FEASIBILITY OF IDENTIFYING ADVERSE HEALTH EFFECTS OF VITAMINS AND ESSENTIAL MINERALS IN MAN

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by

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators actively engaged in research on specific topics in biology and medicine.

This technical report was prepared for the Bureau of Foods, Food and Drug Administration (FDA), by T. Colin Campbell, Ph.D., Senior Scientific Consultant, Richard G. Allison, Ph.D., Staff Scientist, and C. Jelleff Carr, Ph.D., Director Emeritus, in accordance with the provisions of Contract No. FDA 223-75-2090.

The authors and LSRO acknowledge the contributions of the investigators and consultants who assisted in this study (see Section IX). The report reflects the discussions with participants at two ad hoc study groups that met in the Lee Building of FASEB on June 4-5, 1979 and October 15-16, 1979. The LSRO staff obtained additional information and data from a comprehensive literature review of topics discussed at the two ad hoc group meetings. According to policy guidelines of the LSRO Advisory Committee, literature compilations are open for inspection and use by members of the scientific community and the general public for a period of two years beyond the date of report publication.

An attempt has been made to incorporate viewpoints and opinions expressed by meeting participants and other consultants. The report has been reviewed by these consultants; however, the listing of their names in Section IX does not imply that they endorse the conclusions of the study. LSRO accepts responsibility for the contents of this report. The report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to the FDA by the Executive Director, FASEB. While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of individual members of each FASEB constituent Society.

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
SUMMARY

This report concerns the feasibility of identifying adverse health effects in individuals who consume vitamins and essential minerals in excess of quantities demonstrated as beneficial and safe. It focuses upon effects of high doses of nutrients consumed in the absence of underlying illness, and outlines factors relevant to the development of broadly applicable protocols for identifying and studying adverse health effects. Data on known toxicities of essential nutrients, normal dietary and supplemental levels of nutrient ingestion, and laboratory methodologies available for study of potential nutrient toxicities are assessed.

For certain vitamins and essential minerals, clinically significant conditions have been shown to be related to the chronic ingestion of excessive amounts. However, the prevalence of vitamin and mineral toxicities in the U.S. population is unknown and, to date, no common or general indicators of susceptibility to nutrient toxicities have been identified. Age, nutrient composition of the diet, pharmacokinetic phenomena, nutritional status, duration of elevated ingestion, and diseases are among the variables that affect the response of individuals to large doses of nutrients. For most essential nutrients, additional research is needed to increase the sensitivity and reliability of tests for defining and studying nutrient toxicities. It will be necessary to develop a larger database concerning pharmacokinetic, metabolic, and other clinical parameters because adequate data concerning the effects of large doses of nutrients have not been obtained for experimental animals or human beings. Thus, a nutrient-by-nutrient approach should be taken in the design of protocols for identifying and quantitating potential adverse health effects. Currently available data collected in national surveys on the normal consumption of vitamins and essential minerals by the general U.S. population are of limited value for the design of a clinical protocol.

Three variously motivated groups provide an opportunity to study the effect of large nutrient doses: individuals who consume dietary supplements without medical supervision; subjects under experimental treatment with large doses of nutrients for certain medical conditions; and subgroups of the general population consuming diets of unusual or restricted nutrient composition for various reasons, such as religious beliefs and geographic location. Epidemiological principles suggest that studies with unrandomized, self-selected treatment groups cannot be sufficiently definitive to establish a causal relationship between nutrient excesses and subtle, long-term adverse health effects. However, careful study of these individuals would contribute to the protection of public health. Such studies should use epidemiologically acceptable protocols and give full consideration to animal toxicity data.
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I. INTRODUCTION

A. BACKGROUND

The Food and Drug Administration (FDA) is responsible for evaluating and monitoring the safety of foods, establishing regulations, and providing nutrition information to consumers. To meet one aspect of these responsibilities, FDA is considering the feasibility of identifying upper limits of safe use for some nutrients, based upon data demonstrating potential or actual toxicity. The Recommended Dietary Allowances (RDA's) developed by the Food and Nutrition Board (National Research Council, 1980) represent levels of nutrient intake considered adequate for healthy persons. The approach used to set allowances is based on the need to ensure adequate intakes, with little or no consideration of maximum intakes; moreover, current provisions of the Federal Food, Drug, and Cosmetic Act (Food and Drug Administration, 1979a) prohibit the FDA from setting maximum limits on the potency of vitamin and mineral preparations sold as dietary supplements except if "represented for use by individuals in the treatment or management of specific diseases or disorders, by children, or by pregnant or lactating women." The Act further provides that such substances cannot be classified as drugs even when they exceed a level of potency considered adequate for nutrition.

The risks of adverse health effects from nutrient excess and deficiency may represent competing risks amenable to critical analysis given a sufficiently reliable database. Study of individuals exhibiting adverse health effects and those who are uniquely susceptible to adverse effects from excesses of specific nutrients will be useful for such an evaluation. Additional animal, clinical, and epidemiological studies may be required to establish an adequate basis for evaluating the hazards of ingesting excessive quantities of specific nutrients. However, neither the feasibility nor the protocols for such studies in human subjects have yet been established. For this reason, the FDA has requested the Life Sciences Research Office to determine whether the available data, methodologies, and criteria are sufficient to develop study protocols capable of identifying potential or actual adverse health effects resulting from excessive nutrient ingestion by man.

