastric | | Drain(s) | |
|---|---|---|---|---|---|---|
| 1 | 82.00 | (82.00) | 58.50 | (58.50) | 3.90 | (3.90) |
| 2 | 96.00 | (178.00) | 97.00 | (155.50) | 3.90 | (7.80) |
| 3 | 80.00 | (258.00) | 26.30 | (181.80) | 0.00 | (7.80) |
| 4 | 91.30 | (349.30) | 57.20 | (239.00) | 0.00 | (7.80) |
| 5 | 142.00 | (491.30) | 0.00 | (239.00) | 0.00 | (7.80) |

Total Chest Tube Loss: (0.00)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 2 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 16.50 | (16.50) | 0.00 | -19.50 | (-19.50) |
| Day 2: | 16.20 | (32.70) | 0.00 | -19.20 | (-38.70) |
| Day 3: | 14.00 | (46.70) | 6.40 | -10.60 | (-49.30) |
| Day 4: | 14.60 | (61.30) | 17.00 | - 0.60 | (-49.90) |
| Day 5: | 16.00 | (77.30) | 16.70 | - 2.30 | (-52.20) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 144.40 | (144.40) | 0.00 | ( 0.00) | -144.40 | (-144.40) |
| Day 2: | 196.90 | (341.30) | 0.00 | ( 0.00) | -196.90 | (-341.30) |
| Day 3: | 106.30 | (447.60) | 887.00 | (887.00) | 780.70 | ( 439.40) |
| Day 4: | 148.50 | (596.10) | 0.00 | (887.00) | -148.50 | ( 290.90) |
| Day 5: | 142.00 | (738.10) | 0.00 | (887.00) | -142.00 | ( 148.90) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 3 Hospital number: 4713624
Age: 21 (years) Sex: M Survived/Died: S
Mechanism of Injury: MCA* Blunt/Penetrating: B
Diagnosis: LIV LAC,TRANSEC PANC, SB PERFORATION
Injury Severity Score: 27 Glasgow Coma Score: 13
Length of Intensive Care Unit Stay: 60 days
Length of Intensive Care Unit Stay: 29 days
Total Parenteral Nutrition: Y (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 65 | Day 1: | 30 |
| Day 2: | | Day 2: | |
| Day 3: | 105 | Day 3: | 51 |
| Day 4: | | Day 4: | |
| Day 5: | 140 | Day 5: | 68 |
| Day 10: | 130 | Day 10: | 58 |
| Day 15: | 120 | Day 15: | 72 |
| Discharge: | 173 | Discharge: | 79 |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | | Gastric | | Drain(s) | |
|---|---|---|---|---|---|---|
| 1 | 267.00 | (267.00) | 22.50 | (22.50) | 107.30 | (107.30) |
| 2 | 212.00 | (479.00) | 23.80 | (46.30) | 51.90 | (159.20) |
| 3 | 260.40 | (739.40) | 223.20 | (269.50) | 60.50 | (219.70) |
| 4 | 274.80 | (1014.20) | 24.00 | (293.50) | 181.80 | (401.50) |
| 5 | 178.00 | (1192.20) | 0.76 | (294.26) | 1.54 | (403.04) |

Total Chest Tube Loss: (0.00)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 3 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 7.80 | ( 7.80) | 0.00 | -10.80 | (-10.80) |
| Day 2: | 14.20 | (22.00) | 0.00 | -17.20 | (-28.00) |
| Day 3: | 13.40 | (35.40) | 7.20 | - 9.20 | (-37.20) |
| Day 4: | 12.10 | (47.50) | 12.90 | - 2.20 | (-39.40) |
| Day 5: | 13.50 | (61.00) | 19.20 | 2.70 | (-36.70) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 396.80 | ( 396.80) | 0.00 | ( 0.00) | (-396.80) | (-396.80) |
| Day 2: | 287.70 | ( 684.50) | 0.00 | ( 0.00) | (-287.70) | (-684.50) |
| Day 3: | 544.10 | (1228.60) | 556.00 | (556.80) | ( 11.90) | (-672.60) |
| Day 4: | 300.34 | (1528.94) | 0.00 | (556.80) | (-300.34) | (-972.94) |
| Day 5: | 180.30 | (1709.24) | 1000.00 | (1000.00) | ( 819.70) | (-153.24) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 4 Hospital number: 4717419
Age: 29 (years) Sex: F Survived/Died: S
Mechanism of Injury: MVA* Blunt/Penetrating: B
Diagnosis: CHI,SPLENIC/LIV LAC, RETROPERI HEMATOMA
Injury Severity Score: 30 Glasgow Coma Score: 13
Length of Hospital Stay: 28 days
Length of Intensive Care Unit Stay: 16 days
Total Parenteral Nutrition: Y (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 100 | Day 1: | 49 |
| Day 2: | | Day 2: | |
| Day 3: | 105 | Day 3: | 42 |
| Day 4: | | Day 4: | |
| Day 5: | | Day 5: | |
| Day 10: | 160 | Day 10: | 75 |
| Day 15: | 185 | Day 15: | 70 |
| Discharge: | 213 | Discharge: | 79 |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | Gastric | Drain(s) |
|---|---|---|---|
| 1 | 281.00 ( 281.00) | 31.50 ( 31.50) | 0.00 ( 0.00) |
| 2 | 136.00 ( 417.00) | 23.80 ( 55.30) | 0.00 ( 0.00) |
| 3 | 182.00 ( 599.00) | 22.50 ( 77.80) | 0.00 ( 0.00) |
| 4 | 318.00 ( 917.00) | 35.00 (112.80) | 0.00 ( 0.00) |
| 5 | 301.00 (1218.00) | 20.00 (132.80) | 10.00 (10.00) |

