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ZINC STATUS IN THE INTESTINAL BYPASS PATIENT

by

GARY MICHAEL KOSS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Zinc status in the intestinal bypass patient" submitted by Gary Michael Koss, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science in Nutrition.

Elizabeth G. Donald
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Date March 2, 1977

ABSTRACT

The intestinal bypass operation is a surgical procedure used to induce weight loss in morbidly obese individuals. Deficiencies of calcium, potassium, magnesium, and iron have been reported to occur in patients who have undergone the bypass operation. This study was conducted to determine if bypassed patients also develop zinc deficiency.

Zinc status was determined by measuring zinc levels in plasma, liver, muscle and hair in 14 bypassed and 15 control subjects. An atomic absorption spectrophotometer was used to measure the zinc levels in the tissues studied. As some fatty metamorphosis of the liver frequently results from the bypass operation, the amount of fat in the liver was determined. The effect of fat infiltration on liver zinc content was also assessed.

Zinc levels in plasma and hair were significantly lower in experimental subjects than in controls, whereas, levels in liver and muscle samples were similar in both groups. Levels in liver and muscle were within the normal range, whereas, the level in hair was at the lower limit and plasma was less than normal. Although there was a greater amount of fat in the livers of experimental subjects compared with control subjects, this difference was not significant. As liver fat levels increased, zinc levels decreased. This relationship was significant. When fat was

removed from liver samples from both control and experimental subjects, the zinc content was still within a normal range.

Although the mean zinc content in the plasma, liver, muscle, and hair from patients who had undergone the bypass operation was less than levels found in control subjects, for only plasma and hair was the difference significant. Based on the data obtained from this study, the zinc status of the bypassed patient cannot be determined. Further work using more subjects and additional parameters is needed.

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INTRODUCTION

Morbid obesity is a pathologic state that responds poorly to the usual conservative methods of treating the mildly obese patient. The failure of these methods and the pressing nature of the problem [illegible] surgeons to devise operations that will result in and maintain weight loss. The intestinal bypass operation sets aside a sizeable portion (80-90%) of the jejunum and ileum so that no chyme passes through this portion. Weight loss occurs in almost all patients. However, because of the induced malabsorption this procedure is not without complications. Diarrhea and steatorrhea have led to losses of calcium, potassium, and magnesium and thus resulted in low blood levels of these minerals. In view of the foregoing, absorption of other nutrients, particularly other trace elements, may also be depressed. If so, various clinical symptoms may be related to mineral deficiencies which are presently unrecognized because the nutritional status in respect to a number of the trace elements has not been measured in the patient with an intestinal bypass. This study was undertaken to determine the zinc status of intestinal bypass patients as assessed by the determination of the zinc content of plasma, liver, muscle and hair.

LITERATURE REVIEW

The bypass operation

The intestinal bypass operation is a surgical procedure used to induce weight reduction in those who are morbidly obese and who have been unsuccessful in losing weight by conventional means. Morbid obesity has been defined as a weight at least 100 pounds above or double ideal weight (58).

Various operative procedures have been used to exclude portions of the small intestine. These have been summarized in a recent review article by MacLean (36). The objective of such procedures is to decrease absorption of food and thus ultimately to achieve loss of body weight. The most common procedure used locally is an end-to-end jejuno terminal ileostomy: 10 inches of jejunum, measured from the ligament of Treitz, and 20 inches of ileum, measured from the ileocecal valve, are joined end-to-end (58). The jejunal end of the defunctioned bowel is closed and attached to the base of the mesentary and the ileal end is joined end to side to the sigmoid colon. The overall effect of this operation is malabsorption of protein, fat and to a lesser degree, carbohydrate, vitamins, and minerals. The end result is weight loss.

Fatty metamorphosis of the liver occurs in almost all patients following bypass surgery. However, after 300 days

post-operative the number of patients with severe metamorphosis gradually decreases so that by 2000 to 2500 days, about 50% have normal livers on microscopic examination (58).

The importance of zinc in human physiology

In 1934, Todd et al. (74) reported that zinc was necessary for life in animals and suggested that it was probably an essential element for man. Essentiality in man was finally shown in 1963 when Prasad et al. (49) observed a zinc deficiency syndrome in man and when Sandstead et al. (60) reported in 1967 that the syndrome could be treated with zinc.

Zinc is a very important cofactor in a number of enzymes (43). It is essential for the activity of carbonic anhydrase in the red blood cell (RBC). This enzyme is required for the reversible dehydration of carbonic acid, a process critical to the rapid transport and elimination of CO_2. Several enzymes necessary for cellular oxidation, such as human alcohol dehydrogenase are also zinc dependent. The function of zinc in the synthesis of DNA, RNA, and protein has been summarized in the review by Halsted et al. (19). If zinc is lacking, then synthesis of all three substances is inhibited.

The role of zinc in carbohydrate metabolism is still controversial. Quarterman et al. (52) have shown a decreased

glucose tolerance in zinc-deficient rats. Macpinlac et al. (35), on the other hand, did not find any difference in fasting blood sugar, or in glucose tolerance curves between zinc-deficient and control rats. Zinc has been found to influence the in vitro transport and utilization of glucose across rat epididymal fat pad membranes (51). Although insulin contains zinc, it is not known if the zinc is required for the biological activity of insulin (19). The exact role of zinc in carbohydrate metabolism has not yet been elucidated.

Zinc deficiency

The prevalence of zinc deficiency: Zinc deficiency can result from several causes. If the diet is low in zinc, or high in chelating agents, or a combination of both, a deficiency state may occur. The diet consumed by some Iranian villagers (2) is an example of one which can result in a deficiency state. Their diet contains large amounts of cereals and grains and, therefore, is lower in zinc content than one containing more meat (61). The high phytate level in the unleavened, wholemeal bread is thought to chelate zinc thus further reducing the available zinc for absorption (56). Reinhold et al. (54) have questioned the importance of a zinc-phytate complex in contributing to zinc deficiency and have proposed that the binding of zinc by the fiber itself is of greater importance. Zinc

deficiency may also occur secondary to digestive disorders such as malabsorption and steatorrhea (30).