B. SCOPE

The objective of this study is to determine if it is feasible, utilizing currently available methodology, to develop protocols that would be useful in identifying individuals who may exhibit, or who may be uniquely susceptible to, adverse health effects from the consumption of excessive quantities of vitamins or essential minerals. Because evidence of toxicity from dietary intake alone is sparse, the discussions focus upon the possibility
of developing a protocol to examine a subset of the population who may be consuming vitamins and essential minerals in excess of those necessary for normal growth, development, or maintenance of health. The intent is to identify methodology, particularly laboratory studies, useful in determining possible adverse health effects in individuals already consuming one or more nutrients in excess of normal needs. It is recognized that dietary intakes of sodium, potassium, phosphorus, and chloride are usually in excess of nutritional needs and are infrequently ingested as supplements. However, other vitamins and essential minerals including vitamin A, vitamin D, and iron are widely available as supplements and are being consumed as supplements by some individuals.

This report is based upon topics reviewed by two ad hoc working groups convened by LSRO in 1979. The first group discussed adverse health effects of nutrients in man, and the second reviewed possible criteria for safety evaluation of nutrients. Because of the obvious and considerable overlap in these areas, this report incorporates material from both meetings. Topics include epidemiological considerations, variation of biochemical values with dose, the pattern of change with high or long-term nutrient administration, the relationship of these changes to toxic manifestations, and the extent of ethical tests possible on human beings. However, the issues concerned with ethical research with human subjects and the impact of federal regulation on this area of research have been discussed extensively and are not repeated here (McMahon, 1978; U.S. Department of Health, Education, and Welfare, 1980).

Data from limited human investigations and clinical observations suggest that excessive consumption of certain nutrients, for example, fluoride, iodine, and vitamins A and D, may produce clinically evident manifestations of toxicity. Effects of other vitamin and mineral excesses in humans are infrequently described as adverse in the scientific literature. However, even adverse long-term effects of large quantities of nutrients may be difficult to correlate with their intake because, in addition to being delayed, they may be related to genetic and environmental factors: race, endocrine and kidney function, degree of obesity, lifestyle, and other factors difficult to correlate with nutrient intake over a lifetime.

A number of related topics have been excluded from the scope of this study. For example, selected information on certain vitamins and essential minerals is presented to illustrate problems associated with studying nutrient toxicities in man. Neither the choice nor the exclusion of a particular nutrient as an example in the following sections is meant to imply an assignment of priority for further study or an assessment of its potential adverse effects. Detailed discussion of individual vitamin and mineral toxicities has not been attempted because exhaustive reviews are readily available in the current literature (DiPalma

Another LSRO study (Allison et al., 1980) considers the possible development of a comprehensive system of evaluating the safety of nutrients. It addresses the importance of animal feeding studies and the development of newer methodologies of tissue, cellular, and subcellular systems in short-term toxicity testing. The influences of infancy, pregnancy, and extant metabolic disorders related to nutrient toxicity and other nutritional considerations, such as total caloric intake, are beyond the scope of this report.

In summary, the primary emphasis of this report is to outline protocols which may be useful in identifying adverse health effects from excessive consumption of essential nutrients in subsets of the population.
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II. PHYSIOLOGICAL AND PHARMACOKINETIC EFFECTS

A. TOXICITY OF ESSENTIAL NUTRIENT EXCESSES

An essential nutrient, by definition, is required for life; therefore, its toxicity must be evaluated as part of a broad dose-response continuum with full recognition that it cannot be eliminated from the diet. Other substances, if responsible for serious adverse reactions, can potentially be removed from food and the human environment. Such action is clearly not possible with essential nutrients even if adverse health effects are observed at both high- and low-dose intakes. For each nutrient, the characteristics of the dose-response curve and the parameters used to distinguish a toxic dose from a nontoxic dose must be carefully defined because the levels of intake regarded as toxic by certain criteria may be the same levels considered beneficial by other criteria. This is a well recognized problem not related exclusively to nutrients. For example, at the same level of use, pesticides can be considered either beneficial or harmful depending upon the criteria employed.

