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COMPARISON OF THE BIO-AVAILABILITY OF TRACE ELEMENTS IN INORGANIC SALTS, AMINO ACID CHELATES AND YEAST.

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ABSTRACT

The bio-availability of various forms of the trace elements selenium, manganese, zinc and iron has been investigated using rats as the test animal. Ten groups of 5 rats each were used for the study. For each element studied, all rats were fed a commercial trace element deficient diet for 2 to 4 weeks to produce a state of deficiency. Then each group was fed the element supplemented at one of 3 levels in one of 3 forms - inorganic salt, commercial amino acid chelate and yeast. After this period of supplementation, the rats were sacrificed and the concentration of the element determined in the blood and liver. The slope of the plot of element concentration in the diet vs. element concentration in the blood or liver gave the bio-availability.

In the blood the order of decreasing bio-availability is yeast > inorganic salt > amino acid chelate. In the liver the order is yeast > amino acid chelate > inorganic salt. The results show that yeast is the most bio-available form of the element. It is thus concluded that yeast is the best form for trace element supplementation.

INTRODUCTION

Although trace element deficiencies resulting in overt physiological changes are rare in the population of developed nations, the incidence of sub-optimal deficiencies may, in fact, be common. These slight deficiencies are a result of three factors:

1) soil deficiencies of trace elements in certain geographical areas.
2) declining level of trace elements in the soil due to repeated farming and the use of easily leachable inorganic fertilizers.
3) processing of foods prior to consumption.

There are many different forms of trace elements in supplements for human consumption that are available in the marketplace. Inorganic salts such as sulfates and carbonates are the most commonly used form as they are the cheapest. Also common are the organic salts such as citrates and gluconates. Another form which is thought to be more utilized by the body are the amino acid chelates. These are usually formed by hydrolysis of protein and reaction of the resulting amino acid with an inorganic salt to supposedly form a chelate of the metal ion with the ligand amino acids. A third form, yeast, is produced by growing a yeast in a nutrient medium containing the inorganic salt. In theory, the yeast absorbs the element by forming a natural chelate between the metal ion and the proteins and/or amino acids of the yeast.

With the advent of atomic absorption spectroscopy twenty years ago, there has been a large body of literature on the concentration of trace elements in foods and more recently physiological fluids. While laboratory analysis can determine how much of a given material is present in a food, only a well-designed bio-assay can provide information about the bio-availability of that material. In nutrition, a bio-assay is especially important in dealing with essential trace elements because many factors influence their utilization. Among these are digestibility of the carrier food, chemical and physical form

of the element, the body's need for that element, interaction with other nutrients and the role of chelation.

Bio-availability was defined by Fritz (1976) as the ratio between the quality of a nutrient in a sample as determined by animal assay to the quantity determined by chemical analysis. There are several methods to determine bio-availability of trace elements. These include (1) balance studies with either man or laboratory animals, (2) radiotracer techniques or (3) serum metal response following test doses. Although radiotracer studies with mice or animals are the easiest to perform, the results may be criticized because the spiked radiotracer may not behave in the same way as the non-spiked element being tested. This is the case when the form of the element being tested is different from that of the spiked tracer. Animal studies offer the best method of determining bio-availability of a trace element. The Association of Official Analytical Chemists (1975) has adopted a method for measuring bio-availability of iron based on repletion of hemoglobin in anemic rats. In this official method a reference standard is used and other forms of the element compared to it.

MATERIALS AND METHODS

Male weanling Sprague-Dawley rats (45-55 g) were used for the study. The rats were put into 10 groups of 5 and matched according to the average weight of the group. Each group was housed in steel cages and fed water and rat food ad libitum. All groups were fed a trace element deficient diet (ICN Nutritional Biochemicals) for a period of time to induce a depletion of the trace element. Then one group was continued on the deficient diet for the duration of the study. The other 9 groups were fed 3 different levels of trace element supplemented with 3 different forms of the element - inorganic salt, amino acid chelate and yeast. The forms were weighed, thoroughly pulverized, and added to the powdered diet in a laboratory blender.

After the repletion period, all rats were weighed and sacrificed. The liver and the blood were removed and stored at -20°C until analysis.

Selenium (Se) Study

The rats were fed selenium-deficient food for 12 days. The repletion study used the same diet to which 50 ppb, 100 ppb and 200 ppb Se were added. The forms of Se used were sodium selenite Na_2SeO_3, amino acid chelate (Essential Organics, 50 µg/tablet) and yeast (Grow Company, 200 µg Se/g yeast).

