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II. Absorption of zinc from organic and inorganic sources*

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Oysters and herring, two foods reported to be extraordinarily rich in zinc, were used to determine whether such foods could be used in conjunction with serial measurements of plasma zinc concentration to quantify the absorption of zinc in man. A dose of 120 gm of Atlantic oysters contained 108 mg of elemental zinc and produced a mean elevation in plasma zinc of 142 ± 22 μg/dl (mean ± S.E.M.) at 3 hr after ingestion. Both black beans and corn tortillas, at intakes of 120 gm, decreased the bioavailability of zinc from oysters, and inhibition was significantly greater by tortillas than by beans. In the presence of black beans, equivalent doses of elemental zinc as $ZnSO_4 \cdot 7H_2O$ and as oysters produced indistinguishable plasma zinc patterns. In the presence of tortillas, absorption was slightly greater from inorganic zinc than from oysters, but the biological importance of this difference is unclear. A dose of 120 gm of the herring used in this study contained only 2 mg of elemental zinc. When 70 gm of tortillas were ingested with the herring, plasma zinc concentration declined significantly. For Atlantic oyster, the richest known animal source of zinc, no evidence for a distinct, more available pool of dietary zinc, analagous to the "heme iron" pool of dietary iron, was demonstrable. Our data did show the effective use of Atlantic oysters as a source of organic zinc in the study in humans of the absorption and bioavailability of zinc. (J LAB CLIN MED 94:335, 1979.)

Various food constituents can enhance or inhibit the absorption of given nutrients, especially of trace elements. In recent years, recognition has increased that an individual's nutriture with respect to certain trace elements depends on not only the dietary intake but also the inherent bioavailability of the elements from a given diet. This fact is reflected in the dietary recommendations for zinc determined by the Expert Committee of the World Health Organization in 1973.[1] In the case of iron, moreover, a factorial approach has been proposed for calculating its availability from a given diet.[2] Thus meaningful recommendations for the daily intake of some minerals can only be made with a reasonable knowledge of both dietary intake and relative bioavailability of the mineral.

Our interest has been focused on the bioavailability of various forms of zinc, for which few data are available. The studies by O'Dell et al.[3] in experimental animals suggested that dietary zinc of animal origin is more readily available than zinc of plant origin. Workers at the Mayo Clinic, moreover, found a slightly greater proportion of bioavailable zinc from