Luckey and Venugopal (1977) constructed a hypothetical dose-response curve for essential minerals which can be applied broadly to all essential nutrients, integrating three identifiable zones: deficiency, adequacy (normal health), and toxicity. Traditionally, within the zone of adequacy, it is assumed that the observed health and biochemical parameters are characteristic of a normal, healthy person. However, if analyzed as part of a dose-response continuum, the transition from adequacy to toxicity, seldom abrupt, incorporates increasing evidence of effects usually described as abnormal or pharmacological. These abnormal or pharmacological effects may or may not be predictive of recognized adverse health effects but can provide a ready guideline for research studies. Thus, nutrient toxicity represents the integration of responses whose magnitude and probability of occurrence are minimized within the range of adequate intake. Below this range, deficiency symptoms appear, and above this range, toxicity could occur. The accuracy and precision of the values set for the zone interfaces, the breadth of the ranges, duration of exposure prior to a toxic response, and individual responsiveness vary from nutrient to nutrient. Although less attention has been given to the toxic dose than to the measurement of nutrient requirements, the lack of reports on toxicity for many nutrients is suggestive, if tenuous, evidence that the range between toxicity and deficiency may be rather large. For example, an ad hoc committee appointed by the National Nutrition Consortium, Inc. (1978) to examine potential hazards of excessive consumption of vitamins and essential minerals noted that thiamin, riboflavin, niacin, biotin, pantothenic acid, pyridoxine, and vitamin B₁₂ possess "such low toxicity" that they were not reviewed. However, for each essential nutrient, identifying possible adverse health effects depends on appropriateness and sensitivity of the methodology employed.
An estimate of the margin of safety for most food chemicals can be derived from a comparison of the usual or expected level of human exposure and the highest level of that chemical fed to experimental animals without adverse effects. When necessary, regulatory procedures address the need to control the level of a chemical added to the food supply. This can reduce exposure and increase the margin of safety. Developing a wide margin of safety is not as easily accomplished for essential nutrients because reduced intakes may lead to deficiency. These constraints and characteristics of nutrients must be recognized when addressing the probability of an adverse effect resulting from exposure to any nutrient. Companion changes in biochemical and metabolic parameters may or may not represent toxicity.

A perusal of the literature suggests: 1) very little is established concerning the potential toxicities of most vitamins and minerals for humans, and 2) there are wide variations in the types and degrees of response for this diverse group of nutrients (DiPalma and Ritchie, 1977; Goyber and Mehlman, 1977; Hayes and Hegsted, 1973; Herbert, 1979; Luckey and Venugopal, 1977; National Nutrition Consortium, Inc., 1978; Prasad, 1976a, b, 1978; Rechcigl, 1978; Venugopal and Luckey, 1978). Specific endpoints of toxicity, such as organ injury, would be prima facie evidence of toxicity, especially when related directly to high levels of ingestion, elevated blood concentrations, or specific sites of storage and deposition of nutrients. Both ad hoc groups indicated it would be useful to identify variables that either enhance or limit the usefulness of methodology available for the development of protocols.

B. VARIABLES AFFECTING RESPONSE

The age of the individual alters toxic potential for virtually all chemicals. Very few experiments have been undertaken to relate susceptibility to nutrient toxicities with age. In general, body weight and the developmental changes characteristic of growth contribute to a greater sensitivity for the young. For example, children are more susceptible to the toxic effects of cobalt (Caplan and Curtis, 1961; Little and Sunico, 1958) and zinc (Pories et al., 1974) than adults. An exception to this generalization appears in Harrison's (1978) review of the literature on vitamin D toxicity, which concluded that when doses per unit body weight are compared, young growing animals show less evidence of toxicity than adult animals. Elderly persons also represent a group at increased risk of nutrient toxicity for several reasons: likelihood of inadequate diet due to reduced quantity and variety of foods, more frequent use of drugs, higher background of disease, and lowered capacity to respond to metabolic stress. In older individuals, calcium and phosphorus metabolism presents examples of both age and sexual differences, i.e. the absorption and retention of these minerals decrease, but females after menopause are at greater risk of developing osteoporosis than males of comparable age (Krane, 1977).
Nutrient composition of the diet, total nutrient intake, and nutritional status may alter individual nutrient toxicities; an excess of one nutrient may produce a deficiency or cause toxicity of another at a lower level. Nutrient interactions have been best studied in the area of mineral nutrition of experimental animals (Barber et al., 1955, 1956; Gipp et al., 1974; Ritchie et al., 1963; Venugopal and Luckey, 1978). Virtually all nutrients display interactions that may alter physiological response to a given dose of a single vitamin or mineral. For instance, there can be increased susceptibility to toxicity associated with other substances due to altered activity of the microsomal drug-metabolizing enzymes, which are sensitive to nutritional status, and increased susceptibility to bacteria-associated disease due to possible immunosuppression of the humoral immune system by high nutrient levels. Other examples can be envisioned. The proportions of protein, fat, and carbohydrate in the diet and other factors such as phytic acid and poorly digested fibrous materials affect absorption from the gastrointestinal tract (Herman, 1979). The toxic potential may vary by several orders of magnitude for each nutrient if an appropriate combination of variables is employed. Geographic variations in nutrient intake may alter results. For example, high fluoride or sodium content in drinking waters, high selenium in foods produced in regions such as the Dakotas (Allaway, 1973), and regional differences in iodine intake may present significant interaction with the nutrient under investigation.

