Trace-Element Nutrition in Health and Disease:
Contributions and Problems of Analysis

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Most imbalances of trace-element nutrition are characterized by the absence of acute, easily recognizable disease. To diagnose marginal deficiencies or excesses of trace elements, the analyst must use rather sophisticated procedures, and even where an imbalance of trace-element nutrition can be proven, the implications for health are not always evident. Previous attempts to connect deviations of trace-element nutrition with health and disease have suffered from conspicuous gaps in our knowledge; etiologic factors for many chronic disease processes are unknown, with the result that existing hypotheses can be one-sided. In addition, the biological roles of many trace elements are hypothetical, at best. Whereas the short-term consequences of extreme under- or over-exposures are reasonably well-known, it is very difficult to investigate the marginal imbalances and their long-term consequences that are most likely related to existing health problems in man.

The rapid development of analytical methodology in the past decade has created a powerful tool for trace-element research. However, these tools can be dangerous if their technical and general limitations are not clearly understood. It is necessary to appreciate these limitations, not only in technology but also with regard to the interpretation of the results, in view of the ever-increasing demands on the analytical chemist. These demands are created by the discovery of new essential trace elements, by large international trace-element surveys in populations and by the increasing recognition of public health problems resulting from trace-element imbalances.1

Criteria for Trace-Element Analysis

The three criteria that the analysis must meet if results are to be meaningful to health-related studies are specificity, precision, and sensitivity, in that order.

Specificity

The experienced analytical chemist realizes that a printout from even the most sophisticated instrument is only a number, which may or may not be related to amount of the trace element that was fed into the instrument. The validity of analytical results rests upon the utmost effort of the chemist to establish a strong cause-effect relationship between the element to be analyzed and the final signal. What was self-evident to the analytical chemist of two generations ago who had to use tedious separation procedures in order to arrive at gravimetric or colorimetric analyses is easily concealed today by the elegant appearance of sophisticated and automated instruments: the determination of one element in a mixture of many others is a very complex procedure, influenced by a wide variety of factors. These factors become more and more dominant as the concentr-
tion of the element to be determined decreases. An example of reported concentrations of chromium in blood is presented in Table 1. The analytical chemist will immediately regard these values with suspicion. Apparently, they do not represent a biological distribution from very high to very low values (if alone on the basis of a spread of one to one thousand); they show a recent decline of reported values with time. They suggest a continuous modification of analytical techniques, because of more careful prevention of contamination or because the more recent methods are more specific.2 If the life scientist, unfamiliar with the problems of trace-element analysis, should draw any conclusions from the data in Table 1—for example, for a correlation between the geographic distribution of tissue chromium and the incidence of any particular disease—these conclusions would be wrong. There is still no complete consensus as to the actual chromium concentrations in blood.

All trace elements occur in biological material, often even within one tissue, in different forms, and each may have its own biochemical and analytical behavior. Until all these forms and their properties have been identified, the specificity of any one method of elemental analysis remains in doubt. These considerations emphasize the need for a set of strict criteria to assure specificity of analytical determinations. Some of these criteria will be discussed.

Use of more than one method: Even if any given analytical method is well-controlled, the results should not be regarded as factual until they have been found to agree with those obtained by a basically different procedure. The two methods should be different in principle from each other, beginning with sample preparation, digestion, or ashing, and on to the final determination. Preferably, all three common methods of ashing should be used: wet digestion, high-temperature ashing, and low-temperature ashing in an oxygen plasma. The final determination should rest on principles as different as emission spectrometry and absorption spectrometry, neutron activation analysis, x-ray fluorescence, or gas chromatography of chelates. The application of this criterion will alert the researcher to the potential errors contained in any one method.

Routine comparison of results against certified standards: The use of certified standard tissues is imperative for any one laboratory; it becomes essential when more than one laboratory is involved in a cooperative study. The certification of standards must be based on data obtained by two basically different methods, including the ashing procedure. The outstanding influence of ashing techniques on apparent analytical results has been described recently (1). To be of value, the standards must be of identical or near-identical composition with the samples that they will be compared to. This will diminish but not completely eliminate matrix effects. Each category of biological materials (e.g., blood, urine, liver, or hair) presents an entirely new analytical problem; it cannot be taken for granted that a method applicable to one category of tissues is also valid for another. This is true even for different samples of any one tissue; for example, a concentrated versus a dilute urine.