Total Chest Tube Loss: (450.00)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 4 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 5.90 | ( 5.90) | 7.20 | -1.70 | (-1.70) |
| Day 2: | 6.20 | (12.10) | 14.50 | 5.30 | ( 3.60) |
| Day 3: | 8.70 | (20.80) | 14.50 | 2.80 | ( 6.40) |
| Day 4: | 7.80 | (28.60) | 16.20 | 5.40 | (11.80) |
| Day 5: | 11.60 | (40.20) | 15.00 | 0.40 | (12.20) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 402.50 | ( 402.50) | 0.00 | ( 0.00) | -402.50 | (-402.50) |
| Day 2: | 249.80 | ( 652.30) | 0.00 | ( 0.00) | -249.80 | (-652.30) |
| Day 3: | 294.50 | ( 946.80) | 450.00 | ( 450.00) | 155.50 | (-496.80) |
| Day 4: | 453.00 | (1399.80) | 0.00 | ( 450.00) | -453.00 | (-949.80) |
| Day 5: | 421.00 | (1820.80) | 1000.00 | (1450.00) | 579.00 | (-370.80) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 5 Hospital number: 4721445
Age: 22 (years) Sex: F Survived/Died: S
Mechanism of Injury: MVA* Blunt/Penetrating: B
Diagnosis: SPLEEN/LIVER LACERATION, FX TIB/FIB
Injury Severity Score: 59 Glasgow Coma Score: 15
Length of Hospital Stay: 32 days
Length of Intensive Care Unit Stay: 17 days
Total Parenteral Nutrition: N (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 65 | Day 1: | 33 |
| Day 2: | | Day 2: | |
| Day 3: | 65 | Day 3: | 29 |
| Day 4: | | Day 4: | |
| Day 5: | 100 | Day 5: | 37 |
| Day 10: | 110 | Day 10: | 46 |
| Day 15: | 145 | Day 15: | 65 |
| Discharge: | | Discharge: | 96 |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | Gastric | Drain(s) |
|---|---|---|---|
| 1 | 153.00 ( 153.00) | 12.60 ( 12.60) | 6.50 ( 6.50) |
| 2 | 537.00 ( 690.00) | 73.50 ( 86.10) | 7.50 (14.00) |
| 3 | 443.00 (1133.00) | 40.00 (126.10) | 5.50 (19.50) |
| 4 | 283.00 (1416.00) | 98.00 (224.10) | 5.70 (25.20) |
| 5 | 261.00 (1677.00) | 46.00 (270.10) | 5.20 (30.40) |

Total Chest Tube Loss: (1546.60)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 5 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | Nitrogen In | Nitrogen Balance |
|---|---|---|---|
| Day 1: | 12.80 (12.80) | 0.00 | -15.80 (-15.80) |
| Day 2: | 14.60 (27.40) | 0.00 | -17.60 (-33.40) |
| Day 3: | 13.60 (41.00) | 0.00 | -16.60 (-50.00) |
| Day 4: | 19.20 (60.20) | 0.00 | -22.20 (-72.20) |
| Day 5: | 17.40 (77.60) | 0.00 | -20.40 (-92.60) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | Copper In | Copper Balance |
|---|---|---|---|
| Day 1: | 481.42 ( 481.42) | 0.00 (0.00) | -481.42 (- 481.42) |
| Day 2: | 927.32 (1408.74) | 0.00 (0.00) | -927.32 (-1408.74) |
| Day 3: | 797.82 (2206.56) | 0.00 (0.00) | -797.82 (-2206.56) |
| Day 4: | 695.52 (2902.08) | 0.00 (0.00) | -695.52 (-2902.08) |
| Day 5: | 621.52 (3523.60) | 0.00 (0.00) | -621.52 (-3523.60) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 6 Hospital number: 4738704
Age: 39 (years) Sex: M Survived/Died: S
Mechanism of Injury: GSW* Blunt/Penetrating: P
Diagnosis: GSW LAC FROEHEAD/EAR,SB, LIVER,ARMS
Injury Severity Score: 27 Glasgow Coma Score: 15
Length of Hospital Stay: 11 days
Length of Intensive Care Unit Stay: 5 days
Total Parenteral Nutrition: Y (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 110 | Day 1: | 48 |
| Day 2: | | Day 2: | |
| Day 3: | 125 | Day 3: | 49 |
| Day 4: | | Day 4: | |
| Day 5: | 110 | Day 5: | 41 |
| Day 10: | 155 | Day 10: | 69 |
| Day 15: | | Day 15: | |
| Discharge: | 155 | Discharge: | 69 |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | | Gastric | | Drain(s) | |
|---|---|---|---|---|---|---|
| 1 | 87.00 | ( 87.00) | 27.80 | ( 27.80) | 34.20 | ( 34.20) |
| 2 | 129.00 | (216.00) | 28.50 | ( 56.30) | 33.60 | ( 67.80) |
| 3 | 114.00 | (330.00) | 36.00 | ( 92.30) | 129.00 | (196.80) |
| 4 | 121.00 | (451.00) | 171.00 | (263.30) | 32.40 | (229.20) |
| 5 | 160.00 | (611.00) | 32.00 | (295.30) | 90.50 | (319.70) |