The liver and blood were dry ashed according to TAM and LACROIX (1979) except that 50% HNO_3 was used to dissolve the ash. Liver and blood Se were analyzed fluorimetrically according to the AOAC method (1975).

Manganese (Mn) Study

The depletion period lasted 21 days using the Mn deficient diet containing 160 ppb Mn. The food was then supplemented with 2 ppm, 5 ppm and 10 ppm Mn in the form of manganese sulfate ($MnSO_4$), amino acid chelate (Nu-Life Products, 10 mg/tablet) and High Mn Yeast (Grow Company, 0.05 g/Mn g yeast). The liver and blood Mn were measured by atomic absorption spectroscopy after a modification of the AOAC Method (1975). The samples were heated with 2N HCℓ while drying overnight at 105-110°C, then dry ashed at 350-400° C.

Zinc (Zn) Study

Rats were fed a low Zn diet (1.1 ppm) for 30 days. They were then supplemented for 30 days with 50 ppm, 100 ppm and 250 ppm Zn in the form of zinc

sulfate ($ZnSO_4$), amino acid chelate (NF Factors, 15 mg Zn/tablet) and High Zn yeast (Grow Company 0.063 g Zn/g yeast). Liver Zn was determined by atomic absorption spectroscopy after drying in an oven for several hours at 105° C, then dry ashing overnight at 450° C. The ash was reconstituted in 2N HCl. Blood Zn was determined by atomic absorption spectroscopy using the method of STEVENS (1979) in which 0.5 ml of blood was diluted to 100 ml with 0.05% aqueous Triton X-100.

Iron (Fe) Study

The depletion period was 18 days with Fe deficient food (5.8 ppm). Repletion lasted 30 days with food supplemented with 10 ppm, 25 ppm and 50 ppm Fe in the form of iron sulfate ($FeSO_4$), amino acid chelate (Natural Sales Company, 22 mg Fe/tablet) and High Fe yeast (Grow Company, 0.05 g Fe/g yeast). Liver Fe was determined by atomic absorption spectroscopy using the same ashing procedure as for Zn. Blood hemoglobin Fe was determined by atomic absorption spectroscopy using the method of ZETTNER (1967).

Calculations

The bio-availability was calculated using a conventional linear dose response plot. The concentration of element in the diet (x-axis) was plotted vs. the concentration in the blood or liver (y-axis). The slope of the line and the least squares correlation coefficient were calculated using a Texas Instrument SR-51-II calculator. From the slope of the line, the bio-availability was determined. The inorganic salt was used as the standard for comparison purposes.

RESULTS AND DISCUSSION

Selenium Study

The nutritional value of selenium was first described in experimental animals over 20 years ago by SCHWARTZ and FOLTZ (1957). Selenium has been shown to be necessary for growth and fertility in animals. It is also necessary for the prevention of various animal disease conditions including liver necroses. STADTMAN (1980) has reviewed the selenium-dependent enzymes in living systems. In man, glutathione peroxidase is the most important selenium enzyme and it is involved in cellular defense against oxidants. The case for its essentiality in man has been reviewed by SCHROEDER (1970). The Food and Nutrition Board of the National Academy of Sciences (1979) has recommended 50-200 µg of Se/day for human adults.

The results of the dose response assay and bio-availability are shown in Tables 1 and 2. As can be seen, the blood and liver levels rise with increasing dose. This has also been seen by other investigators. Selenium deposition in the blood, muscle, liver, kidney and skin of chickens was shown by SCOTT and THOMPSON (1971) to bear a direct relationship between inorganic Se in the diet in levels up to 0.3 ppm. The order of relative bio-availability was seen to be different in the liver than in the blood. However, in both liver and blood, the yeast Se was the most bio-available. In the liver, Chelate Se was more bio-available than inorganic Se while in the blood the inorganic Se was more bio-available. Natural Se was also found by LATSHAW (1975) to be superior to selenite Se in increasing the Se levels in the muscle and liver of laying hens. We found, in rats, a linear relationship between dose and response. KU (1972) found a linear correlation between muscle Se and dietary Se in pigs.

Table 1. Dose Response Assay for Different Forms of Selenium in the Blood and Liver of Rats Supplemented with Selenium.