Control of contamination: The complex problems related to accidental contamination of samples during collection, preparation, and determination have been described (2). Although they cannot be discussed here, they present a continual potential source of error that demands the constant attention of the analyst. For work with some of the “new” trace elements occurring in very small concentrations it may be necessary to use sample preparation areas protected by laminar flow or even “clean rooms” allowing maximal exclusion of dust-borne contamination.

Precision

Precision is inversely proportional to the coefficient of variation (relative standard deviation) as derived from multiple determinations on a given sample. A high degree of precision is desirable from the analytical as well as the life scientist’s point of view. However the relative importance of this criterion depends on the nature of the project. Obviously, the precision of any method depends on the concentra-

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2 The alternative interpretation has not been ruled out as yet: that the most modern methods may detect only a fraction of the chromium that is present, because of volatilization and the like.

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Table 1. Reported Chromium Concentrations in Blood

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Concentration, µg/liter</th>
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<tbody>
<tr>
<td>Grushko, Y. M.</td>
<td>1948</td>
<td>35</td>
</tr>
<tr>
<td>(Biokhimiya 13: 124)</td>
<td></td>
<td></td>
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<tr>
<td>Urone, P. F., et al.</td>
<td>1950</td>
<td>50</td>
</tr>
<tr>
<td>(Anal. Chem. 22: 1317)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monacelli, R., et al.</td>
<td>1956</td>
<td>180</td>
</tr>
<tr>
<td>Volod’ko, L. V., et al.</td>
<td>1962</td>
<td>200</td>
</tr>
<tr>
<td>Schroeder, H. A., et al.</td>
<td>1962</td>
<td>520; 170</td>
</tr>
<tr>
<td>(J. Chronic Dis. 15: 941)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wolstenholme, N. A.</td>
<td>1964</td>
<td>1,000*</td>
</tr>
<tr>
<td>(Nature 203: 1284)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hambidge, K. M.</td>
<td>1971</td>
<td>7</td>
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<tr>
<td>(In Newer Trace Elements in Nutrition, Dekker, N. Y.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(J. Agr. Food Chem. 19: 398)</td>
<td></td>
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<tr>
<td>Pekarek, R. S., et al.</td>
<td>1974</td>
<td>0.72</td>
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<tr>
<td>(Anal. Biochem. 59: 283)</td>
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<td></td>
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</tbody>
</table>

* Calculated on the basis of 22.4% total solids in blood.
tion of the element in question in the sample. The closer the concentration is to the limit of detectability, the less precision can be expected. Coefficients of variation of approximately 5% are desirable but cannot always be achieved unless an extraordinary effort is made. Any biological function in a population is scattered around a mean, and the standard deviations inherent in biological material are usually greater than those related to the analysis. Small changes in the concentrations of trace elements in biological materials are very difficult to interpret even if determined with perfection. On the other hand, differences in trace-element concentrations that can be interpreted with confidence are usually large enough so that a relative standard deviation of 10% or even 20% is often satisfactory. The biological interpretation of analytical data can be improved not only by better precision of the analysis but by careful choice of meaningful tissues and meaningful tissue fractions. This will be discussed later in more detail. On the other hand, in certain situations utmost precision is required as the basis for an evaluation of the nutritional status and for potential policy decisions regarding food enrichment. In view of the recent establishing of a Recommended Dietary Allowance for zinc by the Food and Nutrition Board of the National Research Council, it is of great importance to know whether the “average” American diet meets the allowance of 15 mg per day for adults. As the majority of reported dietary intakes vary between 11 and 13 mg, a relative standard deviation of 20% or 30% would be compatible with conclusions that the “average” diet is sufficient, while a relative standard deviation of 1% or 2% would indicate that the daily intake is below the recommended allowance. But even a precision of 20–30% is difficult to achieve when more than one laboratory is involved (3).