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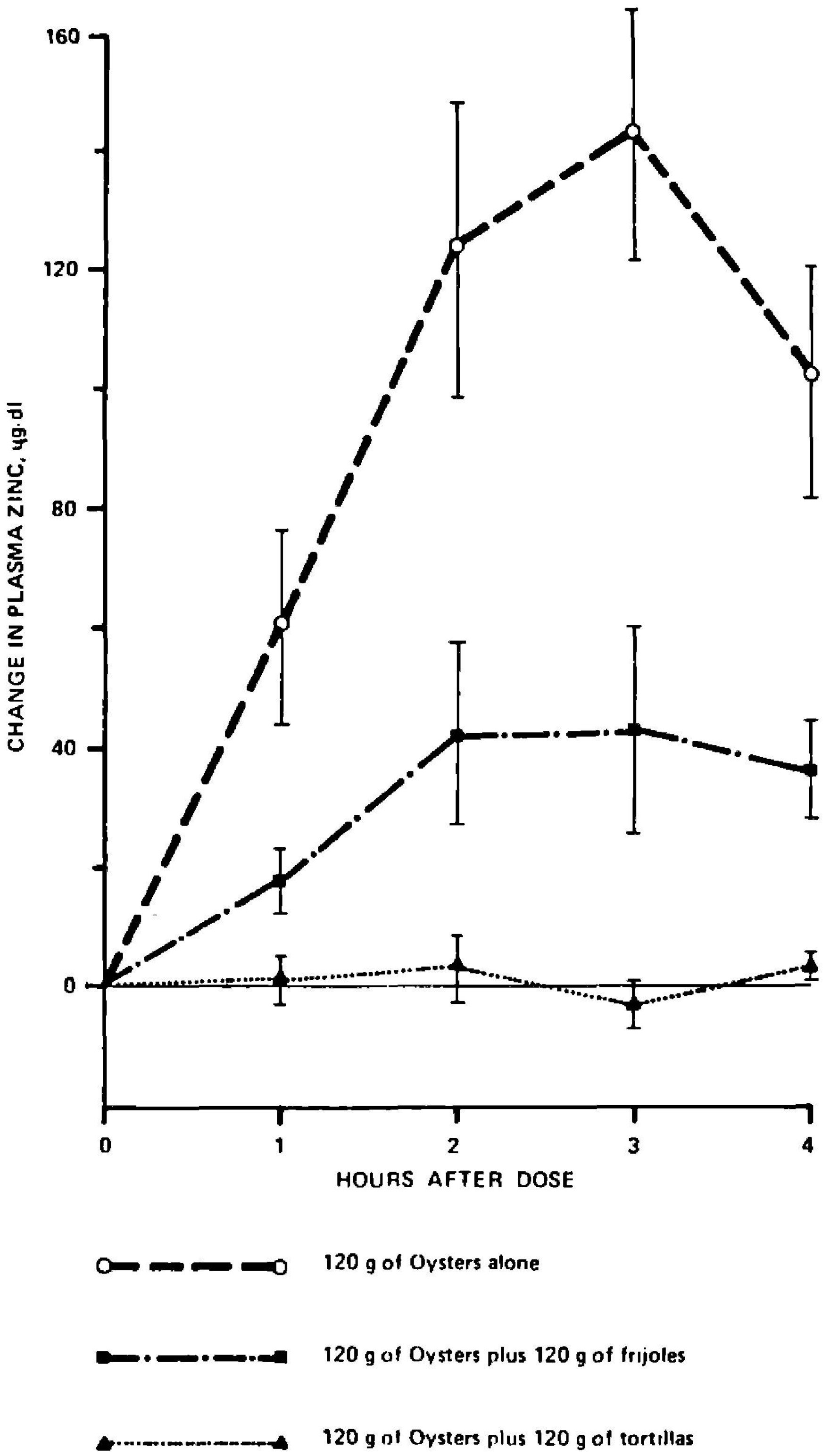

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Fig. 1. Changes in plasma zinc concentration (mean ± S.E.M.) at 60 min intervals over 4 hr following ingestion of 120 gm of oysters, containing 108 mg of elemental zinc. The oysters were consumed alone (N = 6); with 120 gm of black bean gruel (N = 5); or with 120 gm of corn tortillas (N = 4). Statistically significant differences were observed between each of the curves at 2, 3, and 4 hr. Note: *frijoles* = black bean gruel throughout.

meat (hamburger) than from a cereal fortified with zinc oxide.[4] Likewise, it is well documented that iron is more readily absorbed from animal than from plant sources.[5] However, in contrast to our paucity of knowledge of zinc, extraordinary experimental and conceptual advances in the understanding of the absorption of dietary iron have emerged in recent years.[2, 6] Dietary iron apparently exists in two distinct absorption pools of heme and nonheme iron.[7] Roughly half of the iron in fish, fowl, or meat is present in hemoglobin or myoglobin as part of the porphyrin moiety—the heme iron. The remainder of the iron in animal tissue is accounted for by iron in ferritin and hemosiderin—the nonheme iron. The inorganic iron in plants also belongs to the nonheme pool. A number of studies showed

Table I. Probability levels for the difference in mean changes in plasma zinc at corresponding time intervals