The deficiency syndrome was first thought to be limited to males because there is a considerably higher concentration of zinc in the testes than in the ovaries. However, two cases of females with zinc deficiency symptoms have now been documented (17).

Symptoms of zinc deficiency: Clinically, zinc deficiency results in severe iron deficiency anemia, open epiphysis, hepatosplenomegaly, spoon nails, and hypogonadism. Impaired wound healing and growth retardation result from defective collagen deposition, as zinc is required for the production of DNA and RNA needed for collagen synthesis (12). Some individuals exhibit rough skin with hyperpigmentation, and frequently a history of geophagia (49). Loss of taste (hypogeusia) and smell (hyposmia) have been successfully treated with zinc (20, 23). However, not only zinc, but also copper and nickel appear to have a role in the mechanism of taste as all of these trace elements are effective in treating hypogeusia (22).

Factors that affect zinc status

Dietary intake of zinc: The average Canadian diet contains liberal amounts of animal protein and provides 5 to 6 mg of zinc per 1000 kcal (8). This is based on the assumption that 60% of dietary zinc is derived from meat, fish,

and poultry. Therefore, the recommended intake of 9 and 10 mg per day for normal adult females and males, respectively, should be readily met. However, the diet of bypassed patients may not contain 5 to 6 mg of zinc per 1000 kcal as their diets are usually higher in carbohydrate than is the average diet (58). Osis et al. (42) have shown that the zinc content of carbohydrate-rich foods is lower than that of protein-rich foods. Patients who have undergone the bypass operation usually continue to eat higher than normal amounts of food [1]; thus, a high carbohydrate diet may or may not lead to marginal zinc intakes as patients consuming a sufficient quantity of such a diet could still meet the recommended intake for zinc because of the large quantity of food eaten.

Intestinal absorption of zinc: The site and mechanism of zinc absorption has been studied in a number of different species but not in the human. In the rat, the major site of absorption is the duodenum followed by the ileum and then the jejunum (9). Studies by Methfessel and Spencer (37) showed that absorption in the ileum and jejunum was nearly identical. Absorption from the stomach, cecum, and colon is very low and does not contribute materially to the overall absorption of zinc.

Evans et al. (10) have proposed the following sequence

1. Personal communication, P.A. Salmon.

as the mechanism of absorption: 1) the pancreas secretes a zinc-binding ligand into the intestinal lumen; 2) zinc binds to the ligand in the lumen; 3) the zinc-ligand complex is actively transported through the intestinal microvillus and into the epithelial cell; 4) in the epithelial cell, zinc is transferred to binding sites on the basolateral plasma membrane; 5) metal-free albumin interacts with the plasma membrane and removes zinc from the receptor sites. The quantity of metal-free albumin available at the basolateral membrane determines the amount of zinc removed from the intestinal epithelial cell and thus regulates the quantity of zinc that enters the body. Although the protein, α_2-macroglobulin, contains 30 to 40% of the total zinc content of serum, it is thought to play only a minor role, if it participates at all, in zinc absorption (44). Therefore, according to the theory of Evans et al. (10) zinc absorption is inversely proportionally to serum zinc levels.

The level of zinc in the diet has a variable effect upon zinc absorption. Furchner and Richmond (14) showed that the previous zinc status of an animal affected the amount of dietary zinc absorbed. Rats were fed various levels of zinc for 28 days and then given an oral dose of ^{65}Zn. Animals fed diets low in zinc retained more of the ^{65}Zn than did those whose zinc nutriture was better. The authors studied one human male and found similar results.

The subject retained less ^{65}Zn following 30 days of zinc supplementation. Becker and Hoekstra (5) state that by supplying higher levels of dietary zinc, the total amount absorbed can be slightly increased while at the same time the percent absorbed decreases.

A number of other dietary factors also affects the absorption of zinc. The addition of calcium and phytate to a low-zinc diet fed chicks and rats decreases the availability of zinc (5, 27). The effect of calcium is mediated by phytic acid. Zinc is made unavailable by either co-precipitation by or adsorption to a calcium-phytate complex (41). Diets containing proteins of plant seed origin, such as soy protein, have a lower zinc availability than do diets containing animal proteins such as casein (39). Plant seed proteins contain substantial amounts of phytic acid, while animal proteins contain none. Spencer et al. (68) fed diets containing low and high amounts of calcium to 5 men. They found that the varying levels of calcium did not affect absorption of ^{65}Zn. They also noted that the diet was low in phytic acid and that this could explain why zinc absorption was unchanged.

Reinhold et al. (54) have recently presented evidence that fiber in unleavened, wholemeal breads may be responsible for binding large amounts of zinc. Zinc appears to bind firmly to both bran and cellulose and does not seem to be released in the intestine by the digestive enzymes.

zinc bound to starch or protein is released.

A substance found in liver extract (62), and the amino acid cysteine (40) can chelate zinc and thus increase zinc availability in chicks when added to a diet containing soy proteins. Copper also increases zinc availability; however, the reason for the increase is not known as the copper:zinc interaction is poorly understood (76). Iron, on the other hand, decreases the absorption of zinc (76). This may occur because of the similarities in "patterns of absorption" of ^{59}Fe and ^{65}Zn as found in ligated intestinal segments from rats.

Losses of zinc from the body: Roman (57) has shown that in the normal individual, the urinary loss of zinc is about 0.5 mg/24 hours, which is a small proportion of the amount excreted from the body. The main excretory route of zinc appears to be via the gastrointestinal tract (68). In normal individuals, dietary zinc can vary widely without affecting the zinc level in urine (66). Studies conducted by McCance and Widdowson (34) showed human subjects to be in balance at all levels of zinc intake. Increasing the dietary intake did not affect the amount of zinc excreted in the urine but materially increased the amount of zinc lost via the feces. In a much more recent study Spencer et al. (68) administered ^{65}Zn orally and also found only a small amount of zinc excreted in the urine.

The absorption of zinc may be altered in the patient with a bypass operation as they frequently suffer from steatorrhea (45). This has been reported to be responsible for a decrease in the absorption of calcium and magnesium (4), and therefore could similarly affect the absorption of zinc (30). Eventually, this could result in a decrease in body stores of zinc.