Form of Se	Se in Food (ppb)	Average Blood Se (ppb)	Average Liver Se (ppb)
Na_2SeO_3	50	463 ± 174	490 ± 64
Na_2SeO_3	100	790 ± 245	727 ± 43
Na_2SeO_3	200	1249 ± 356	933 ± 331
Chelate	50	332 ± 219	503 ± 223
Chelate	100	560 ± 308	750 ± 129
Chelate	200	811 ± 483	1129 ± 76
Yeast	50	708 ± 253	651 ± 287
Yeast	100	947 ± 361	908 ± 162
Yeast	200	1633 ± 226	1597 ± 160

Table 2. Relative Bio-availability of Different Forms of Selenium.

Form of Se	Slope of Plot	Coefficient Correlation	Relative Bio-availability
Na_2SeO_3	5.15 (Blood)	0.9956	100% (Blood)
Chelate	3.10 (Blood)	0.9868	60% (Blood)
Yeast	6.23 (Blood)	0.9966	122% (Blood)
Na_2SeO_3	2.83 (Liver)	0.9736	100% (Liver)
Chelate	4.12 (Liver)	0.9976	146% (Liver)
Yeast	6.39 (Liver)	0.9977	226% (Liver)

Manganese Study

Manganese (Mn) deficiency has been demonstrated in many animals and is observed in man in association with a Vitamin K deficiency (DOISY, 1974). The main manifestations of Mn deficiency are impaired growth, skeletal abnormalities, disturbed or depressed reproductive functions, ataxia of the newborn and defects in lipid and carbohydrate metabolism are displayed in all species studied (UNDERWOOD, 1977). The National Academy of Sciences (1979) has recommended 2.5-5.0 mg Mn/day for a human adult. However, recent studies in Japan, England and the United States (UNDERWOOD, 1977) have shown that the average daily intake is 2-3 mg, on the low end of the recommended scale. The low intake is due in part to the fact that whole cereal grains which are high in Mn such as wheat (38 ppm) are processed to produce white flour containing only 5 ppm Mn (ZOOK, 1970).

The results for the Mn study are shown in Tables 3 and 4. The yeast was the most bio-available form of the 3 forms tested. The order of bio-availability in blood was yeast > inorganic Mn > chelate. In liver, the order was yeast > chelate > inorganic Mn. The extreme variability of the blood and and liver concentrations of members of a group has been seen by other workers (HURLEY, 1974). They found that at low levels of Mn (less than 40 ppm) the response of individuals may vary greatly due to differences in genetic background. BRITTON (1966) found in agreement with this study that a linear relationship existed between dietary Mn and tissue levels in mice. The present study appears to be the first to examine the relative bio-availability of different forms of Mn.

Zinc Study

Zinc (Zn) is probably the most studied of the trace elements. Growth retardation was observed in the original demonstration of Zn deficiency in

Table 3. Dose Response Assay for Different Forms of Manganese in the Blood and Liver of Rats Supplemented with Manganese.

Form of Mn	Mn in Food (ppm)	Average Blood Mn (ppm)	Average Liver Mn (ppm)
$MnSO_4$	2	35.0 ± 5.8	72.8 ± 22.6
$MnSO_4$	5	45.7 ± 10.8	94.4 ± 47.6
$MnSO_4$	10	65.7 13.5	150.8 ± 49.6
Chelate	2	32.6 ± 7.4	82.8 ± 25.2
Chelate	5	42.5 ± 6.5	130.6 ± 40.0
Chelate	10	54.1 ± 4.7	191.6 ± 50.8
Yeast	2	44.5 ± 16.1	121.2 ± 37.4
Yeast	5	64.6 ± 14.8	159.6 ± 25.8
Yeast	10	89.0 ± 10.9	223.6 ± 40.2
Mn deficient food	0.016	26.0 ± 4.8	44.4 ± 18.0

Table 4. Relative Bio-availability of Different Forms of Manganese.

Form of Mn	Slope of Plot	Correlation Coefficient	Relative Bio-availability
$MnSO_4$	3.95 (Blood)	0.9995	100%
Chelate	2.80 (Blood)	0.9955	71%
Yeast	6.15 (Blood)	0.9900	156%
$MnSO_4$	10.29 (Liver)	0.9961	100%
Chelate	14.43 (Liver)	0.9952	142%
Yeast	16.76 (Liver)	0.9672	163%

rats (TODD, 1934). Nutritional dwarfism and hypogonadism are the main clinical manifestations of Zn deficiency in humans (PRASAD, 1962). Also, appetite and taste are adversely affected by a Zn deficiency. Zn is also an important constituent of many enzymes. The recommended daily allowance is 15 mg Zn/day for a male adult and 25 mg/day for lactating women. SANDSTEAD (1973) has stated that diets of some infants, pregnant women and college women and institutionalized invididuals are deficient in Zn.