Experimental pairs			*1 hr*	*2 hr*	*3 hr*	*4 hr*
120 gm of oysters alone	vs.	120 gm of oysters + 120 gm of black beans	N.S.	$p < 0.02$	$p < 0.01$	$p < 0.02$
120 gm of oysters alone	vs.	120 gm of oysters + 120 gm of tortillas	$p < 0.05$	$p < 0.005$	$p < 0.005$	$p < 0.005$
120 gm of oysters + 120 gm of black beans	vs.	120 gm of oysters + 120 gm of tortillas	N.S.	N.S.	$p < 0.05$	$p < 0.02$
120 gm of oysters + 120 gm of black beans	vs.	475 mg of $ZnSO_4 \cdot 7H_2O$ + 120 gm of black beans	N.S.	N.S.	N.S.	N.S.
120 gm of oysters + 120 gm of tortillas	vs.	475 mg of $ZnSO_4 \cdot 7H_2O$ + 120 gm of tortillas	N.S.	N.S.*	N.S.	N.S.
120 gm of herring in tomato sauce alone	vs.	120 gm of herring in tomato sauce + 70 gm of bread and 10 gm butter	N.S.	N.S.	N.S.	N.S.
120 gm of herring in tomato sauce alone	vs.	120 gm of herring in tomato sauce + 70 gm of tortillas	N.S.	N.S.	$p < 0.05$	$p < 0.02$
120 gm of herring in tomato sauce + 70 gm of bread and 10 gm butter	vs.	120 gm of herring in tomato sauce + 70 gm of tortillas	N.S.	N.S.	N.S.	N.S.

*Four individuals participated in both oyster and $ZnSO_4 \cdot 7H_2O$ studies with tortillas; with the Student t test for paired samples, a significant difference was found at 2 hr ($p < 0.02$).

that heme iron is more readily absorbed than nonheme iron and is not affected by dietary substances that either inhibit or promote the absorption of inorganic iron from the diet.[8, 9]

Because the zinc in animal tissue is generally present in a protein-bound form, we wondered whether (1) two absorption pools for zinc could be distinguished as well or (2) dietary zinc of animal origin would be analogous to hemosiderin-associated or ferritin-associated iron and have absorption characteristics similar to those for inorganic zinc. Oysters of Atlantic, but not Pacific, origin[10] have zinc concentrations approaching 1 mg/gm and represent the richest known food sources of this nutrient. With oysters, however, we cannot assume that most of the zinc is incorporated into metalloproteins. Bodansky[11] demonstrated over half a century ago that up to 50% of oyster zinc is dialyzable. More recently, it was shown that of the zinc in oysters, a minor part is associated with the zinc metalloenzymes, carbonic anhydrase, alkaline phosphatase, carboxypeptidase A, malic dehydrogenase, and α-D-mannosidase, and that the major part was dialyzable and hence less firmly bound.[12] The zinc content of herring has been reported to exceed 0.4 mg/gm.[13, 14]

Recently, the change in plasma zinc concentration after ingestion of pharmacological doses of inorganic zinc salts has been used to determine the influence of dietary substances on zinc bioavailability.[15-18] Smith et al.[19] ingeniously used the postprandial rise in plasma zinc following ingestion of oysters to evaluate patients with familial hyperzincemia. In the present study, we have attempted to develop a method for the use of zinc-rich foods