A third type of variable affecting individual response may be designated as pharmacokinetic phenomena. Examples of factors that influence pharmacokinetics would be the physical and chemical form of the nutrient, including its nature in dietary items as well as the size, number, and duration of supplemental doses. The influence of physical and chemical form of the nutrient is illustrated by the type and degree of toxic responses to different forms or dosage modes of vitamin A, vitamin D, and iron salts (Select Committee on GRAS Substances, 1978b, 1980a, b). Körner and Völlm (1975) found that with comparable doses of vitamin A given orally to 132 humans of various ages, hypervitaminosis A appeared earlier when the vitamin was given in the emulsified form rather than in an oily form; this is presumably related to the greater absorption of the emulsified form. In infants and children, doses of vitamin D of 2000-5000 IU/kg of body weight given daily for several months were far more toxic than a single dose of 100,000 IU/kg (Harrison, 1978). A single oral dose of 1250 mg of cobalt metal powder was tolerated by young rats, but daily doses of 30 mg given for 30 days (900 mg total) were lethal (Prasad, 1978). The time between dosing is another significant kinetic variable. Hillman (1956) found that a 40-year-old, 75-kg male subject ingesting 1 million IU of vitamin A/day during an initial 13-day period exhibited different signs and symptoms than he experienced during a second 25-day period, despite similarities in plasma vitamin A pharmacokinetic profiles.
A fourth group of variables affecting toxicity may be related to the use of drugs and the presence of various diseases, many of which have a genetic etiology. For example, idiopathic hypercalcemia in infants is thought to be an inborn error of metabolism (Kenney et al., 1963). Such infants may exhibit lower tolerances for vitamin D-containing supplements than normal infants (Harrison, 1978). Individuals with Wilson's disease manifest copper toxicity with near normal dietary intakes of 2-5 mg daily (National Nutrition Consortium, Inc., 1978). Although individuals with hemochromatosis may be at higher risk with consumption of iron supplements, the hypothesis has not been evaluated experimentally in man or in appropriate animal models (Select Committee on GRAS Substances, 1980b). These are examples of diseases that may be related to inborn errors of metabolism and which demonstrate increased sensitivities to nutrient toxicities. Data obtained on nutrient status and on toxic responses in a clinical setting must be carefully evaluated for such confounding factors.

These variables may alter individual susceptibility to nutrient toxicity. Obviously, individuals and subsets of the population may differ greatly in sensitivity to these and other unknown variables, causing consternation in the investigator who wishes to identify the number of people at risk. However, such knowledge may assist in the identification of particularly sensitive individuals.
III. HUMAN EXPERIENTIAL DATA

Evidence of consumption of toxic levels of vitamins and essential minerals from dietary sources alone is sparse; but the population consuming supplemental vitamins and minerals may be presumed to be a logical one to consider epidemiologically. The prevalence of vitamin and mineral supplement consumption in the United States is not accurately known. A survey conducted for the FDA in 1972 (National Analysts, Inc., 1972) showed that a large proportion of the American population believed that such supplements were valuable. Supplements are taken primarily without medical supervision (Jukes, 1979). In determining the significance of using these products, a much more detailed analysis of consumption will be required, emphasizing 1) the number and characteristics of users, and 2) the quantities and composition of supplements consumed compared with nutrients derived from food sources.

A. HUMAN CONSUMPTION DATA

Estimates of the number of consumers currently using supplements range from 34% in a survey of women in four western states (Standal et al., 1979) to 55-59% of total consumers, according to FDA surveys during the period 1973-1975 (Food and Drug Administration, 1976). The FDA surveys indicated that the most likely users of supplements were female homemakers 18-34 years old whose nutrition knowledge was considered "high" and whose children were under 18 years of age. Similar findings on attitudinal variables of consumer behavior for nutrition supplements were reported by Saegert and Saegert (1976). In addition to these usage data, the second Health and Nutrition Examination Survey, when completed, will provide estimates of frequency of use of vitamin and mineral supplements in a large national sample, with frequency-of-use categories listed either as "none", "regular use", or "irregular use". Because this is a large, geographically representative sample (approximately 25,000 subjects), a more accurate assessment of usage patterns should be possible although precise data on quantities consumed will not be known.

Standal et al. (1979) obtained data from a sample of 334 women residing in four states (California, Washington, Nevada, and Hawaii) and found that 30% of the subjects supplemented their diet with four water-soluble vitamins (thiamin, riboflavin, niacin, and ascorbic acid) at daily levels ranging from 340% of the RDA for niacin to 780% for thiamin. Vitamin A supplements were used by 24% of the women at a mean intake of 300% of the RDA. Although the fractional distribution was not given, the levels of intake spanned a range from 33-2080% of the RDA for vitamin A, and from 7-5600% for thiamin supplements. The data do not indicate the proportion of supplement users at various levels of intake.
Another study that may relate to this latter question is the unpublished finding of the first Health and Nutrition Examination Survey showing that approximately 30 of 20,749 (0.15%) subjects vigorously pursued a diet with large quantities of vitamin A activity (National Center for Health Statistics, 1978). These individuals selected quantities of foods such as eggs, liver, and carrot products containing 300,000-400,000 IU of vitamin A equivalents (as retinol) per capita per day; a large fraction of these equivalents was in the form of the relatively nontoxic carotene.