Total Chest Tube Loss: (801.48)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 6 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 7.20 | ( 7.20) | 0.00 | -10.20 | (-10.20) |
| Day 2: | 5.10 | (12.30) | 0.00 | - 8.10 | (-18.30) |
| Day 3: | 7.00 | (19.30) | 10.80 | .80 | (-17.50) |
| Day 4: | 11.70 | (31.00) | 19.20 | 4.50 | (-13.00) |
| Day 5: | 9.92 | (40.92) | 19.20 | 6.28 | (- 6.72) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 309.30 | ( 309.30) | 0.00 | ( 0.00) | -309.30 | (-309.30) |
| Day 2: | 351.40 | ( 660.69) | 0.00 | ( 0.00) | -351.40 | (-660.69) |
| Day 3: | 439.30 | (1099.98) | 753.00 | ( 753.00) | 313.70 | (-346.99) |
| Day 4: | 542.80 | (1642.78) | 0.00 | ( 753.00) | -542.80 | (-889.78) |
| Day 5: | 442.80 | (2085.57) | 1000.00 | (1753.00) | 557.20 | (-332.58) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 7 Hospital number: 4741989
Age: 66 (years) Sex: F Survived/Died: S
Mechanism of Injury: MVA* Blunt/Penetrating: B
Diagnosis: TRANSSECTED AORTA
Injury Severity Score: 75 Glasgow Coma Score: 15
Length of Hospital Stay: 66 days
Length of Intensive Care Unit Stay: 38 days
Total Parenteral Nutrition: N (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 80 | Day 1: | 33 |
| Day 2: | | Day 2: | |
| Day 3: | 100 | Day 3: | 33 |
| Day 4: | | Day 4: | |
| Day 5: | 95 | Day 5: | 49 |
| Day 10: | | Day 10: | |
| Day 15: | 115 | Day 15: | 46 |
| Discharge: | 115 | Discharge: | 61 |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | Gastric | Drain(s) |
|---|---|---|---|
| 1 | 115.00 (115.00) | 8.40 ( 8.40) | 184.00 (184.00) |
| 2 | 118.00 (233.00) | 22.00 (30.40) | 44.40 (228.40) |
| 3 | 94.00 (327.00) | 10.50 (40.90) | 36.00 (264.40) |
| 4 | 63.00 (390.00) | 3.60 (44.50) | 45.00 (309.40) |
| 5 | 103.00 (493.00) | 0.00 (44.50) | 48.70 (358.10) |

Total Chest Tube Loss: (900.00)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 7 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 12.60 | (12.60) | 0.00 | -15.60 | (-15.60) |
| Day 2: | 12.00 | (24.60) | 0.00 | -15.00 | (-30.60) |
| Day 3: | 14.20 | (38.80) | 0.00 | -17.20 | (-47.80) |
| Day 4: | 9.10 | (47.90) | 0.00 | -12.10 | (-59.90) |
| Day 5: | 6.80 | (54.70) | 0.00 | - 9.80 | (-69.70) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 487.40 | ( 487.40) | 0.00 | (0.00) | -487.40 | (-487.40) |
| Day 2: | 364.40 | ( 851.80) | 0.00 | (0.00) | -364.40 | (-851.80) |
| Day 3: | 320.50 | (1172.30) | 0.00 | (0.00) | -320.50 | (-1172.30) |
| Day 4: | 295.30 | (1467.60) | 0.00 | (0.00) | -295.30 | (-1467.60) |
| Day 5: | 331.70 | (1799.30) | 0.00 | (0.00) | -331.70 | (-1799.30) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 8 Hospital number: 4748307
Age: 27 (years) Sex: M Survived/Died: S
Mechanism of Injury: MVA* Blunt/Penetrating: B
Diagnosis: CHI,PULM CONTUSION,TRANSSECTED AORTA
Injury Severity Score: 75 Glasgow Coma Score: 15
Length of Hospital Stay: 38 days
Length of Intensive Care Unit Stay: 17 days
Total Parenteral Nutrition: Y (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 115 | Day 1: | 37 |
| Day 2: | | Day 2: | |
| Day 3: | 125 | Day 3: | 49 |
| Day 4: | | Day 4: | |
| Day 5: | 155 | Day 5: | 61 |
| Day 10: | 140 | Day 10: | 42 |
| Day 15: | 200 | Day 15: | 111 |
| Discharge: | 115 | Discharge: | 61 |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | | Gastric | | Drain(s) | |
|---|---|---|---|---|---|---|
| 1 | 83.00 | ( 83.00) | 31.00 | ( 31.00) | 2.75 | ( 2.75) |
| 2 | 88.00 | (171.00) | 50.00 | ( 81.00) | 4.08 | ( 6.83) |
| 3 | 51.00 | (222.00) | 58.00 | (139.00) | 4.00 | (10.83) |
| 4 | 47.00 | (269.00) | 25.00 | (164.00) | 10.20 | (21.03) |
| 5 | 78.00 | (347.00) | 20.00 | (184.00) | 3.60 | (24.63) |