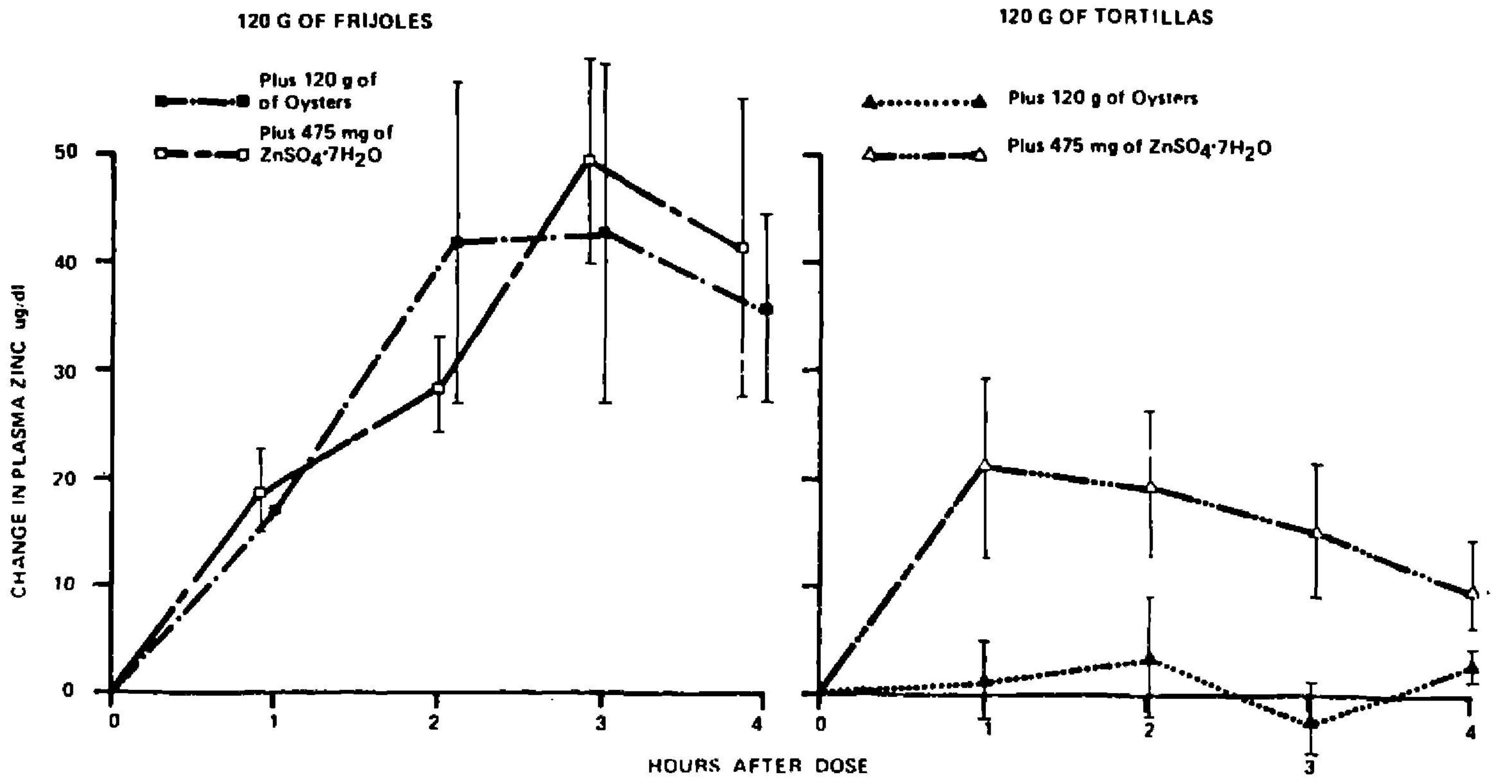

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Fig. 2. Changes in plasma zinc concentration (mean ± S.E.M.) at 60 min intervals over 4 hr following ingestion of various zinc-enriched meals. For the curves to the left, the meals consisted of 120 gm of oysters with 120 gm of black bean gruel (N = 5) or 475 mg of $ZnSO_4 \cdot 7H_2O$ with 120 gm of black bean gruel (N = 5). The curves to the right illustrate changes in plasma zinc following consumption of the same doses of either oysters (N = 4) or zinc sulfate (N = 5), with 120 gm of corn tortillas. In each instance, the zinc source provided 108 mg of elemental zinc. The asterisk indicates that a difference, significant at the $p < 0.02$ level, was observed by paired analysis of the four individuals who consumed both meals of tortillas.

in absorption studies and to compare the absorption of zinc from organic and inorganic sources in the presence of inhibitors of zinc bioavailability.