This brief commentary reflects the paucity of data available on which to base an estimate of the numbers of persons in the general population who are ingesting large quantities of nutrient supplements. To obtain adequate data, innovative and relatively expensive research will be required. Alternatively, an acceptable estimate of quantities consumed may be obtained by use of a telephone survey (such as has been initiated by FDA) or similar type of survey, which might include a determination of how frequently those who ingest dietary supplements change both the supplement composition and consumption pattern. Appropriate information on total exposure to nutrients from all sources, including dietary sources, would aid in interpreting data on supplement consumption.

Currently available intake data are of questionable and very limited value for the objective of this study. In order to increase the usefulness of intake data, it would be desirable to specify the clinical and metabolic characteristics of the individual, the levels of consumption, the identities and chemical forms of the nutrients, and the supplement usage schedules. On the other hand, survey studies on intake with probability samples would be useful for tracking patterns of nutrient consumption in the general population. Moreover, it would be appropriate to identify subsets of the population either suspected or known to be vulnerable, both in terms of nutrient level and susceptibility to toxic manifestations.

B. EPIDEMIOLOGICAL PRINCIPLES

To evaluate the causal significance of the association between health effects and the ingestion of a potentially harmful substance such as a nutrient in excessive dosage, it is necessary to include the following judgmental criteria:

1. The consistency of the association.
2. The strength of the association.
3. The specificity of the association.
4. The temporal relationship of the association.
5. The congruency with knowledge about biological mechanisms.
Data to be evaluated by these criteria must come from epidemiological data that include clinical, pathological, and experimental evidence from a large number of people. The final conclusion about a causal relationship between the substance and its effects will rest on informed judgment after consideration of dosage, time of consumption, age of the subject, influence of disease, drugs, or other confounding factors. In many respects, these issues resemble the problem faced by investigators attempting to discover if causal relationships exist between smoking and health (U.S. Department of Health, Education, and Welfare, 1964), between coronary heart disease and diet plus smoking, and more recently, between exposure to suspected environmental carcinogens and cancer.

If statistical analysis of the epidemiological data can demonstrate that the four essential criteria are met, the possibility of a causal association for a particular nutrient would be strengthened. Because long-term, adverse effects are established in only a few instances of nutrient excesses, it is probable that these relationships are subtle, if they exist at all. The more protracted the adverse effects, the more complex is the equation and the less certain are the results. For example, even for the significance of sodium ingestion in hypertension, analysis of epidemiological evidence is not definitive (Select Committee on GRAS Substances, 1979b; Tobian, 1979).

The most difficult of the five judgmental criteria to meet is the specificity of the association. The well-recognized difficulty in the above analytical epidemiological studies is the impossibility of assigning treatment randomly, thus confounding factors have been uncontrolled. The ad hoc groups discussed the feasibility of designing studies that would solve this difficulty and recognized that such studies may have ethical constraints.
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IV. METHODOLOGY FOR THE STUDY OF NUTRIENT TOXICITIES

The methodologies currently used for the evaluation of nutritional status include biochemical and clinical procedures which can be examined for their usefulness in identification of nutrient toxicities. Many tests, designed primarily to detect deficiencies, have been validated in numerous nutrition surveys and are useful for the evaluation of either population groups or individual subjects (Sauberlich, 1975a). However, those useful for detecting and studying nutrient toxicities may be limited to tests that measure or indicate tissue nutrient saturation. For example, the measurement of urinary 2-pyridone/N^1-methyl nicotinamide ratios reflects the development of niacin deficiency and would probably be of little value for the detection of niacin toxicity.

A somewhat similar compendium of assays for minerals was briefly discussed by Luckey and Venugopal (1977). Appropriate analyses are capable of detecting and estimating picogram (10^-12 g) levels of minerals in biologic materials. At these levels, however, considerable error can result from contamination of the sample or the measurement process. The instrumental techniques include atomic absorption, fluorimetry, emission spectroscopy, neutron activation, gas-liquid chromatography, anodic stripping, x-ray fluorescence, electron microprobe, and spark-source mass spectroscopy. In most cases, these methodologies have yet to be applied broadly in a clinical survey of nutrient status.

A profile which correlates with level of intake and duration of exposure may be established for some nutrients; the interpretation of tissue saturation kinetics as related to toxic symptoms will be critically important. The specific fluid and tissue specimens selected will depend both on accessibility and on the nutrient under study. For example, different tissues will saturate at different dosages, and a key question will be to determine which of these saturated sites indicates adequacy and which produces damage. This type of analysis will likely be different for each nutrient although many essential nutrients share common features. Water-soluble vitamins may span a narrow dosage range when all sites become saturated and beyond which excretion readily occurs, while saturation of different sites for fat-soluble vitamins may occur over a broader range of dosages beyond which toxic symptoms would be more likely to occur because of the more limited excretory mechanisms. An important consideration when studying many of the essential minerals is the fact that toxicity may result at doses far below those that saturate easily studied tissues.
A. TOXIC MANIFESTATIONS REPORTED

1. Iron

Acute toxicity of ingested iron occurs almost exclusively from large overdoses of readily available iron-containing preparations, e.g. hematinics. Small children are by far the most frequent victims of severe effects. Vomiting (possibly bloody) generally occurs within 30 minutes of ingestion followed by diarrhea (possibly bloody), lethargy, pallor, tachycardia, rapid ventilation, shock, coma, and convulsions (National Research Council, 1977b).