Sensitivity

Sensitivity is highly desirable, but perhaps the least important of the three categories. A nondetectable concentration of a given element is useless only when it is reported as “zero.” On the other hand, a statement that an element occurs in a tissue in concentrations of less than the stated limit of detectability will remain valid and may have considerable importance to the life scientist. (Of course, even amounts that cannot be detected may contain many billions of atoms of the element in question.) Such statements will not confuse the present knowledge and will leave the way open for the development of more sensitive methods in the future. Sensitivity, however, becomes an important problem when concentrations have to be measured that are close to the limit of detectability. In this case an inordinate amount of human judgment and interpretation is involved that may prove to be invalid. Many of the discrepancies illustrated in Table 1 can be attributed to the use of flame atomic absorption spectroscopy, which currently is adequate for measuring chromium in many tissues but not in blood, because of its low sensitivity. In some situations it is possible to preconcentrate the element to be analyzed by suitable extraction and concentration procedures. Where this is not possible, any results close to the limit of detectability should be treated and interpreted with great caution.

The impressive development of analytical methodology within the past decade has resulted not only in increased sensitivity but, more important, in greater simplicity of operation. This has been a great stimulus to medical and biological research, but it also has introduced the potential dangers of oversimplification and overconfidence. In some cases trace-element determinations are left to technicians who operate the equipment, and the results are passed on to the life scientist who interprets and uses the results without knowing exactly how they were derived. It must be emphasized that trace-element analysis today is as difficult as it was 20 years ago, because the improvements in sensitivity and apparent ease of operation are balanced by the demands for accurate analyses in the nanogram and subnanogram range. Therefore, the task still requires the expert, competent, highly trained analytical chemist to obtain the data and the close cooperation between him and the life scientist to interpret them.

Application of Trace-Element Analysis to the Study of Disease

The following discussion rests on the assumption that reliable and sensitive analytical methods are available for the measurement of the trace elements in question. Correlation of trace-element concentrations in any tissue with a disease process does not give any basis for establishing a cause–effect relationship unless supported by additional data. For example, an excessive or deficient concentration of an element in a diseased kidney is not necessarily the cause of kidney disease but may well be the consequence of either a deficient excretion mechanism or of faulty reabsorption. The loss of nickel from the myocardium after acute infarction and its increase in plasma is well recognized as a consequence of myocardial damage, and the loss of chromium into the blood and urine of insulin-requiring diabetics is believed to be a consequence of insulin administration. Even the well-investigated accumulation of copper in certain tissues in hepatolenticular degeneration and of iron in hemochromatosis is not the primary cause for the disease but rather an effect of deficient hemostatic and transport phenomena, although they are responsible for specific tissue lesions. The problem of relating analytical data per se to disease states is almost insurmountable unless precise experimental data demonstrate a cause–effect sequence. Only after it had been established that exposure of experimental animals to excessive concentrations of lead resulted in specific symptoms was it possible to use lead analyses for the diagnosis of plumbism. Long-term expo-
sures of experimental animals to increased cadmium results in hypertension. This is a solid basis for the hypothesis that excessive cadmium intake may be one causative factor for hypertension in man; it justifies the extensive application of cadmium analysis in this disease. On the other hand, the administration of insulin leads to a significant increase of chromium excretion in experimental animals and man so that low tissue concentrations of chromium in the insulin-dependent diabetic are to be considered a reflection of the disease but not its cause.

For practical purposes it can be suggested that concentrations of trace elements in grossly pathological tissue are extremely difficult, if not impossible, to interpret. On the other hand, values obtained from mildly abnormal tissue can be of value if a strong hypothesis, backed up by animal experiments, allows a cause–effect relationship to be established.

**Application of Trace-Element Analysis in Health**

**Detection of Causative Factors before Disease is Apparent**

The desirability of detecting biochemical changes long before the open manifestation of chronic degenerative diseases has been emphasized (4). This is certainly true for trace elements. To find deviations from normal at a time before serious tissue damage has set in eliminates the possibility that tissue damage may have been the cause for the changes of trace-element composition. It must be realized, however, that even under ideal conditions of a longitudinal study, if abnormal trace-element concentrations are found in a certain tissue before the onset of clinical disease, this does not establish per se a cause–effect relationship. Abnormal concentrations of an element may be completely innocuous. It is also possible that an abnormal concentration of one element, meaningless by itself, is an indication for abnormal concentrations of others, directly related to the disease, that have not been looked for. (In this connection it is important to realize that at any given time our knowledge of the number and function of essential trace elements is necessarily incomplete.) As was mentioned in the preceding section, analytical data gain importance and their interpretation becomes more reliable when results of carefully controlled animal experiments show that the element in question—either by excess or deficiency—has some effect on health processes.