Sweat losses of zinc average about 500 µg/l (24), but can be as much as 2.5 mg/day under heavy sweating conditons (75).

Sex and age of subjects: Lindeman et al. (29) and Reinhold (53) suggested that plasma levels of zinc are lower in females than males but Halsted et al. (18) did not find a significant difference between the sexes. Klevay (26) reported that sex had no effect on level of zinc in hair. Netsky et al. (38) showed that the zinc content of liver, muscle, and hair is not affected by age, while the effect of age on plasma zinc levels is still controversial (18, 26).

The assessment of zinc status

Fox (13) stated that in the sixties, typical daily intakes of zinc in North America were considered to be adequate as there were no known zinc deficient population groups. In 1972, Hambidge et al. (20) reported low levels of zinc in hair, combined with anorexia, poor growth, and

poor taste acuity in supposedly healthy, normal children in Denver, Colorado. More recently, Sandstead (59) conducted studies estimating the dietary availability of zinc. His findings suggest that some infants, pregnant women, teenage and college women, institutionalized individuals and some living on nutritionally poor diets because of restricted income can have a marginal to deficient intake of this element.

No one simple and reliable method for the assessment of zinc status is presently known (13), thus several parameters should be studied. The balance technique has been a classical method used to study nutritional requirement of various nutrients. This technique, although difficult, may provide a good basis for the assessment of zinc status, with positive retention of zinc by human subjects being indicative of zinc deficiency. Using ^{65}Zn, Prasad (47) showed that plasma zinc turnover was increased, the 24 hour exchangeable pool decreased, and cumulative excretion of ^{65}Zn in urine and stool was low in zinc-deficient subjects. Unfortunately, the half-life of ^{65}Zn is 245 days, and therefore this isotope is not regularly available for clinical use (47, 48).

The zinc content of various body tissues and fluids can be used to establish status (13). Plasma zinc levels can reflect the current status of an individual as the turnover rate of ^{65}Zn in plasma is very rapid (67). Spencer

et al. (67) have shown that 13 minutes after a test dose of ^{65}Zn is administered, 20% of the test dose is retained in the body. After 24 hours, only 1.3% of the test dose remains. Although the measurement of plasma zinc is very simple, there are a number of factors which can affect these levels (47). The effect of food intake has not really been settled. Davis et al. (7) found that the oral administration of 50 g of glucose to normal, fasted adults resulted in a rapid fall in plasma zinc levels followed by a return to original levels within 2 hours. McBean and Halsted (32), however, have reported that plasma levels were similar whether blood samples were taken during fasting or in a postprandial state. Zinc levels in plasma have been shown to be temporarily lowered in conditions such as infections (28), pregnancy (18), surgical procedures (28), and other stressful situations such as myocardial infarction (28). Fox (13) concluded that further work need to be done to establish conditions that affect plasma zinc levels in the normal adult.

The zinc content of hair is thought to reflect the zinc status for the period of time over which the hair grew (13). Strain et al. (69) showed that supplementation of a zinc deficient diet with zinc sulfate resulted in a marked elevation of zinc levels in hair. They concluded that hair analysis offered a simple and reliable method of assessing zinc stores. Hambidge et al. (20) found low levels

of zinc in hair collected from supposedly healthy children in Denver. Levels increased with zinc supplementation. Klevay (26) tested the hypothesis that hair could be used for the assessment of zinc nutriture in humans by studying the relationship between the zinc content of hair, red blood cells, and plasma. He found no statistically significant relationship between the zinc content of the three parameters studied. As noted in a review article (1) a correlation would not necessarily be expected as hair reflects past nutriture whereas plasma reflects more recent nutriture, current metabolism or both. McBean et al. (33), also failed to find a positive correlation between zinc levels in hair and plasma samples collected from Iranian children. Plasma zinc levels were judged to be low and yet zinc content in hair from the same subjects was high. The authors state that their data did not support the conclusion that zinc content of hair is a reliable parameter to be used to judge zinc stores.

Samples obtained from muscle or liver would reflect the zinc status of these tissues. Such tissues must be surgically obtained and are thus more difficult to procure than either hair or plasma. Analysis of the zinc content of muscle and liver, from individuals who died accidently, have been done by Tipton and Cook (73) and McBean et al. (31). In all cases, the amount in muscle was slightly greater than the amount in liver. The effect of dietary

change on zinc levels in these tissues has not been studied in the human.

Siegel et al. (64) studied the turnover rate of intravenously administered ^{65}Zn in a number of tissues including liver and skeletal muscle. Of the tissues studied, liver retained the highest amount of the dose administered whereas muscle had the lowest level, some 10 to 30 times less than liver. The turnover rate of ^{65}Zn in liver was judged to be slow as some 4% of the dose was still evident after 174 days. The small amount of ^{65}Zn accumulated by skeletal muscle did not appear to change significantly with time. Spencer et al. (67) did a similar experiment and found that after 71 days ^{65}Zn concentration in liver was still one-quarter of the amount present the first day. About 2% of the administered dose was deposited in skeletal muscle and this level was maintained for a prolonged period of time.

Smith et al. (65) have reported that liver zinc levels in the rat did not decrease with a dietary deficiency.

EXPERIMENTAL PROCEDURE

Subjects

Twenty-nine subjects participated in the experiment. Fifteen were controls, of which 6 were male. The 14 experimental patients were all females.

Patients who served as controls varied in age from 17 to 57 with a mean age of 33. Subjects were classified into 3 groups (Table I). Group A was composed of 3 patients who underwent major upper abdominal surgery, thereby making possible the collection of liver and muscle samples for zinc analysis. Plasma and hair samples were also collected from these patients. Group B were post-mortem individuals who had died suddenly. Hair, muscle and liver samples were collected from this group. McBean et al. (31) had found no significant difference in zinc levels in post-mortem samples of liver and muscle from diseased and non-diseased subjects. Group C were healthy, non-hospitalized individuals. Plasma and hair samples were obtained from this group.

Experimental patients were those who had previously undergone an end-to-end jejuno terminal ileostomy bypass operation (58) and were again hospitalized for varying reasons. They ranged in age from 22 to 50 years, with a mean age of 37 (Table II). Liver and muscle samples were obtained during abdominal surgery which took place from 240 to 4770 days

Table I. Description of Control Patients and Tissues Sampled.