Total Chest Tube Loss: (1000.00)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 8 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 12.80 | (12.80) | 0.00 | -15.80 | (-15.80) |
| Day 2: | 16.30 | (29.10) | 0.00 | -19.30 | (-35.10) |
| Day 3: | 17.30 | (46.40) | 6.40 | -13.90 | (-49.00) |
| Day 4: | 20.70 | (67.10) | 19.20 | - 4.50 | (-53.50) |
| Day 5: | 22.00 | (89.10) | 19.20 | - 5.80 | (-59.30) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 316.75 | ( 316.75) | 0.00 | (0.00) | -316.75 | (- 316.75) |
| Day 2: | 342.08 | ( 658.83) | 0.00 | (0.00) | -342.08 | (- 658.83) |
| Day 3: | 313.00 | ( 971.83) | 0.00 | (0.00) | -313.00 | (- 971.83) |
| Day 4: | 275.60 | (1247.43) | 0.00 | (0.00) | -275.60 | (-1247.43) |
| Day 5: | 301.60 | (1540.03) | 1000.00 | (0.00) | 698.40 | (- 549.03) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 9 Hospital number: 4753075
Age: 72 (years) Sex: F Survived/Died: D
Mechanism of Injury: MVA* Blunt/Penetrating: B
Diagnosis: PARTIAL TRANSEC AORTA, FX ARM, BURNS AVULSION ABD
Injury Severity Score: 41 Glasgow Coma Score: 15
Length of Hospital Stay: 36 days
Length of Intensive Care Unit Stay: 36 days
Total Parenteral Nutrition: Y (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 30 | Day 1: | 17 |
| Day 2: | | Day 2: | |
| Day 3: | 70 | Day 3: | 20 |
| Day 4: | | Day 4: | |
| Day 5: | 65 | Day 5: | 27 |
| Day 10: | 80 | Day 10: | 32 |
| Day 15: | 70 | Day 15: | 43 |
| Discharge: | 80 | Discharge: | 36 |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | Gastric | Drain(s) |
|---|---|---|---|
| 1 | 143.00 (143.00) | 25.20 ( 25.20) | 14.00 (14.00) |
| 2 | 120.00 (263.00) | 18.80 ( 44.00) | 7.00 (21.00) |
| 3 | 138.00 (401.00) | 24.00 ( 68.00) | 10.50 (31.50) |
| 4 | 140.00 (541.00) | 102.00 (170.00) | 15.00 (46.50) |
| 5 | 158.00 (699.00) | 16.80 (186.80) | 2.82 (49.32) |

Total Chest Tube Loss: (0.00)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 9 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 13.15 | (13.15) | 0.00 | -16.15 | (-16.15) |
| Day 2: | 7.88 | (21.03) | 0.00 | -10.88 | (-27.03) |
| Day 3: | 6.70 | (27.73) | 1.50 | - 8.20 | (-35.23) |
| Day 4: | 7.40 | (35.13) | 6.20 | - 4.20 | (-39.43) |
| Day 5: | 6.50 | (41.63) | 12.20 | 2.70 | (-36.73) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 182.20 | (182.20) | 0.00 | (0.00) | -182.20 | (-182.20) |
| Day 2: | 145.80 | (328.00) | 0.00 | (0.00) | -145.00 | (-328.00) |
| Day 3: | 172.50 | (500.50) | 189.00 | (189.00) | 16.00 | (-311.50) |
| Day 4: | 244.82 | (745.32) | 0.00 | (189.00) | -244.82 | (-556.32) |
| Day 5: | 177.62 | (922.94) | 1000.00 | (1189.00) | 822.38 | ( 266.06) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 10 Hospital number: 4772166
Age: 76 (years) Sex: F Survived/Died: D
Mechanism of Injury: MVA* Blunt/Penetrating: B
Diagnosis: PARTIAL TRANSEC AORTA, PULM/CARDIAC CONTUSION
Injury Severity Score: 33 Glasgow Coma Score: 15
Length of Hospital Stay: 19 days
Length of Intensive Care Unit Stay: 19 days
Total Parenteral Nutrition: Y (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 48