Materials and methods

Healthy adult subjects, both male and female, were studied after having been informed of the nature and purpose of the investigation. Studies were conducted in accordance with the provisions of the Declaration of Helsinki. Subjects fasted overnight at least 6 hr; venous blood was sampled before and hourly for 4 hr after ingestion of the test meal. During the test, subjects were allowed only water by mouth. Whole blood (3 ml) was collected with zinc-free materials and treated with zinc-free oxalate. Plasma was separated by centrifugation, and the zinc concentration was measured by atomic absorption spectrophotometry with a modification of the method of Sinha and Gabrieli[20] involving a 5:1 dilution of plasma with deionized, distilled water. The zinc contents of oysters and herring also were measured by atomic absorption spectrophotometry after products were homogenized and replicate aliquots of the homogenate were digested with nitric and perchloric acids.

Canned whole raw oysters from the waters off New Jersey (Treats from the Deep Oysters*; Bivalve Packing Co., Bivalve, N. J.) were used in standard doses of 120 gm wet weight. They were consumed alone, with 120 gm of black bean gruel (*Phaseolus vulgaris*), or with 120 gm of tortillas made from lime-soaked white corn. Canned smoked herring in tomato sauce (Gosch, Herring Fillets in Tomato Sauce; Fisch-Union, Cuxhaven, Federal Republic of Germany) also was used in standard amounts of 120 gm wet weight, alone, with 70 gm of white bread and 10 gm of butter, or with 70 gm of corn tortillas. Plasma zinc levels also were determined after subjects consumed 120 gm meals of black

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beans or corn tortillas extrinsically labeled with inorganic zinc as heptahydrated zinc sulfate (J. T. Baker Chemical Co., Phillipsburg, N. J.). An amount of zinc sulfate with a content of zinc equivalent to that of 120 gm of oysters was dissolved in a minimal volume of water and added directly to the foods.

Data were expressed as the change in plasma zinc concentration (mean ± S.E.M.) from the fasting, pretest level at each hourly sampling. The differences between experimental groups were compared by use of the Student's t test.

Results

For quadriplicate samples of oysters, mean zinc content was 900 ± 28 μg/gm wet weight (mean ± S.D.), equivalent to 108 mg of elemental zinc in the 120 gm meal of oysters. The increase in level of plasma zinc that followed the ingestion of oysters alone was depressed by both other foods, and the depression was significantly greater with tortillas than with black beans (Fig. 1, Table I). We did not compare the absorption of zinc between oysters and an aqueous dose of 108 mg because that dose of elemental zinc in solution probably would produce disagreeable symptoms.

The 108 mg of zinc as zinc sulfate (475 mg of $ZnSO_4 \cdot 7H_2O$) was tolerated when mixed into foods, however. In Fig. 2, the plasma zinc concentration curves resulting from the ingestion of this dose of zinc sulfate when mixed with the respective foods, black beans and tortillas, are compared with the previous results obtained with the same foods and equivalent doses of zinc from oysters. There were no significant differences between the absorptive behavior of inorganic zinc salts and oyster zinc with either food except for the 2 hr postingestion rise in plasma zinc with tortillas. Four subjects ate tortillas with both oysters and zinc sulfate, and paired analysis of the data showed a significantly greater depression after oyster zinc ($p < 0.02$) at that sampling time point. A single subject participated in all experimental meals tested (Fig. 3). Also, this subject consumed twice the standard dose, or 240 gm of oysters (containing 216 mg of elemental zinc), alone as a single meal. As shown, the area under the curve representing plasma zinc concentration is approximately twice that produced in the same individual after consuming the standard 120 gm oyster meal.

The zinc contents of our oysters and herring were determined subsequent to the absorption studies using these foods. Surprisingly, triplicate analysis of the herring in tomato sauce revealed a zinc concentration of only 16.9 ± 13 μg/gm (mean ± S.D.), equivalent to 2 mg/120 gm of herring. Consistent with this low zinc content of the fish, plasma concentration was stable following ingestion of the herring alone (Fig. 4). Postprandial zinc concentration declined progressively when 70 gm of white bread and 10 gm of butter were consumed along with the herring and declined further to reach statistical significance at 3 and 4 hr after eating when 70 gm of tortillas were consumed with the herring (Fig. 4, Table I).