Subject	Age (years)	Sex	Status	Tissue			
				Liver	Muscle	Hair	Plasma
Group A[a]							
1	52	M	hernia operation	✓	✓	✓	✓
2	46	M	sigmoid resection	✓	✓	✓	✓
3	27	F	cholecystectomy	–	–	✓	✓
Group B[b]							
4	19	M	cerebral edema with subdura hematoma	✓	✓	✓	–
5	17	M	"Stone" heart syndrome	✓	✓	✓	–
6	39	M	alcholic hepatitis	✓	✓	✓	–
7	57	F	renal carcinoma	✓	✓	✓	–
Group C[c]							
8	22	F	healthy	–	–	✓	✓
9	27	F	"	–	–	✓	✓
10	50	F	"	–	–	✓	✓
11	23	F	"	–	–	✓	✓
12	45	F	"	–	–	✓	✓
13	24	F	"	–	–	✓	–
14	23	M	"	–	–	–	✓
15	27	F	"	–	–	–	✓

[a] Hospitalized surgical patients

[b] Post-mortem subjects

[c] Non-hospitalized controls

Table II. Description of Experimental Patients and Tissues Sampled.

Subject	Age (years)	Sex	Days Post Operative[a]	Tissue			
				Liver	Muscle	Hair	Plasma
16	22	F	240	/	/	/	/
17	25	F	619	/	/	/	/
18	38	F	653	/	-	/	/
19	45	F	912	/	/	/	/
20	45	F	1139	/	/	/	/
21	38	F	2072	/	/	/	/
22	33	F	2247	/	/	/	/
23	27	F	2264	/	/	/	/
24	35	F	2290	/	/	/	/
25	27	F	2427	/	/	/	/
26	41	F	2950	/	/	/	/
27	50	F	3180	/	/	/	/
28	37	F	3875	/	/	/	/
29	44	F	4770	/	/	/	/

[a] Days since the bypass operation

after the bypass operation. Plasma and hair samples were also collected.

Collection and storage of tissues used to assess zinc status

The zinc status in the bypassed patient was determined by measuring zinc levels in liver, plasma, muscle, and hair. Zinc content of the liver was determined in both fat-containing and defatted, lean liver tissue, as fatty metamorphosis of the liver is one of the consequences of the intestinal bypass operation.

All containers and glassware which would come in contact with tissue were soaked overnight in 2M nitric acid and then rinsed 5 times with deionized water to render them zinc-free. As rubber stoppers contain high amounts of zinc, they were not used (16). Containers were stoppered with Parafilm.

Eight milliliter samples of blood were drawn by venous puncture with a glass syringe. Samples were collected from experimental subjects and hospitalized control subjects (Group A, Table I) between 8 and 9 a.m. following a 15 hour overnight fast and before the patient arose and became physically active. Samples from control subjects in Group C (Table I) were collected at 11 a.m. which was at least 3 hours following breakfast and after sedentary activity. If there was any indication of hemolysis, the blood sample was discarded and replacement samples were drawn as

the RBC has a much higher concentration of zinc than does the plasma (70). One-tenth of a milliliter of zinc-free sodium citrate (40 mg/0.1 ml) was used as the anticoagulant (21). Red blood cells were removed by centrifugation and the plasma stored at -20°C until used. Plasma, rather than serum, was used since the concentration of zinc in serum is 16% higher than plasma because of release of zinc from platelets during clotting (71).

One-half gram samples of liver and muscle (rectus abdominus) were obtained by surgical removal and stored at -20°C until used (11).

At the same time blood samples were drawn from subjects, 0.1 g samples of hair were collected from the nape of the neck. Each sample contained recently grown hair; no more than 2.5 cm distance from the scalp. Hair samples were stored in paper envelopes until used.

Measurement of zinc

Zinc levels in all tissues studied were measured using an "Evans Electroselenium Ltd." (EEL) atomic absorption spectrophotometer (see Appendix I A).

A. In plasma: The procedure of Prasad et al. (50) was used to determine the amount of zinc in plasma. Plasma was deproteinated with trichloroacetic acid (TCA), (see Appendix I B) and zinc measured in the TCA fraction of the

digest. Samples were assayed in duplicate.

B. In liver: Zinc levels in liver were determined using a modification of the procedure of Evenson et al. (11). Modification involved extraction of tissue fat with a Soxhlet micro-extraction apparatus (see Appendix I C) and a scaling up of Evenson's procedure so that larger tissue samples could be utilized (see Appendix I D). To remove the contaminating effect of red blood cells, liver samples were first washed with 10% reagent-grade formalin which contained no detectable zinc (38). Excess formalin was removed by placing the tissue sample on a disc of cellulose filter paper. The washed sample was then digested with nitric acid and zinc measured in the digest. Samples were assayed in triplicate.

C. In muscle: Zinc levels in muscle were determined using a modified version of the method described by Evenson et al. (11). Modifications are described in Appendix I D. All samples were assayed in triplicate.

D. In hair: Hair samples were washed according to the procedure of Reinhold et al. (55) (see Appendix I E). A modification of the method described by Pomeroy et al. (46) was used to determine zinc levels. Hair samples were acid digested and zinc measured by means of atomic absorption spectrophotometry. Samples were assayed in duplicate.

E. Preparation of a calibration curve: The absorbance of standard solutions containing 0.5 ppm to 6.0 ppm of zinc in 0.5 gradations were determined. Zinc concentration was plotted against absorbance. To account for small fluctuations in the spectrophotometer, the concentration of zinc in the standard solutions was determined both before and after the test samples were assayed. Absorbance values reported are an average of the two assays. As the standard curve changed from one set of analyses to another, a new curve was made each time a group of samples was run.

The standard solutions of zinc (see Appendix I F) were prepared with nitric acid and used for the assessment of zinc in muscle, liver and hair, while trichloroacetic acid (TCA) (25) was used to prepare the standard solutions for the assessment of zinc in plasma. Ten millimolar HNO_3 and 0.01% TCA were used for blanks, respectively.