Chronic toxicity may occur in susceptible individuals after long-continued iron overload from dietary sources or transfusions. Widespread parenchymal deposits of iron occur in various organs, especially the liver. Major manifestations are skin pigmentation, hepatomegaly, diabetes, cardiac abnormalities, endocrine changes, and arthropathy (National Research Council, 1977b).

A number of laboratory tests have been suggested to detect the presence of iron overload; the most commonly employed are tabulated below (adapted from Edwards et al., 1977).

<table>
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<th>Test</th>
<th>Unit</th>
<th>Normal Mean Value</th>
<th>95% Confidence Levels</th>
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<tr>
<td>Serum iron</td>
<td>µg/dl</td>
<td>107</td>
<td>45 -169</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>percent</td>
<td>35</td>
<td>20 - 50</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td></td>
<td>94</td>
<td>27 -329</td>
</tr>
<tr>
<td>female</td>
<td></td>
<td>34</td>
<td>9 -125</td>
</tr>
<tr>
<td>Hepatic iron concentration</td>
<td>µg/100 mg</td>
<td>9</td>
<td>1 -25</td>
</tr>
<tr>
<td>Hepatic-parenchymal cell</td>
<td>wet liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stainable iron</td>
<td>grade (0-5)</td>
<td>0.2</td>
<td>0.0- 1</td>
</tr>
<tr>
<td>Urinary iron excretion</td>
<td>mg/24 h</td>
<td>1.2</td>
<td>0.4- 2</td>
</tr>
</tbody>
</table>

The most significant of these tests is probably the amount of iron detected in the liver, either by microscopic evaluation of stainable iron or by chemical analysis. Both methods require liver biopsy, which cannot be performed routinely. Of the remaining tests, serum ferritin seems the method of choice for screening because of the separated ranges of concentrations in iron deficiency anemia (<12 ng/ml), normal state (12-330 ng/ml), and iron overload (up to 10,000 ng/ml) (Jacobs and Worwood, 1975). Serum iron and transferrin saturation, generally available clinical
tests, might also be considered and should be used for confirmation. Since high levels of serum ferritin may also be present in various liver diseases and leukemias, its use for determining iron overload appears questionable (Jacobs and Worwood, 1975).

2. Iodine

Effects of excessive consumption and absorption of iodine are reflected primarily by alterations in thyroid metabolism (Nagataki, 1974). Wolff (1969) distinguished among "small to moderate" intakes, intermediate quantities with physiological consequences, and "excessive" amounts of 2 mg or more daily, and defined four degrees of excess iodine intake based on increasing quantities consumed. The first degree of excess results in temporary increases in absolute iodine uptake by the thyroid and may involve reduction of thyroid iodine clearance. The second degree of excess may inhibit iodine release from hyperactive thyroid glands or from the thyroid when iodine release has been stimulated by thyroid-stimulating-hormone. Thirdly, progressively larger doses inhibit organic binding of iodine; that is, the classical Wolff-Chaikoff effect. Finally, excess iodine chronically administered or consumed results in complete saturation of the iodide-transport mechanism; large doses (40 mg/day or more) can produce complete inhibition of thyroid hormone synthesis in many euthyroid individuals (Fisher and Carr, 1974).

Excess iodine produces a number of dermatologic responses particularly in immunologically sensitive individuals. Marked increase in salivary and bronchial secretions of most individuals is the basis for therapeutic use of iodide (Vidor, 1978). However, effects on serum protein-bound iodine (PBI), total serum iodine, urinary excretion, and thyroid hormone status are more objective measures of excess iodine intake.

The minimal daily intake is considered the intake level that prevents iodine-deficiency goiter (approximately 1 μg/kg body weight for adults). The RDA for adolescents and adults is 150 μg/day (National Research Council, 1980). The iodine content of typical United States diets is 4 to 13 times the RDA for the several age groups of the population (Vanderveen, 1979). The Committee on Food Protection of the National Research Council (1970) accepted as safe a daily intake of iodine of 50-1000 μg based on studies of balance and excretion per 24 hours. Wolff (1969) concluded that a total intake of 2000 μg/day may be a level of intake which should be considered toxic. In a recent review of iodine nutrition, Vidor (1978) referenced this conclusion of Wolff (1969) but stated that iodine intakes of 100-300 μg/day are desirable and intakes between 500-1000 μg/day would more adequately ensure against iodine deficiency. However, he suggested that the upper safe limit of iodine intake, that is, a level beyond which thyroid inhibition occurred, remained to be established.
The status of iodine nutriture in euthyroid individuals can be assessed clinically by means of PBI analysis, measurement of total serum iodine, or urinary iodine excretion (Interdepartmental Committee on Nutrition for National Defense, 1963; Sauberlich et al., 1974). The total serum iodine exceeds the PBI value by an amount approximately equal to the inorganic iodide of the serum. Serum PBI values for euthyroid adults range from 4.0-8.0 μg/dl (Pileggi, 1964); however, Nelson et al. (1971) have suggested 3.4-8.2 μg/dl is the normal range. Sterling (1974) has noted that accuracy of PBI and total serum iodine measurements is limited by methodology to ±0.5 μg/dl. Because serum PBI measures only organically bound iodine, total serum iodine measurement may be a more realistic measure of serum iodine content. In addition, determining PBI values requires special, almost research level, procedures not widely available in many laboratories.