The results of the Zn supplementation study are shown in Tables 5 and 6.

Table 5. Dose Response Assay for Various Forms of Zinc in the Blood and Liver of Rats Supplemented with Zinc.

Form of Zn	Zn in Food (ppm)	Average Blood Zn (ppm)	Average Liver Zn (ppm)
Zn deficient food	1.1	2.56 ± 0.08	6.16 ± 1.37
$ZnSO_4$	50	2.77 ± 0.09	6.85 ± 0.99
$ZnSO_4$	100	2.98 ± 0.24	8.09 ± 1.67
$ZnSO_4$	250	3.41 ± 0.53	8.96 ± 1.12
Chelate	50	2.76 ± 0.57	6.75 ± 1.10
Chelate	100	3.06 ± 0.60	8.32 ± 2.10
Chelate	250	3.41 ± 0.97	9.60 ± 1.82
Yeast	50	2.79 ± 0.25	7.26 ± 1.23
Yeast	100	3.45 ± 0.47	9.29 ± 1.38
Yeast	250	3.98 ± 0.74	11.33 ± 1.21

In this study, the order of bio-availability of Zn is yeast > chelate > inorganic Zn. However, the blood bio-availabilities' of Zn were almost identical for inorganic Zn and the chelate. A linear response of blood and liver concentration to diet concentration was seen in this study. KIRCHGESSNER (1972) also found a linear relationship in rats.

Table 6. Relative Bio-availability of Different Forms of Zinc.

Form of Zn	Slope of Plot	Correlation Coefficient	Relative Bio-availability
$ZnSO_4$	0.00334 (Blood)	0.9527	100%
Chelate	0.00336 (Blood)	0.9786	101%
Yeast	0.00576 (Blood)	0.9639	172%
$ZnSO_4$	0.0110 (Liver)	0.9507	100%
Chelate	0.0142 (Liver)	0.9672	129%
Yeast	0.0206 (Liver)	0.9736	187%

Iron Study

The demands of the body for iron are greatest during three periods - the first two years of life, the period of rapid growth and throughout the child-bearing age in women.

Iron deficiency in children results in the development of fatigue, anorexia depressed growth and decreased resistance to infection. The clinical symtom of Fe deficiency is anemia. The National Academy of Sciences (1974) recommends from 10-18 mg Fe/day. Fe fortification of cereals, flour, and bread is common in the United States to prevent deficiencies. But CALLENDER (1973) states such a fortification can only be expected to prevent some women who are marginally deficient from developing Fe deficiency.

The results from the Fe study are shown in Tables 7 and 8.

Table 7. Dose Response Assay for Various Forms of Iron in the Blood and Liver of Rats Supplemented with Iron.

Form of Fe	Fe in Food (ppm)	Average Blood Hb (mg/100 ml)	Average Liver Iron (ppm)
Fe Deficient Food	5.8	48.3 ± 15.3	29.2 ± 2.3
$FeSO_4$	10	65.5 ± 16.4	39.9 ± 1.4
$FeSO_4$	25	107.9 ± 15.7	43.1 ± 3.4
$FeSO_4$	50	116.5 ± 25.7	51.5 ± 4.5
Chelate	10	71.0 ± 13.8	37.9 ± 6.7
Chelate	25	88.0 ± 11.1	40.1 ± 5.3
Chelate	50	91.2 ± 10.1	45.5 ± 3.7
Yeast	10	69.5 ± 13.1	40.6 ± 6.5
Yeast	25	104.7 ± 18.9	44.6 ± 3.8
Yeast	50	118.8 ± 17.8	55.9 ± 4.1

Table 8. Relative Bio-availability of Different Forms of Iron.

Form of Fe	Slope of Plot	Correlation Coefficient	Relative Bio-availability
$FeSO_4$	1.39 (Blood)	0.9282	100%
Chelate	0.79 (Blood)	0.8785	57%
Yeast	1.41 (Blood)	0.9510	101%
$FeSO_4$	0.405 (Liver)	0.9537	100% (Liver)
Chelate	0.290 (Liver)	0.9303	72% (Liver)
Yeast	0.491 (Liver)	0.9695	121% (Liver)