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 75 | Day 1: | 36 |
| Day 2: | | Day 2: | |
| Day 3: | 90 | Day 3: | 42 |
| Day 4: | | Day 4: | |
| Day 5: | 100 | Day 5: | 47 |
| Day 10: | 90 | Day 10: | 57 |
| Day 15: | | Day 15: | |
| Discharge: | | Discharge: | |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | Gastric | Drain(s) |
|---|---|---|---|
| 1 | 116.00 (116.00) | 70.00 ( 70.00) | 0.00 (0.00) |
| 2 | 93.00 (209.00) | 87.00 (157.00) | 0.00 (0.00) |
| 3 | 137.00 (346.00) | 43.50 (200.50) | 0.00 (0.00) |
| 4 | 86.00 (432.00) | 37.50 (238.00) | 0.00 (0.00) |
| 5 | 88.00 (520.00) | 42.50 (280.50) | 0.00 (0.00) |

Total Chest Tube Loss: (573.00)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 10 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 11.00 | (11.00) | 0.00 | -14.00 | (-14.00) |
| Day 2: | 11.00 | (22.00) | 0.00 | -14.00 | (-28.00) |
| Day 3: | 12.20 | (34.20) | 3.20 | -12.00 | (-40.00) |
| Day 4: | 11.10 | (45.30) | 15.40 | 1.30 | (-38.70) |
| Day 5: | 10.90 | (56.20) | 15.40 | 1.50 | (-37.20) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 300.60 | ( 300.60) | 0.00 | ( 0.00) | -300.60 | (- 300.60) |
| Day 2: | 294.60 | ( 595.20) | 0.00 | ( 0.00) | -294.60 | (- 595.20) |
| Day 3: | 295.10 | ( 890.30) | 137.00 | (137.00) | -158.10 | (- 753.30) |
| Day 4: | 238.10 | (1128.40) | 0.00 | (137.00) | -238.10 | (- 991.40) |
| Day 5: | 245.10 | (1373.50) | 0.00 | (137.00) | -245.10 | (-1236.50) |

APPENDIX F continued

PART A: CLINICAL INFORMATION

Patient number: 11 Hospital number: 4799664
Age: 71 (years) Sex: F Survived/Died: D
Mechanism of Injury: FELL 2 LEVELS Blunt/Penetrating: B
Diagnosis: SMOKE INHALATION, SUBCAPS SPLENIC LAC
Injury Severity Score: 38 Glasgow Coma Score: 12
Length of Hospital Stay: 10 days
Length of Intensive Care Unit Stay: 10 days
Total Parenteral Nutrition: Y (Y=yes;N=no)
Time of Entry into Study (in hours post admission): 24

PART B: LABORATORY VALUES

(Note: absence of value indicates not measured)

SECTION I: SERUM COPPER AND CERULOPLASMIN

| | Serum Copper (micrograms/100 ml) | | Ceruloplasmin (milligrams/100 ml) |
|---|---|---|---|
| Day 1: | 126 | Day 1: | 76 |
| Day 2: | | Day 2: | |
| Day 3: | 180 | Day 3: | 82 |
| Day 4: | | Day 4: | |
| Day 5: | 170 | Day 5: | 76 |
| Day 10: | 148 | Day 10: | 84 |
| Day 15: | | Day 15: | |
| Discharge: | | Discharge: | |

SECTION II: SOURCES OF COPPER LOSS
Daily losses measured in micrograms/24 h
Cumulative losses are in micrograms and are in parentheses

| Day | Urine | | Gastric | | Drain(s) | |
|---|---|---|---|---|---|---|
| 1 | 122.00 | (122.00) | 29.30 | ( 29.30) | 0.00 | (0.00) |
| 2 | 157.30 | (279.30) | 148.20 | (177.50) | 0.00 | (0.00) |
| 3 | 76.00 | (355.30) | 65.40 | (242.90) | 0.00 | (0.00) |
| 4 | 184.80 | (540.10) | 12.00 | (254.90) | 0.00 | (0.00) |
| 5 | 106.00 | (646.10) | 6.40 | (261.30) | 0.00 | (0.00) |

Total Chest Tube Loss: (557.20)

APPENDIX F continued

PART B: LABORATORY VALUES FOR PATIENT NUMBER 11 continued

SECTION III: NITROGEN BALANCE
Daily values measured in grams/24 h
Cumulative values measured in grams and are in parentheses

| | Urine Urea Nitrogen | | Nitrogen In | Nitrogen Balance | |
|---|---|---|---|---|---|
| Day 1: | 12.60 | (12.60) | 0.00 | -15.60 | (-15.60) |
| Day 2: | 17.20 | (29.80) | 1.30 | -18.90 | (-34.50) |
| Day 3: | 7.00 | (36.80) | 9.30 | - 0.70 | (-35.20) |
| Day 4: | 11.70 | (48.50) | 10.90 | - 3.80 | (-39.00) |
| Day 5: | 13.60 | (62.10) | 6.10 | -10.50 | (-49.50) |