Urinary excretion of iodide, measured in reference to creatinine excretion, is the least accurate of the three measures, but in the clinical setting or nutritional survey, it may be the most efficient and practical for estimating daily iodine intake or turnover. Excretion levels below 50 μg iodine/g creatinine are accepted as an indicator of deficiency, but no level indicative of excess has been established (Interdepartmental Committee on Nutrition for National Defense, 1963; Sauberlich et al., 1974; U.S. Department of Health, Education, and Welfare, 1972). Bruhn and Franke (1978) have developed an ion selective method for analysis of iodide ion content of fluid milk samples as an indirect method for estimating iodine content. The applicability of this technique to measurement of urinary iodide has not been determined; however, ion selective electrodes may provide accurate, rapid, and inexpensive methods useful for the study of iodine and other essential minerals in their ionic forms.

About 250-350 μg iodine/g creatinine is the average daily excretion for adults. Data from the Ten-state Nutrition Survey (U.S. Department of Health, Education, and Welfare, 1972) indicate that of 5901 adults, 17 to 59 years old, 34% had iodine excretion rates indicative of intakes of at least 300 μg iodine/day and 16% had intakes of a least 500 μg/day. Iodine excretion for 6.5% of the population was in excess of 800 μg iodine/g creatinine/day. However, neither this nutrition survey (U.S. Department of Health, Education, and Welfare, 1972) nor previous studies (Interdepartmental Committee on Nutrition for National Defense, 1963) provides any insight on levels of excretion which might be indicative of toxicity.

For clinical purposes, measurement of total serum iodine and urinary excretion is feasible and relatively accurate. High serum content and high urinary excretion of iodine are indicative of high intake levels from excessive dietary and other iodine sources such as pharmaceutical preparations (Nagataki, 1974). Chronic ingestion of more than 2000 μg/day by adults results in
increased serum and urinary values in euthyroid subjects. In certain subjects iodine goiter and/or myxedema is produced with accompanying palpable, possibly visible thyroid enlargement. Other signs and symptoms, such as tachycardia, protruding eyes, and increased responses to stimuli, associated with excess iodine intake have been reviewed and cataloged by Vidor (1978). For the most part, dermatologic and respiratory system responses are not definitive, and subjective manifestations are not diagnostic without analysis for serum iodine and/or urinary excretion. There are, of course, a small number of individuals who are hypersensitive and who may have life-threatening reactions to excess iodine exposure (Vidor, 1978).

3. **Fluoride**

Muhler (1970) estimated that dietary intake of fluoride ion, exclusive of that added to drinking water, ranged from 0.3–3.1 mg/person/day. Osis et al. (1974) and Kramer et al. (1974) have reported similar data on dietary intake in the U.S. population. The Committee on Biological Effects of Atmospheric Pollutants (National Research Council, 1971) has estimated dietary intake to be 0.2–1.0 mg/day. Fluoridation of public water supplies is a widely accepted practice in the United States and provides approximately 1 ppm fluorine with seasonal adjustments as required (National Nutrition Consortium, Inc., 1978). Fluoride is also added to dentifrices, and certain other dental products. Topical application to the teeth is also practiced. The primary reason for such practices is prevention of dental caries by fluoride incorporation into teeth during their formation (National Research Council, 1971).

When consumed at high levels, fluoride is known to be toxic. Exposures to 2.5–5.0 g fluoride within 2–4 hours are lethal to man (National Research Council, 1971). Tooth mottling occurs in children when fluoride concentrations in food and water are 2–8 ppm (National Research Council, 1971). However, toxicity such as skeletal fluorosis apparently requires chronic ingestion and involves daily intakes of 20–80 mg. Osteosclerotic changes are the typical sequelae of chronic excess consumption. The manifestations of excess consumption have been reviewed in considerable detail by the Committee on Biological Effects of Atmospheric Pollutants (National Research Council, 1971). The National Nutrition Consortium, Inc. (1978) has concluded that there is a 4– to 5-fold margin of safety between the amount of fluoride providing optimal protection against dental caries and that producing observable osteosclerotic lesions, and approximately a 15– to 20-fold margin of safety between early osteosclerosis and crippling fluorosis resulting from calcification of ligaments in the neck and vertebral column.
Absorption of fluoride is rapid and apparently passive (National Research Council, 1971). Similarly, urinary excretion is also rapid, but over 99% of the amount retained is deposited in calcified structures. Thus, the most definitive indication of excess fluoride ingestion is an increase in skeletal fluoride. Autopsy data indicate bone concentrations of fluoride can reach 679-3500 ppm in adults with signs of fluorosis (National Research Council, 1971).