F. Analysis of data: Means and standard error of the mean were calculated for each parameter measured. Doubtful observations were rejected according to the procedure described by Brumblay (6). To test the significance of differences between tissue zinc levels of the control and the experimental groups, an unpaired t-test was used. The correlation coefficient "r" was used to express the relationship between days since the bypass operation and tissue zinc levels as well as between per cent liver fat and liver zinc levels.

RESULTS AND DISCUSSION

Zinc levels in plasma

Control subjects had a mean plasma zinc level of 76 µg/100 ml (Table III). This value falls within the range of 72 to 115 µg/100 ml which Halsted and Smith (18) reported as normal but is lower than the mean of 96 µg/100 ml which these authors had found. Subjects 1-3 (Group A) had a mean plasma level of 76 µg/100 ml which was identical to the mean found in samples taken from Group C (subjects 8 - 15). Blood from subjects in Group A had been drawn before 9 a.m. and following a 15 hour fast, and no activity, whereas, that taken from Group C was drawn at least 3 hours after breakfast and following sedentary exercise. Food consumption and exercise seemed to have no affect on zinc plasma levels in these subjects.

Experimental subjects had a mean plasma zinc level of 63 µg/100 ml (Table III), which is below the 72 µg/100 ml suggested by Halsted and Smith as the lower limit of normal (18). Whether plasma was obtained before or after surgery did not affect plasma zinc levels as the mean of 58 µg/100 ml for plasma obtained 7 to 10 days after surgery was not significantly lower than the mean of 71 µg/100 ml for plasma obtained before surgery (Table III).

The mean plasma level of 63 µg/100 ml for experimental subjects was significantly less ($P < 0.01$) than the 76 µg/100 ml

Table III. Levels of Zinc in the Plasma of Control and Experimental Subjects.

Control		Experimental		
Subject	Zinc µg/100 ml	Subject	Zinc µg/100 ml	Sample Obtained
1	87	16	56	A[a]
2	66	17	51	A
3	75	18	56	A
8	79	19	56	A
9	81	20	65	B[b]
10	76	21	75	A
12	71	22	63	B
14	78	23	70	B
15	75	24	51	B
		25	61	A
		26	51	A
		27	83	B
		28	93	B
		29	54	A
Mean±SEM[c]	76±2		63±3	

[a] After surgery

[b] Before surgery

[c] Standard error of the mean

obtained for control subjects. Correlation between days since the bypass operation and plasma zinc levels for the experimental subjects was not significant (r=0).

Zinc levels in liver

Freeze-dried liver samples obtained from control and experimental subjects contained mean zinc levels of 213 and 175 μg/g, respectively (Table IV). These values are similar to the mean of 179 μg/g found in normal subjects by McBean et al. (31) and fall within the range of 91 to 323 μg/g considered by these workers to be normal. The mean levels of zinc found in control and experimental subjects were not significantly different from each other.

Zinc levels in dried liver, from which the fat had been extracted, were 236 μg/g in the control subjects and 220 μg/g in the experimental subjects (Table IV). These values were not significantly different from each other. Zinc levels in fat-extracted liver from both control and experimental subjects were significantly higher ($P < 0.01$) than values found in fat-containing liver. However, these values were still within the normal range reported by McBean et al. (31).

Although the amount of fat in liver was greater in experimental than control subjects, 21% vs 10% (Table IV), this difference was not significant. Salmon (58) has reported that the percentage of individuals with fatty

Table IV. Zinc Levels and Percent Fat in Liver.

Subject	Days Post-op[a]	Zinc (μg/g)[b]		Fat %
		Fat-containing	Fat-free	
CONTROLS				
1		216	229	6
2		240	264	9
4		195	220	11
5		215	237	9
6		188	216	13
7		222	251	11
Mean±SEM[c]		213±10	236±10	10±1
EXPERIMENTAL				
16	240	82	198	58
17	619	120	156	14
18	653	224	254	12
19	912	205	228	10
20	1139	134	208	36
21	2072	212	268	21
22	2247	235	268	13
23	2264	233	255	9
24	2290	89	143	39
25	2427	152	206	26
26	2850	173	207	16
27	3180	277	301	8
28	3875	158	199	21
29	4770	158	189	16
Mean±SEM[c]		175±15	220±11	21±4

[a] Days since the bypass operation

[b] Freeze-dried samples

[c] Standard error of the mean

metamorphosis is especially marked during the first year post-op and gradually decreases to more normal levels some 1000 to 1500 days following bypass surgery. Five experimental subjects in the present study had had the bypass operation for fewer than 1200 days. They had a mean level of 26% fat in the liver compared with 19% for the 9 subjects who had had the operation for greater than 1200 days. This difference was not statistically significant.

No significant correlation between days since the bypass operation and zinc levels was found for either fat-containing ($r=0.22$) or fat extracted, freeze-dried ($r=0.05$) liver samples. Correlation between per cent liver fat and zinc levels in fat-free, dried samples from experimental subjects ($r=-0.48$) was also not significant. In fat-containing, experimental liver samples, correlation between per cent liver fat and zinc levels ($r=-0.79$) was significant at $P < 0.01$. As the amount of fat in the liver increased, the amount of zinc in the liver decreased (Figure 1).

Zinc levels in muscle

Freeze-dried muscle samples from control subjects had a mean zinc concentration of 185 µg/g (Table V). This falls within the range of 94-264 µg/g considered to be normal as reported by McBean et al. (31) and is slightly