SECTION IV: COPPER BALANCE
Daily values measured in micrograms/24 h
Cumulative values measured in micrograms and are in parentheses

| | Copper Out | | Copper In | | Copper Balance | |
|---|---|---|---|---|---|---|
| Day 1: | 262.74 | ( 262.74) | 0.00 | ( 0.00) | -262.74 | (- 262.74) |
| Day 2: | 416.94 | ( 679.68) | 175.00 | (175.00) | -241.94 | (- 504.68) |
| Day 3: | 252.84 | ( 932.52) | 0.00 | (175.00) | -252.84 | (- 757.52) |
| Day 4: | 308.24 | (1240.76) | 0.00 | (175.00) | -308.24 | (-1065.76) |
| Day 5: | 223.84 | (1464.60) | 0.00 | (175.00) | -223.84 | (-1289.60) |

*MVA: Motor Vechicle Accident
MCA: Motorcycle Accident
GSW: Gun Shot Wound

# APPENDIX G

## BLOOD & BLOOD PRODUCT ADMINISTRATION

| Patient # | Blood & Blood Products* PRBC | PLTS | FFP | Estimated Blood Loss |
|---|---|---|---|---|
| 1 | 3 | - | - | 1 liter |
| 2 | 2 | - | - | NA |
| 3 | 10 | 10 | 2 | 7 liters |
| 4 | 7 | - | 2 | NA |
| 5 | 21 | 20 | 14 | 15 liters |
| 6 | 1 | - | - | 1.5 liter |
| 7 | 6 | - | 2 | NA |
| 8 | 6 | - | - | 1.5 liter |
| 9 | 8 | - | - | NA |
| 10 | 1 | 10 | - | NA |
| 11 | 2 | - | - | NA |

*PRBC: Packed red blood cells
PLTS: Platelets
FFP: Fresh frozen plasma

# APPENDIX H

## INDIVIDUAL HEMATOLOGICAL PARAMETERS AND SERUM ALBUMIN*

| Study Day | WBC | HgB | Hct | MCV | MCH | MCHC | Albumin |
|---|---|---|---|---|---|---|---|
| | $mm^3$ | g/100ml | % | $\mu g^3$ | $\mu g^3$ | % | g/100ml |
| (Pt #1) | | | | | | | |
| 1 | 34.6 | 13.0 | 38.7 | 91.4 | 30.7 | 33.6 | 3.4 |
| 2 | 11.0 | 10.5 | 31.5 | 90.2 | 30.1 | 33.4 | 2.1 |
| 3 | 10.6 | 9.3 | 27.2 | 88.2 | 30.4 | 34.2 | 2.1 |
| 4 | 10.2 | 9.3 | 27.2 | 88.9 | 31.4 | 34.2 | - |
| 5 | 10.5 | 11.8 | 34.3 | 90.1 | 31.0 | 34.4 | - |
| 10 | 13.0 | 12.1 | 34.7 | 89.1 | 31.0 | 34.8 | - |
| 15 | 12.6 | 11.3 | 34.3 | 89.7 | 29.7 | 33.1 | 3.4 |
| DC | 10.5 | 10.6 | 30.4 | - | - | - | 3.0 |
| (Pt #2) | | | | | | | |
| 1 | 13.0 | 10.1 | 29.0 | 86.1 | 30.0 | 34.8 | - |
| 2 | 9.0 | 9.2 | 27.8 | 86.0 | 28.6 | 33.2 | - |
| 3 | 9.0 | 9.8 | 27.9 | 87.1 | 30.6 | 35.2 | - |
| 4 | 14.2 | 9.2 | 27.1 | 88.0 | 29.9 | 33.9 | 2.3 |
| 5 | 14.9 | 8.7 | 26.0 | 86.7 | 29.0 | 33.4 | 2.2 |
| 10 | 13.2 | 10.6 | 30.3 | 86.7 | 30.4 | 35.0 | 2.7 |
| 15 | - | - | | - | - | - | - |
| DC | - | 9.2 | - | - | - | - | 3.4 |
| (Pt #3) | | | | | | | |
| 1 | 25.0 | 11.3 | 33.0 | 93.8 | 32.1 | 34.2 | 2.8 |
| 2 | 15.7 | 11.2 | 31.3 | 88.7 | 31.7 | 35.8 | 2.1 |
| 3 | 15.3 | 9.3 | 27.3 | 88.7 | 30.2 | 34.0 | 2.3 |
| 4 | 14.9 | 11.5 | 33.3 | 91.0 | 31.4 | 34.5 | 2.5 |
| 5 | 18.1 | 11.3 | 33.2 | 90.8 | 30.9 | 34.0 | 2.7 |
| 10 | 36.4 | 10.6 | 30.8 | 90.4 | 31.1 | 34.4 | 2.9 |
| 15 | - | 9.7 | 28.1 | - | - | - | 2.1 |
| DC | 9.0 | 11.2 | 33.9 | 88.5 | 29.2 | 32.9 | 4.3 |
| (Pt #4) | | | | | | | |
| 1 | 22.2 | 10.3 | 28.4 | 86.3 | 31.2 | 36.1 | 3.0 |
| 2 | 19.5 | 9.6 | 27.4 | 88.0 | 31.0 | 35.2 | 2.3 |
| 3 | 16.2 | 9.6 | 28.3 | 88.5 | 30.0 | 33.9 | - |
| 4 | 20.5 | 10.3 | 29.5 | 89.5 | 31.4 | 35.0 | 2.3 |
| 5 | 21.7 | 10.1 | 29.7 | 89.5 | 30.4 | 34.0 | 1.9 |
| 10 | 43.5 | 11.5 | 33.2 | 89.6 | 31.0 | 34.6 | 2.5 |
| 15 | 29.7 | 9.1 | 26.4 | 90.7 | 30.3 | 33.3 | 2.3 |
| DC | 11.7 | 11.6 | 35.4 | 91.9 | 30.1 | 32.8 | - |