**What is Normal?**

To designate an analytical value "abnormal" requires that the range for normal values can be established and defined. It is of the utmost importance to realize that the definition of normal is not necessarily identical with that of average (5). The term "average" is a definition of facts, derived from a sufficient number of measurements in a population. The term "normal," on the other hand, is based on the goal of optimal or near-optimal function and of perfect health. To differentiate between the terms average and normal is particularly important at a time when population groups are exposed to rapid changes of life styles, food intake patterns, and environmental exposure. It is well recognized that changing dietary habits have led to a decreased iron intake and to a considerable incidence of iron deficiency in some industrialized populations. This is well expressed in the analytical results of several population surveys. The resulting averages and distribution curves are certainly valid, but they cannot be used as a definition of normalcy. The same is true for the average serum cholesterol concentrations and for the impairment of glucose tolerance with age in industrialized societies. Many questions of far-reaching consequences may be asked: What is the importance of the postnatal negative balances of several essential trace elements, of the drastic decline of zinc concentrations in hair during the first three years of life, of the decrease in chromium and silicon in certain tissues throughout the lifetime? Until causes such as nutritional deficiencies can be ruled out, these developments should not be accepted as normal. To establish a valid "norm" for an analytical variable is much more difficult and more meaningful than to determine an average. In the following, a few ideas will be discussed that I believe will be helpful in establishing "normal" values.

**Meaningful tissue:** To obtain useful information from trace-element analysis, one must select a meaningful tissue, one that reflects deficient or excessive environmental exposure or, alternatively, one that is functionally linked to a disease process under investigation (e.g., hypertension-kidney; diabetes-pancreas; etc.). Some knowledge of the metabolism and site of action of trace elements is helpful in the selection of meaningful tissues; this knowledge can often be obtained in animal experiments. Theoretically, tissues can be discussed under four categories: (a) regulatory sites; (b) tissues in which the element has an essential function; (c) tissues related to transport, storage, and excretion; and (d) sequestering tissues for excessive exposures. The tissues of the first category are responsible for maintenance of homeostasis by regulating absorption or excretion of an element or its synthesis into a biologically active molecule. Such sites have been identified for only few elements. One such example is the intestinal mucosa which, if the mucosal block theory is accepted, is the primary regulator of iron absorption. Iron analysis in this tissue then could be expected to be a sensitive indicator of the nutritional status and of the balance between requirement and dietary supply. The thyroid is the primary regulatory organ for iodine, in its biologically active form, and exquisitively sensitive methods have been developed with use of this organ as an indicator for the status of iodine nutriture. The liver is believed to be the site where inorganic manganese is transformed into transmanganese-bound metal, where inorganic vanadium is incorporated into trans-
ferrin and, perhaps, the site where chromium binding to glucose tolerance factor is regulated.

The second category consists of those tissues in which the element under study has an essential biological function. Examples of this are erythrocytes as sites of the function of iron in hemoglobin, of zinc in carbonic anhydrase, and liver and muscle tissue for all those elements that are essential co-factors of enzymes in these organs. Trace-element analyses in these tissues may, but do not necessarily, give information on the nutritional status. Homeostatic regulation may be so strong that even in clearly defined deficiency situations the value for a trace element remains almost unchanged. Erythrocyte iron and hemoglobin measurements give no information about marginal iron deficiency; they become indicators only after the available body reserves of iron have been exhausted. Homeostatic regulation of zinc concentrations in the liver of some species is so strong that zinc concentrations in severely deficient animals are not significantly different from normal controls (6).