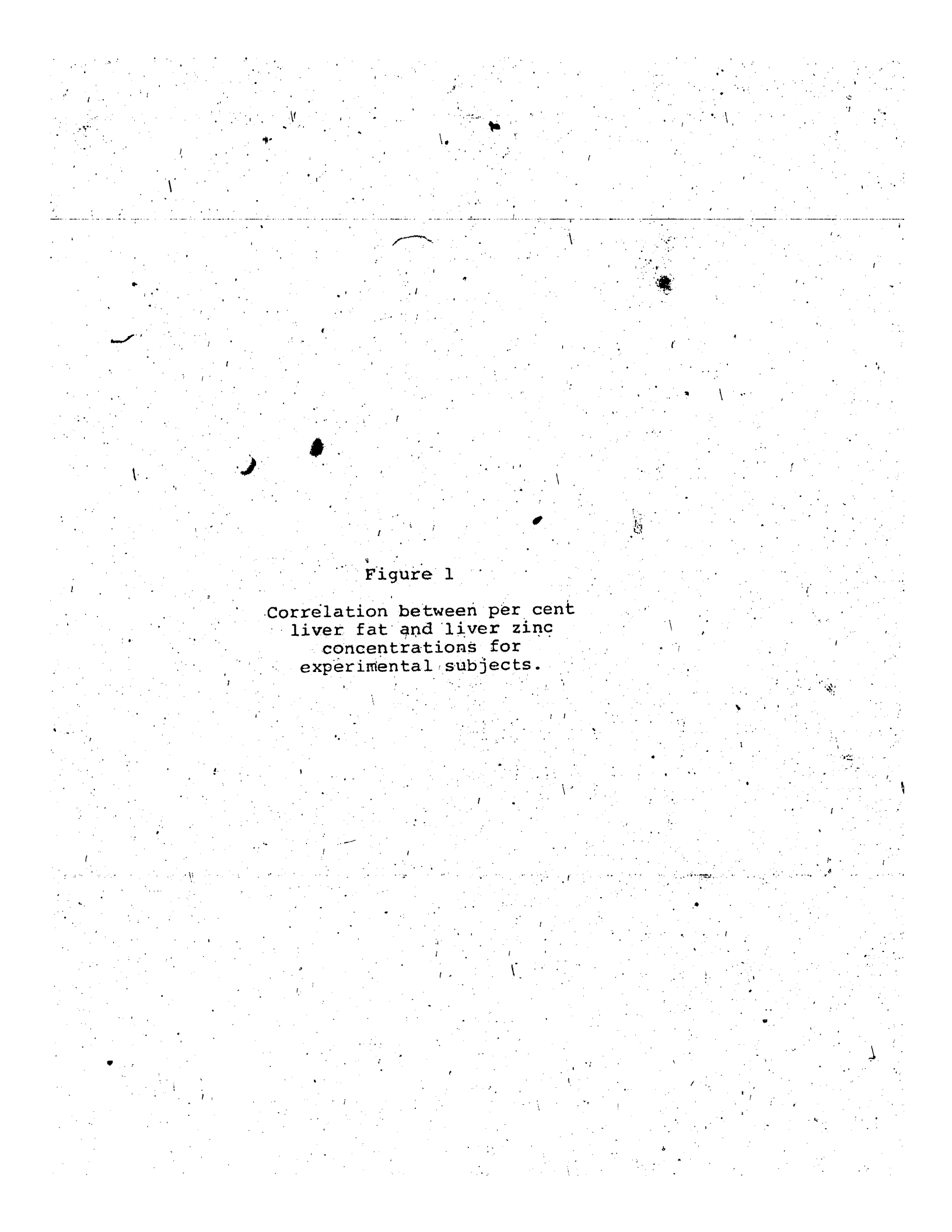

Figure 1

Correlation between per cent liver fat and liver zinc concentrations for experimental subjects.

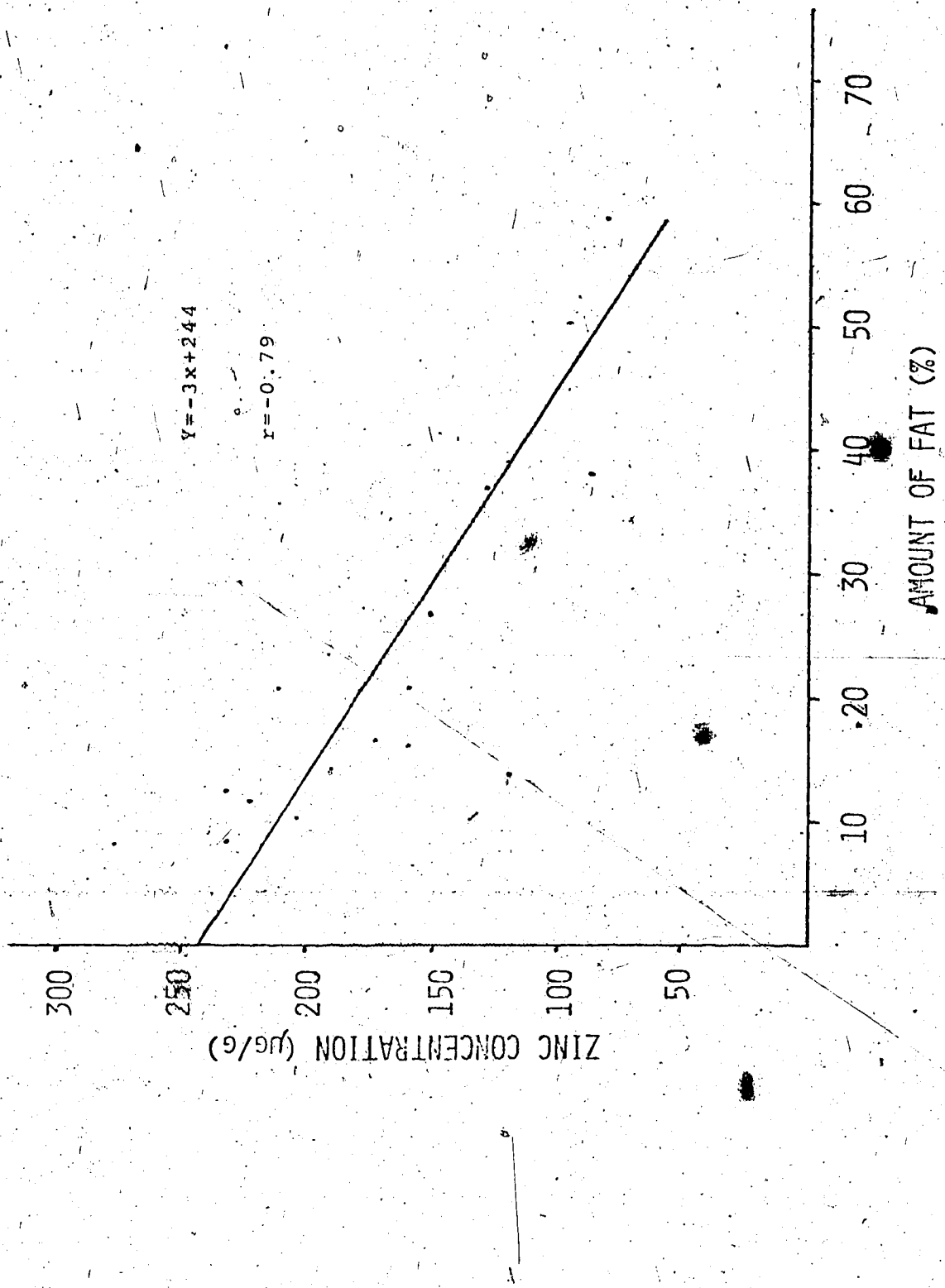
Y=-3x+244
r=-0.79
ZINC CONCENTRATION (μg/g)
300
250
200
150
100
50
10
20
30
40
50
60
70
AMOUNT OF FAT (%)