APPENDIX H continued

| Study Day | WBC | HgB | Hct | MCV | MCH | MCHC | Albumin |
|---|---|---|---|---|---|---|---|
| | $mm^3$ | g/100ml | % | $\mu g^3$ | $\mu g^3$ | % | g/100ml |
| (Pt #5) | | | | | | | |
| 1 | 6.6 | 11.2 | 33.0 | 87.0 | 29.6 | 34.0 | 2.1 |
| 2 | 18.3 | 12.4 | 35.5 | 88.5 | 30.9 | 34.9 | - |
| 3 | 14.7 | 16.6 | 47.2 | 87.8 | 31.0 | 35.3 | 2.0 |
| 4 | 17.9 | 13.5 | 39.8 | 89.2 | 30.3 | 33.9 | - |
| 5 | 15.3 | 14.7 | 42.8 | 88.3 | 30.3 | 34.3 | 2.2 |
| 10 | 27.7 | 14.8 | 42.9 | 89.8 | 31.0 | 34.5 | 2.4 |
| 15 | 25.6 | 12.1 | 37.6 | 90.0 | 28.9 | 32.2 | 2.3 |
| DC | 20.4 | 11.4 | 36.0 | 89.7 | 28.4 | 31.7 | 3.3 |
| (Pt #6) | | | | | | | |
| 1 | 13.2 | 13.7 | 41.4 | 91.3 | 30.2 | 33.1 | 3.0 |
| 2 | 10.3 | 11.1 | 33.5 | 91.4 | 30.2 | 33.1 | - |
| 3 | 9.4 | 8.8 | 26.9 | 90.8 | 29.7 | 32.7 | - |
| 4 | 9.1 | 8.8 | 27.0 | 91.7 | 29.9 | 32.6 | - |
| 5 | 8.5 | 9.1 | 26.5 | 90.4 | 31.3 | 34.4 | 2.6 |
| 10 | - | - | - | - | - | - | - |
| 15 | - | - | - | - | - | - | - |
| DC | 10.0 | 8.6 | 25.7 | 91.3 | 30.5 | 33.4 | 2.9 |
| (Pt #7) | | | | | | | |
| 1 | 10.6 | 12.7 | 36.9 | 87.7 | 30.2 | 34.4 | - |
| 2 | 13.4 | 10.1 | 30.9 | 88.7 | 29.3 | 33.1 | - |
| 3 | 16.0 | 9.6 | 28.7 | 88.3 | 29.5 | 33.5 | - |
| 4 | 14.6 | 9.9 | 30.4 | 89.3 | 28.9 | 32.3 | 2.2 |
| 5 | 13.7 | 9.3 | 28.8 | 89.4 | 28.4 | 32.3 | 2.5 |
| 10 | 20.5 | 8.8 | 27.2 | 90.2 | 29.2 | 32.4 | 3.5 |
| 15 | 16.7 | 7.8 | 24.1 | 90.8 | 29.4 | 32.4 | 2.7 |
| DC | 8.4 | 10.4 | 31.9 | 90.3 | 29.5 | 32.6 | 2.9 |
| (Pt #8) | | | | | | | |
| 1 | 13.8 | 10.2 | 30.4 | 87.1 | 29.8 | 34.2 | 3.3 |
| 2 | 14.1 | 12.9 | 38.5 | 87.8 | 29.5 | 33.5 | 2.6 |
| 3 | 14.8 | 10.4 | 31.0 | 88.5 | 29.7 | 33.6 | 2.3 |
| 4 | 16.9 | 8.4 | 25.3 | 88.1 | 29.3 | 33.2 | 2.7 |
| 5 | 30.8 | 9.4 | 28.1 | 87.7 | 29.4 | 33.5 | 2.9 |
| 10 | 42.6 | 12.6 | 38.3 | 89.7 | 29.5 | 32.9 | 2.3 |
| 15 | 49.3 | 10.8 | 32.8 | 93.0 | 30.7 | 33.0 | 2.5 |
| DC | 18.0 | 13.0 | 38.5 | 89.4 | 30.2 | 33.7 | |