The third group, tissues involved in transport and storage, consists of the body fluids, including urine, and the marrow and cortex of bone. Blood serum or plasma are the most frequently analyzed samples, but analytical results for them are also among the most difficult to interpret. Plasma carries newly absorbed trace elements and those being transported to their target organs; thus it may reflect the recent dietary intake. Concentrations may be tightly controlled by hormonal regulation (e.g., for the bulk element, calcium) so that near-normal concentrations are maintained even in the presence of severe nutritional deficiency. Or plasma may not be in equilibrium with the important tissue stores at all, as is the case with chromium. For all these reasons it is necessary to determine the metabolic behavior of a trace element under study in plasma by the use of experimental animals, in order to determine exactly what information can be expected from analysis of this fluid. Analyses for many trace elements in urine are beset with technical difficulties, caused by high concentrations of salts and their day-to-day variation with urine volume. A new matrix problem is presented every time a sample is analyzed. In spite of these difficulties, urine can be an ideal material for the study of trace-element metabolism, particularly in those cases where urine is the main excretory route and where excretion is comprised of more than a constant, obligatory loss, as is true for chromium. Even for zinc, which is mainly excreted via the gastrointestinal tract, determinations of urinary concentrations have been helpful, when combined with analyses of other tissues. A decrease in or depletion of a trace element in storage organs—such as of iron in bone marrow and other tissues of the reticulo-endothelial system—can very sensitively indicate marginal deficiencies, long before any functional consequences are noticeable. Storage tissues are defined as those that contain the element in a form that is easily and immediately exchangeable with other tissues when the need arises. Thus, bone cortex by this definition is a storage tissue for calcium but not for zinc.

Finally, the sequestering tissues immobilize elements and take them out of equilibrium with the rest of the organism. To this group belong the lung and kidney, part of the reticulo-endothelial system, nails, hair, and the stratum corneum of the skin. All of these tissues can be used as indexes of chronic over-exposure. Particularly, hair has been shown to be a meaningful and representative tissue for at least three trace elements: zinc, copper, and chromium. It has the advantage of being easily obtainable, of integrating nutritional intake over a period of time, and of allowing a retrospective study of individual time periods, when analytical methods are sensitive enough for analysis of cut segments. For at least two trace elements, zinc and chromium, hair analysis is a promising tool for determining even marginal deficiency, if the trace-element deficiency is not complicated by severe malnutrition in which the growth of hair may be so impaired that the trace-element concentration does not change.

The choice of a tissue or tissues from these four groups admittedly is often dictated by necessity. If it can be influenced by a thorough knowledge of the element under investigation and by an understanding of the health-related problem to be solved, the results will gain much in meaning and interpretation. For certain problems analytical data for any one tissue may not carry enough weight, and combinations of different categories of tissue may be necessary.

Meaningful fraction: The separation of a tissue or body fluid into different fractions holds considerable promise for a better interpretation of trace analytical data. That essential trace elements occur in tissues and in body fluids in different fractions or compartments is well known. The physiological function of some of these fractions has been identified and analysis for part rather than for all of an element is being used to great advantage for the diagnosis of trace-element nutrition. The determination of protein-bound iodine, of transferrin-bound iron, and of ceruloplasmin-bound copper is routine; these analyses furnish information that is much more meaningful than would be furnished by analysis for the total element. Carrier proteins have been postulated or identified for several trace elements, but their function as well as the conditions governing the exchange of metal from these proteins to other carriers of low molecular weight in plasma are not known. It can be hoped that ongoing research will define one or more of those fractions as being sensitive indicators of the nutritional status for several of the elements that have gained increasing public-health importance, such as zinc.

The identification of meaningful fractions is not only desirable but essential for those trace elements that occur in the organism in compounds of widely different biological activity—for example, cobalt and
chromium. To perform its only known physiological function, cobalt must be present as part of the vitamin B12 molecule. If serum concentrations of cobalt present as part of vitamin B12 and as total cobalt are compared (approximately 10 vs. 8500 nanograms per liter), the futility of attempts to use any data on total cobalt concentration as a basis for an assessment of vitamin B12 nutriture is immediately apparent; no existing analytical method would be able to detect the minimal changes in total cobalt concentration that would be caused by even drastic declines in vitamin B12 concentrations. A similar although less drastic situation exists for chromium. This element is present in the organism in form of a biologically active compound, termed "glucose tolerance factor," which has not yet been completely identified but most likely is a dinicotinato-chromium complex. This compound (or type of compounds) composes a fraction of the total chromium, a different fraction from one organ to another. The rest consists of a variety of chromium compounds that physiologically and analytically are not different from simple, inorganic complexes such as tetraaquo- or hexaaquo-compounds. The chromium in glucose tolerance factor is quite volatile and can be easily lost during sample preparation, whereas the simple chromium compounds are stable and can be easily determined. Chromium nutritional status is not—or only minimally—reflected in total serum chromium concentrations; it is not surprising that preliminary experiments indicate that determination of the chromium in glucose tolerance factor furnishes more meaningful results.