Table V. Levels of Zinc in the Muscle of Control and Experimental Subjects

Control		Experimental	
Subject	Zinc[a] µg/g	Subject	Zinc[b] µg/g
1	175	16	206
2	198	17	155
4	174	19	143
5	186	20	160
6	184	21	166
7	191	22	156
		23	195
		24	137
		[illegible]	205
		26	223
		27	149
		28	142
		29	183
Mean±SEM[b]	185±5		171±7

[a] Freeze-dried samples

[b] Standard error of the mean

lower than the mean value of 197 µg/g found by these authors. Experimental subjects had a mean of 171 µg/g (Table V) which is also considered to be normal. Levels in control and experimental subjects were not significantly different.

Correlation between days since the bypass operation and muscle zinc levels was not significant (r=0.02).

Zinc levels in hair

Hair samples from control subjects had a mean of 193 µg/g (Table VI). This is considered to be normal as it falls within the range of 150-250 µg/g suggested by Pomeroy et al. (46). Hair from experimental subjects had a mean zinc content of 155 µg/g (Table VI). This level is low when compared to controls and is significantly different at a $P < 0.025$.

Correlation between days since the bypass operation and hair zinc levels was not significant (r=0).

Zinc status of bypass patients

Zinc levels in plasma and hair taken from the intestinal bypass patients were significantly lower than levels found in controls. Whether the decreased levels in these tissues was due to an inadequate intake of zinc by these subjects is not known as a record of food intake was not obtained. All subjects consumed ad libitum diets.

Table VI. Levels of Zinc in the Hair of Control and Experimental Subjects.

Control		Experimental	
Subject	Zinc[a] µg/g	Subject	Zinc[b] µg/g
1	186	16	108
2	255	17	83
3	175	18	157
4	183	19	105
5	178	20	150
8	221	21	110
9	184	22	119
10	180	23	176
11	169	24	221
12	159	25	180
13	232	26	149
		27	207
		28	208
		29	190
Mean±SEM[b]	193±10		155±11

[a] Freeze-dried samples

[b] Standard error of the mean

Experimental patients ate relatively large quantities of food and thus could have been consuming adequate amounts of zinc. However, a decrease in zinc absorption because of the shortened intestine could have resulted in the lower levels of zinc found in plasma and hair.

Stress or trauma can cause a decrease in plasma zinc levels (28). All experimental subjects had undergone recent abdominal surgery. However, Sefton et al. (63) showed that the level of zinc in the plasma usually returned to normal 2 to 3 days following surgery; therefore, surgery should not have greatly affected levels in this study as plasma samples were obtained either before or 7 to 10 days after surgery.

The level of zinc in hair samples taken from experimental patients was significantly lower than in control subjects. Hair gives an indication of previous zinc intake (33), but does not necessarily indicate zinc status at the time of sampling. Gilbert et al. (15) have shown that ^{65}Zn turnover rate in hair from the rat is very slow. They found that 100 days after the injection of ^{65}Zn, 7% of the dose was still present in the hair compared with 0.4% and 2.2% of the test dose in liver and muscle, respectively. Ballou and Thompson (3) showed the same results. They found that even after 300 days, 0.01% of the test dose was still in the hair, whereas no detectable levels of the test dose could be found in liver and muscle. They were also able to

show that chronic feeding of ^{65}Zn resulted in a continuous uptake of zinc by hair suggesting that the turnover rate of zinc in hair is very slow.

In the present study, hair samples were clipped from the nape of the neck and were not longer than 2.5 cm in length, as measured from the scalp. Depending upon rate of hair growth, these samples should indicate recent zinc status, perhaps some 1 to 3 months previous to sampling.

Only a few experimental subjects had low zinc levels in both plasma and hair. The results of this study (r=0.36) would agree with the suggestion of McBean et al (33), that there is no correlation between plasma and hair zinc concentrations.

Levels of zinc in muscle and liver samples in experimental subjects were similar to levels found in controls and would indicate an adequate state of zinc nutrition. Ballou and Thompson (3) showed that liver, when compared to muscle, rapidly takes up ^{65}Zn. One hour after administration of ^{65}Zn 4.8% and 0.12% of the test dose was found in liver and muscle, respectively. Turnover of ^{65}Zn in the liver is very rapid compared with muscle, as after 100 days, the amount of the test dose retained had dropped from 4.8% to 0.02% in liver compared to a drop from 0.12% to 0.01% in muscle.

Zinc levels decreased as the amount of fat increased in liver samples obtained from experimental subjects; thus,

there was a negative correlation between per cent fat and liver zinc levels. Zinc levels in fat-extracted liver samples from experimental subjects were significantly higher ($P < 0.01$) when compared to fat-containing liver samples. A significant difference was also found in the control subjects. However, the zinc levels in both fat-containing and fat-extracted liver samples from the experimental, as well as control subjects were normal (31).

Zinc levels in plasma, liver, muscle, and hair of experimental subjects were very scattered and thus poorly correlated. This lack of correlation agrees with the statement by McBean et al. (31) that a decrease in plasma zinc, as would be found in deficiency states, has not yet been shown to be reflected by decreases of zinc in other tissues. Gilbert and Taylor (15) found that almost twice as much of a test dose of ^{65}Zn was taken up by liver than by leg muscle in rats. Zinc was then rapidly lost from liver to plasma whereas that in muscle did not completely equilibrate with plasma for several days. Thus plasma zinc seems to more freely exchange with zinc in liver than in muscle. Differences in turnover rate could help explain why plasma levels correlate poorly with zinc levels in soft tissues.