APPENDIX H continued

| Study Day | WBC | HgB | Hct | MCV | MCH | MCHC | Albumin |
|---|---|---|---|---|---|---|---|
| | $mm^3$ | g/100ml | % | $\mu g^3$ | $\mu g^3$ | % | g/100ml |
| (Pt #9) | | | | | | | |
| 1 | 15.0 | 13.4 | 38.8 | 89.8 | 31.0 | 34.5 | 3.1 |
| 2 | - | 13.0 | 39.1 | - | - | - | 1.6 |
| 3 | 13.8 | 12.9 | 37.8 | 88.3 | 30.1 | 34.1 | 1.8 |
| 4 | 12.6 | 11.7 | 34.3 | 87.8 | 29.9 | 34.1 | - |
| 5 | 13.3 | 12.2 | 36.6 | 88.6 | 29.5 | 33.3 | - |
| 10 | 14.1 | 8.7 | 24.6 | 91.6 | 32.3 | 35.4 | 1.5 |
| 15 | 18.6 | 11.1 | 33.6 | 89.2 | 29.5 | 33.0 | 2.1 |
| DC | 20.5 | 9.7 | 28.3 | 93.0 | 31.9 | 34.3 | - |
| (Pt #10) | | | | | | | |
| 1 | - | 9.6 | 27.9 | 94.4 | 31.7 | 33.6 | 2.6 |
| 2 | 6.7 | 11.0 | 32.4 | 93.2 | 31.6 | 33.9 | - |
| 3 | - | 10.4 | 32.3 | - | - | - | 2.3 |
| 4 | 6.0 | 11.3 | 31.9 | 89.8 | 31.8 | 35.3 | - |
| 5 | 7.9 | 9.9 | 30.1 | 92.2 | 30.4 | 32.9 | 1.7 |
| 10 | 10.5 | 9.2 | 27.3 | 90.3 | 30.5 | 33.7 | 1.9 |
| 15 & DC | 10.0 | 12.0 | 34.1 | 89.5 | 31.5 | 35.2 | - |
| (Pt #11) | | | | | | | |
| 1 | 20.0 | 15.4 | 43.9 | 85.0 | 29.8 | 35.0 | 3.1 |
| 2 | 20.8 | 11.2 | 31.9 | 84.6 | 29.7 | 35.1 | - |
| 3 | 16.4 | 8.3 | 24.9 | 85.7 | 28.5 | 33.3 | 1.9 |
| 4 | 18.2 | 9.6 | 28.0 | 86.7 | 29.7 | 34.3 | 1.5 |
| 5 | 18.9 | 10.4 | 30.4 | 85.7 | 29.3 | 34.2 | 1.4 |
| 10 & DC | 43.4 | 9.5 | 26.9 | 86.2 | 30.4 | 35.3 | 1.2 |

*Units and Normal Values

WBC: 4,800-10,800/$mm^3$
Hgb: 14.0-18.0 g/100 ml (male)
12.0-16.0 g/100 ml (female)
Hct: 42-52% (male)
37-47% (female)
MCV: 80-94 $\mu g^3$
MCH: 27-31 $\mu g^3$
MCHC: 33-37%
Alb: 3.5-5.2 g/100 ml

VITA

Susan Ferguson Clark was born September 14, 1955 in Washington, DC. In 1977 she received a B.S. degree in Human Nutrition and Foods from Virginia Polytechnic Institute and State University, Blacksburg, Va. The same year she entered the Masters program at the University of Kentucky, Lexington, Ky. and was the recipient of an M.S. with honors in Clinical Nutrition in August, 1978. She was credentialed a Registered Dietitian in 1979. From 1978 to 1980 she worked as the Clinical Nutrition Coordinator of the University of Kentucky's Diabetes Program in the Department of Medicine with an adjunct faculty appointment in the Department of Clinical Nutrition. Then, from 1980 to 1981, she assumed a Clinical Instructor faculty appointment in the Department of Clinical Nutrition's graduate program at the University of Kentucky. In 1982 until 1983 she worked as a Clinical Nutrition Research Associate for the University of Michigan Hospitals, Department of Surgery in Ann Arbor, MI. From May 1983 to May 1984 she worked as a Critical Care Instructor at the University of Michigan Hospitals, Department of Dietetics with an adjunct faculty appointment at the University of Michigan's School of Public Health, Department of Human Nutrition. In September 1984, she enrolled in the doctoral program at Virginia Polytechnic Institute and State University, Blacksburg, Virginia and is presently working to complete requirements for a PhD degree in Human Nutrition and Foods.

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