Discussion

The use of the change in plasma zinc concentration as an index of zinc bioavailability has certain limitations. It depends upon the rate of zinc uptake in the upper intestine and possibly does not reflect the net absorption of zinc over the entire length of the intestine. The clearance of absorbed zinc from the plasma and a possible enterohepatic circulation of zinc, or, at least, a meal-associated release of zinc into the intestine in pancreatic secretions, are imponderable variables influencing the magnitude of plasma zinc elevation. Moreover, the amount of zinc required to elevate the circulating zinc concentration to clearly detectable levels is greater than that present in most meals; exceptions would be

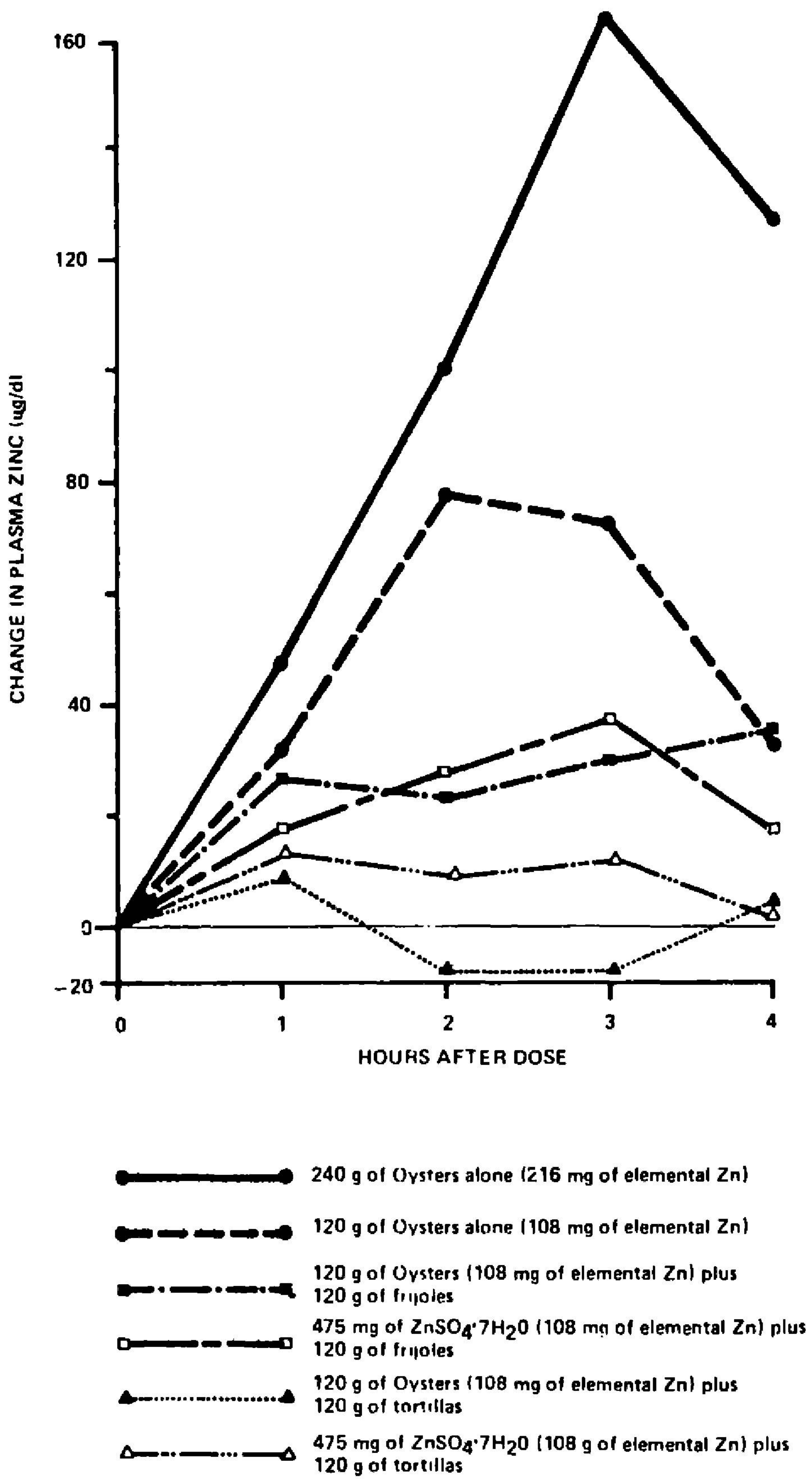

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Fig. 3. Changes in plasma zinc concentration following ingestion of each of the five meals enriched with 108 mg of elemental zinc for one individual who participated in all studies. In addition, in the uppermost curve are shown changes following ingestion of 240 gm of oysters alone, containing 216 mg of elemental zinc.

meals containing foods such as oysters (or herring of high zinc content) in which over 50 mg of elemental zinc are consumed in a single sitting. Our data indicate that Atlantic oyster could be used as test meals to produce a predictable increment in the concentration of zinc in plasma in studies in humans of the absorption of zinc from animal sources.

The data suggest that most of the zinc from at least one animal source, oysters, forms part of a *common* dietary pool. The zinc in oysters apparently resembled inorganic zinc in its intestinal absorption in the presence of other foods. When black beans were added to the meal, the responses to oyster zinc and zinc sulfate were indistinguishable; when