To apply the concept of meaningful fraction is equally important in the trace-element analysis of foods. The availability of many cationic trace elements for intestinal absorption is poor and, in addition, it can vary considerably, depending on the tissue in which they are present. Zinc, iron, and chromium are better absorbed from meat than from vegetable products and even relatively high intakes of these elements from the latter sources may be of little value nutritionally. The much-needed information concerning biological availability for absorption is being accumulated in time-consuming animal and human experiments and agreement on the resulting data is not unanimous. Attempts in the past to identify chemical fractions in food to be used as an index of biological availability (e.g., the dipyrindyl-reactive fractions of iron) have not been successful. On the other hand, a recent study of food chromium, by using a simple step of ethanol extraction, arrived at a significant correlation between ethanol-extraction chromium and the biological effect of the extract (7). If such meaningful fractions can be identified for other trace elements in tissues and foods, they will not only be of value for diagnostic purposes but they will also make the analytical chemist's task easier: because deviations from normal in these meaningful fractions can be expected to be greater than those in the total, the demands on the precision of the analytical methods will be decreased and the reliability of the results greatly enhanced.

Correlation with function: This concept, in addition to being a helpful tool for the selection of meaningful tissues and meaningful fractions, is the ultimate criterion that governs the weight to be given to, and indeed the interpretation of, analytical data for the study of health-related problems. There are outstanding analytical studies that have not yet been correlated with biological function and are therefore controversial in their interpretation. They would gain major public-health importance if, by correlation with physiological function, their significance for health could be identified. For example, the well-known loss of several essential trace elements by the newborn could be interpreted as physiologically normal if no impairment in performance were found after exhaustive searching. Alternatively it could reflect a deficiency resulting from an increased requirement, not met by dietary intakes, if an impaired function were consistently associated with it and if this impairment could be prevented by supplementation with the element under study. To decide which alternative is correct is of far-reaching consequences in the field of iron nutrition in view of the extreme difficulty of correcting a pre-existing deficiency by nutritional means.

A recent study by Hambidge et al. (8) established the age-related changes of zinc concentration in hair in a supposedly normal population. Zinc concentrations in hair decline sharply after birth, remain low for two or three years, and then increase slowly toward the original high values. Although this study did not define the causes and the significance of these changes, 10 children (approximately seven percent of the total) were found for whom these values were significantly below the average for their age group. Subsequent clinical studies showed clearly that certain functions for which zinc is essential were impaired in these children and that the impairment was restored to normal by supplementing the zinc in the diet. Because the assessment of a function could be correlated with the analytical results of hair analysis, these results became meaningful and could serve by themselves as indicators of suboptimal zinc nutriture.

The age-related decline in chromium concentrations in several tissues of subjects in the U.S. is clearly demonstrated. It gains potential clinical importance by its correlation with the increased incidence of impaired glucose tolerance with age, although a cause-effect relationship has not yet been established. In contrast, the demonstrated trend of several heavy metals to accumulate in tissues with age has not been correlated with functional aspects except at excessive concentrations and the significance of such accumulation is not clear.

Physiological functions that can be easily measured and correlated with results of trace analyses
are well known for the long-established essential elements, for example basal metabolic rate and hematopoiesis. Growth performance, appetite, and taste acuity are good indicators of the zinc status, as is intravenous glucose tolerance for chromium nutrition. The activity of glutathione peroxidase (EC 1.11.1.9) in erythrocytes promises to be a sensitive indicator of selenium status, and a decrease of serum clotting factors not responsive to vitamin K supplementation may be related to manganese deficiency. Several functions have been described by recent research as being representative of the “newer trace elements” (9).