Mean zinc levels in the plasma, liver, muscle, and hair from individuals who had undergone the bypass operation were less than levels found in control subjects. However, as only zinc levels in plasma and hair from the experimental

subjects were significantly different from controls, no conclusion as to the zinc status of bypass patients can be drawn. Further work with more subjects, using additonal parameters to assess zinc status, is needed.

SUMMARY

1. The amount of zinc in plasma, liver, muscle, and hair of control subjects was normal when compared with values found in the literature. Zinc levels in liver and muscle from intestinal bypass patients were also normal; however, values for plasma and hair were less than the normal range for these parameters.

2. Levels of zinc in plasma and hair from bypassed subjects were significantly less than values found for control subjects, $P < 0.010$ and $P < 0.025$, respectively. There was no significant difference in zinc levels found in liver and muscle from these 2 groups.

3. Liver samples from bypassed patients contained 21% fat compared to 10% fat from control subjects. This difference was not significant.

4. As the amount of fat in the liver increased, the amount of zinc decreased. With the removal of fat from liver tissue, the amount of zinc per gram of tissue increased. Zinc content of the fat-free tissue was still normal for both control and experimental subjects and the differences between these 2 groups was not significant.

5. The fat content of liver in subjects who had undergone the bypass operation for less than 1200 days was 26% whereas

those who had had the bypass operation for more than 1200 days had a value of 19%. This difference was not statistically significant.

6. Because only plasma and hair and not liver and muscle zinc levels suggested that zinc status in the intestinal bypass patient was inadequate, additional studies are required to fully assess status.

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APPENDIX

Appendix I

A. Instrument details for the "EEL" atomic absorption spectrophotometer (72)

wavelength setting: 211 mμ (2118 Å)

slit setting: 0.07 mm or less

lamp current range: 1 (with a Westinghouse hollow cathode lamp)

fuel: oxidizing: use 10 psi air pressure and 8 psi acetylene pressure to give a clear blue flame

warm-up time: approximately 30 minutes with the burner lit

B. Determination of plasma zinc levels

Eight milliliters of blood were collected with zinc-free glass syringes. Blood was delivered into an acid-cleaned test-tube which contained 0.1 ml of 40% sodium citrate. Plasma was obtained by centrifuging the blood at 3,000 rpm for 20 minutes.

Plasma was deproteinized by adding 1 ml of 10% trichloroacetic acid (TCA) to each 2 ml of plasma and then heating the mixture at 90° for 5 minutes. The sample was again centrifuged for 20 minutes at 3,000 rpm. The supernatant was decanted and

saved. To the precipitate, 1 ml of 10% TCA was again added, and the mixture heated and centrifuged as described above.

The supernatant solutions from each sample were combined and used in the atomic absorption spectrophotometer to determine zinc. The TCA used contained trace amounts of zinc; therefore, zinc was determined in 2 ml of TCA and this value was subtracted from the zinc content found in each plasma sample.

C. Fat extraction of liver samples

One-tenth of a gram of liver was accurately weighed out, washed in 10 ml of formalin and then freeze-dried for 10 hours. The dry weight of the tissue was then recorded. The dried tissue sample was placed in a 10x50 mm cellulose thimble which was then placed into a Soxhlet micro-extraction apparatus.

Twenty-five milliliters of anhydrous ethyl ether was added and the tissue extracted for 4 hours at 38°. Preliminary work had shown the ether extract to be zinc-free.

D. Determination of zinc in fat-free liver samples

A freeze-dried liver sample was placed in a 16x150 mm test tube which had a # 13 ground-glass joint. Three milliliters of 2 M zinc-free HNO_3 was added and the sample was digested at 80° for 24 hours. The HNO_3 was evaporated with a Buchi flash evaporator until only the digest residue remained which was a viscous yellow liquid. Four milliliters of 10 mM

zinc-free HNO_3 was added to the digest residue with vigourous mixing. To remove any insoluble particles, the sample was centrifuged at 3,000 rpm for 20 minutes and the supernatant decanted. Zinc was determined in the supernatant by flame atomization.

E. Determination of zinc in hair

One-tenth gram of hair was cut from the nape of the neck. The hair was washed in 100 ml of a 0.1% (v/v) solution of soap (Lux Liquid) at room temperature for 10 to 20 minutes with occasional stirring. The soap solution was then decanted through filter paper and replaced by another 100 ml of soapy solution. This solution in turn was decanted after 20 minutes of soaking and the hair rinsed in 4-10 exchanges of approximately 50 ml each of deionized, demineralized water until no foaming could be detected in the rinse water. Dyed hair samples were rinsed until no dye was evident in the wash water. The hair was then immersed in 2 successive portions of about 25 ml of 95% ethanol for 5 to 10 minutes each, and finally in 2 successive portions of 25 ml of ether for a similar time. The hair was removed from the ethyl ether and dried over anhydrous calcium chloride for 1 day.

Duplicate samples of 0.05 gram of the dried hair were accurately weighted in an acid-washed 25 ml erlenmeyer flask. To each flask was added 2.5 ml of concentrated HNO_3. The flask was placed on a hot plate in a fume hood and the hair

dissolved by gentle boiling. When the volume was decreased to about half, the solution was cooled and 2 ml of perchloric acid was added. Gentle boiling was continued until only 1 to 2 ml remained and the solution was colourless. After cooling, the solution was brought to 25 ml in a volumetric flask. A 10 ml sample from this flask was used to determine the level of zinc in the hair. A blank was run because of the high zinc levels in the perchloric acid.

F. Preparation of stock solution for standard curves

One-half gram of high purity zinc was dissolved in 100 ml of 50% by volume concentrated nitric acid. The solution was cooled and then made to 500 ml with deionized, demineralized water. Serial dilutions were used to prepare the working concentrations for the standard curve. Ten per cent TCA was used for the stock standard for zinc measurement in plasma.