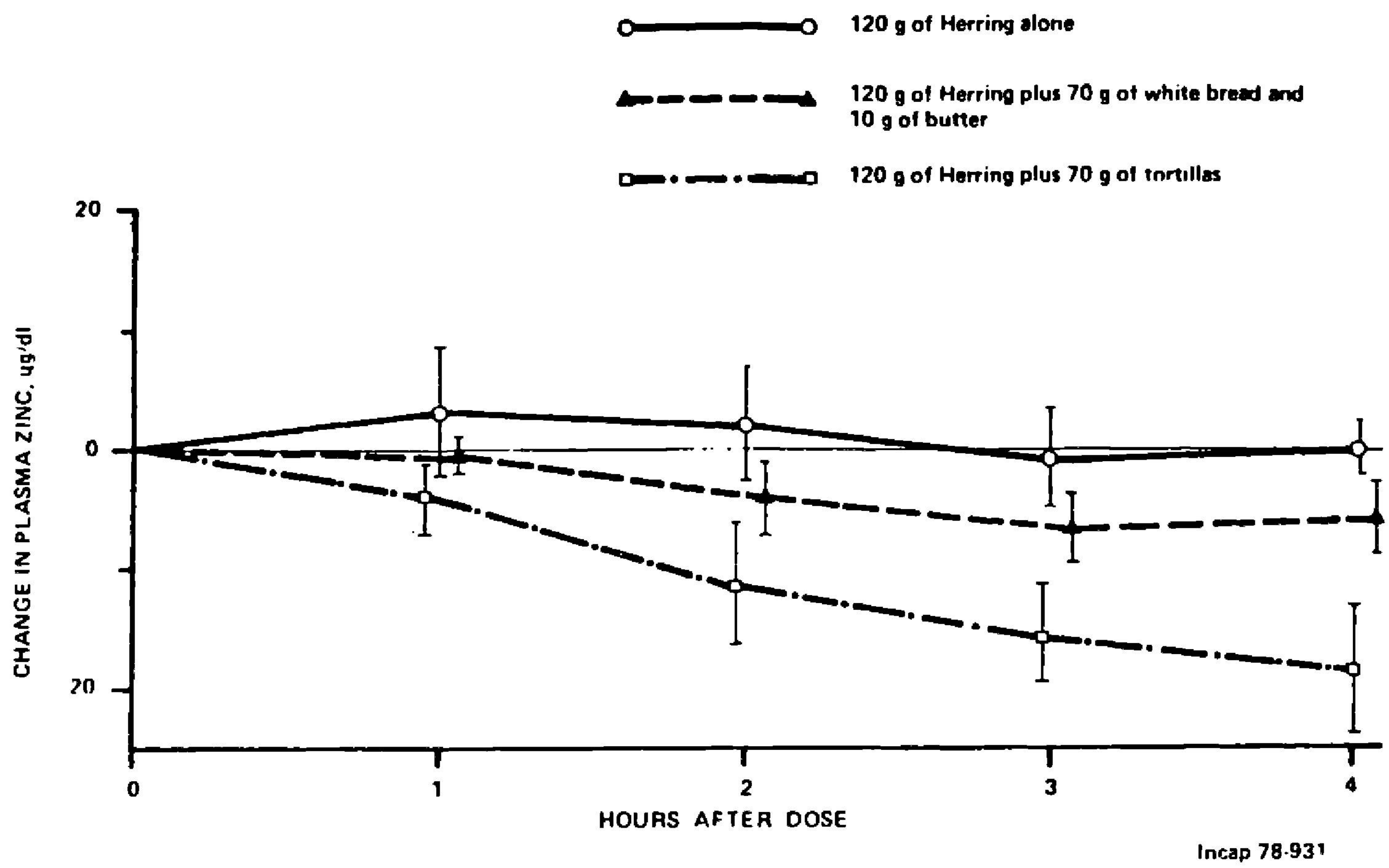

Fig. 4. Changes in plasma zinc concentration (mean ± S.E.M.) following the ingestion of meals containing 120 gm of herring in tomato sauce, containing 2 mg of elemental zinc. The herring was consumed alone (N = 5); with 70 gm of white bread and 10 gm of butter (N = 5); or with 70 gm of corn tortillas (N = 5). The postprandial decline in plasma zinc concentration at 3 and 4 hr was significant with the tortilla-containing meal.

tortillas were added, absorption was slightly higher from the inorganic zinc salt than from the oysters. The biological significance of this statistical difference, however, is uncertain. Thus our results in man are consistent with those of Shah et al.[21] in rats, which did not differ in zinc absorption between lyophilized oyster meat and zinc carbonate. Our data also show that, on a gram-for-gram basis, corn tortillas are more potent inhibitors of zinc absorption than are black beans. The mechanism by which these two traditional Guatemalan foods depress absorption of zinc is not fully understood. Both foods contain phytates. The fiber content is also exceptionally high; in studies at Berkeley, a content of 93 gm of dietary fiber in a 2900 kcal (12.1 MJ) daily ration of a diet based on corn and beans in a proportion of 5:1 was determined.[22] Finally, since the tortillas are prepared from lime-soaked corn, their calcium content would be expected to range between 230 and 240 mg in 120 gm.[23] Although some investigators reported that dietary calcium interfered with zinc absorption,[24, 25] the data are conflicting.[26]

Unfortunately, the zinc content of the herring that we used in these studies was considerably lower than published values.[13, 14] The data from the tests with herring are valuable, nonetheless, because they demonstrate differential elevation of plasma zinc following different oral zinc doses from foods; this gives further credence to the use of the change in plasma zinc concentration as an index of zinc absorption. Data from the tests with herring also showed that addition of tortillas to the meal significantly depressed postprandial plasma zinc conentration. A postprandial fall in zinc concentration has been documented by others,[15, 27-29] but the mechanism has not been delineated. When ingested with the herring, 70 gm of tortillas depressed plasma zinc level more than did 70 gm of white bread. That finding further shows that the nature and composition of the diet influences the magnitude of the postprandial decline in plasma zinc concentration.

Although zinc of animal origin appears to be more biologically available than that from plant sources to the rat,[3] no evidence for differential absorptive behavior, analogous to that of heme iron, was detected with Atlantic oysters used as the source of dietary zinc. Nonetheless, we successfully used oysters as a source of zinc for measurement of its absorption in man, and our studies suggest that it is a useful technique for investigating the effects of dietary components on the bioavailability of zinc.

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