None of the physiological factors discussed here is uniquely specific for any trace element, because all are influenced by various external and hormonal factors. As soon as any of these nonmineral influences becomes dominant (for example, severe folate deficiency with its effect on hematopoiesis), the correlation between biological function and trace-element analysis becomes meaningless.

The concept of near-maximal function: The selection of a meaningful tissue, a meaningful fraction, and the correlation of analytical values with a physiological function by themselves do not allow an interpretation of analytical results as “average” or “normal”. This interpretation, however, is particularly important when the analytical values serve as standards for subsequent health or nutrition surveys and when they may be used ultimately as an argument for or against public-health measures of far-reaching consequences (e.g., enrichment of foods). It has been emphasized, for example, that the skewness in the distribution curve for hemoglobin concentrations in a population is caused by the presence of two population subgroups, one deficient and one normal (10). Because the distribution curves of the two subgroups overlap, it is difficult if not impossible to classify individuals as belonging into either group, on the basis of their hemoglobin data or the corresponding iron concentrations alone. Such a differentiation is possible by increasing the iron concentration through dietary supplementation and correlating these values with the function specific for iron, hemoglobin formation. Low hemoglobin concentrations that are caused by iron deficiency will increase with increasing intake and tissue concentration of iron; those that are normally low and not related to iron status will not. Keeping in mind the sigmoid form of most biological dose–response curves, one may define a normal value for such an element as a value that responds to increased supplementation with only insignificant increases of the specific function. The near-maximal rather than the maximal function is preferred as a criterion because the latter does not allow to distinguish between adequate and excessive concentrations. The system to be studied can involve the whole organism; ideally it could be an easily accessible tissue, or a metallo-enzyme for which a metal:protein ratio can be determined. Removal of the metal, either in the test tube or by nutritional means, results in a decreased function that, within limits, is reversible by addition of the essential element. The degree of unsaturation then would be a measure of the deviation from normal. For some systems (e.g., serum alkaline phosphatase, EC 3.1.3.1), a saturation test can be performed in vitro. Other useful systems of this type comprise the protoporphyrin content of erythrocytes as a measure of iron unsaturation and perhaps the chemotactic response of polymorphonuclear leukocytes to insulin as an indicator of saturation with chromium. “Normal” values would then be defined as those associated with near saturation of the system resulting in a near-optimal function.

Trace Elements and the Quality of the Environment

If it is the goal of all research ultimately to improve the quality of life, the question arises as to the impact of trace-element analysis on the maintenance or improvement of our environment. In the past, the interpretation of the then-existing limited knowledge of trace elements and the policies resulting from this interpretation were quite simple. The “bad” elements, mainly the heavy metals, were justly considered health hazards whenever they occurred at the then-detectable concentrations. On the other hand, the “good” elements, for example iron or iodine, were beneficial to health when given to deficient subjects in excess of the average exposure. Since then much has changed: elements previously belonging to the “bad” group have been identified as being biologically essential (e.g., selenium and chromium). Secondly, the great progress of analytical chemistry has resulted in much greater sensitivity of the analysis and many of the “bad” elements are now found to be ubiquitous (e.g., mercury). Finally, the efforts of industrial hygiene and the changing nutritional practices have resulted in the almost complete disappearance of gross over- or underexposures. The unscientific differentiation between “toxic” and “beneficial” elements is being replaced by the concept of the universal biological dose–response, which postulates that both beneficial and toxic effects are primarily a function of the concentration but not necessarily of the elements’ nature (11). The question as to which element is good or bad has been replaced by the search for the optimal amount, and much of the modern health-related research is concerned with slight deviations from this optimal value that result in marginal deficiencies or marginal overexposures. Their impact on the quality of a life can be considerable. To detect these imbalances, to define their consequences, and to recommend corrective measures are the ultimate goals of trace-element research.

Conclusion

I have emphasized the difficulties of arriving at a meaningful trace-element analysis and a valid interpretation. These difficulties can be overcome only by the closest collaboration between the analytical
chemist and the life scientist. The former must be more than a procurer of data, the latter more than their interpreter; each must be fully aware of the other's thinking, work, and problems. Coordination of the efforts of various laboratories such as is being provided by the Trace Element Program of the World Health Organization and of the International Atomic Energy Agency is a necessary condition for success; it should be supported and gratefully acknowledged.

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