In normal individuals, plasma fluoride levels range from 0.013-3.48 ppm and are markedly affected by the level of fluoride intake. Plasma fluoride occurs in free ionic form as well as a protein-bound fraction, and its determination is not a routine laboratory procedure for estimation of fluoride status. Urinary fluoride concentration is generally accepted as the best clinical indicator of fluoride status in normal individuals. The fluoride specific ion electrode is the method of choice for urine analysis and has been used clinically for surveying populations for fluoride exposure. Smith and Hodge (1959) concluded that osteosclerosis is not evident when fluoride concentration in fat-free dry bone is less than 4000 ppm. Zipkin et al. (1958) have demonstrated that bone concentrations of 4000 and 6000 ppm fluoride are associated with urinary concentrations of 5 and 8 ppm, respectively. Thus, urinary concentrations approaching 5 ppm would be indicative of the lower limit of toxicity.

4. **Vitamin A**

Adverse health effects occur in persons ingesting excessive quantities of vitamin A (Jenkins, 1978; National Nutrition Consortium, Inc., 1978; Select Committee on GRAS Substances, 1980a). Hypervitaminosis A is a syndrome usually associated with serum vitamin A levels exceeding 100 μg/dl in children or adults who have received vitamin A supplementation. Many of the signs and symptoms such as desquamation and peeling of skin, erythema, anorexia, and vomiting are nonspecific. Other effects include increased cerebrospinal fluid pressure, hyperostosis corticalis, and disturbed hair growth. Additionally, serum vitamin A concentrations are always increased, serum calcium and the activity of serum alkaline phosphatase are often elevated, and occasionally, the activities of serum glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase are higher than normal. All of these effects are generally reversible after supplementation is stopped. Experimental animal models available for studying vitamin A toxicity suggest that the calf and pig show comparable susceptibility to humans while the rat, dog, and cat can tolerate larger daily doses (Hayes and Hegsted, 1973).

Körner and Völlm (1975) reviewed the literature and found 517 reported cases of vitamin A intoxication. The reported levels of vitamin A intake that produce toxic effects are variable; however, this does not necessarily mean that tolerance to vitamin A
varies over a wide range of the population. One of the most important factors is the form of vitamin A administered. The symptoms of hypervitaminosis A after administering highly available aqueous emulsion can appear in one-sixth the time required after a less readily absorbed oily solution (Körner and Völlm, 1975). Prior to 1960, acute hypervitaminosis A was often iatrogenic in infants and children of 1 month to 3 years of age, particularly in Latin countries where 350,000-400,000 IU of vitamin A were given daily. However, cases of chronic hypervitaminosis A showed an age-independent incidence and were more likely to result from "self-medication". Analysis of these case reports suggests that hypervitaminosis A occurs after the liver storage of retinol and its esters exceeds 10,000 IU/g tissue, a level 10-times the estimated normal concentration.

The concentration of vitamin A in the plasma or serum of a fasting subject is a commonly employed laboratory measurement and is considered the only practical biochemical indicator of vitamin A status (National Nutrition Consortium, Inc., 1978; Sauberlich et al., 1974). Normal values are 20–60 μg/dl with values above 100 μg/dl considered indicative of toxicity (Hayes and Hegsted, 1973). Values exceeding 30 μg/dl are associated with appreciable reserves in liver tissue and correlate well with daily vitamin A intake though not necessarily linearly (Patwardhan, 1969; Sauberlich et al., 1974). Within the normal range of serum vitamin A values, the levels for healthy human subjects increase with age; values for men are about 20% higher than for women (Abraham et al., 1974; Leitner et al., 1960; U.S. Department of Health, Education, and Welfare, 1972). There are few data correlating vitamin A intake with either serum levels or the pattern of storage in liver tissue when serum levels are above 60 μg/dl.

Sauberlich et al. (1974) concluded that the following methods were less useful for assessing vitamin A status: radioimmunoassay of plasma retinol-binding protein, urinary excretion of vitamin A or its metabolites, and urinary excretion of two lysosomal enzymes, aryl sulfatase and acid phosphatase. Because a reliable blood serum assay correlates well with vitamin A intake, this technique would be useful for identifying individuals who may be risking hypervitaminosis A by supplementing their dietary intake of vitamin A. However, the most commonly used methods for vitamin A analysis require approximately 3 ml of serum.

5. **Vitamin D**

The hormonal action of vitamin D and its metabolites increases both calcium and phosphate uptake by the small intestine, enhances alkaline phosphatase activity in brush border cells, and promotes the synthesis of calcium-binding protein (DeLuca, 1976). Vitamin D toxicity is manifested primarily as hypercalcemia resulting from the increased absorption of calcium (Food and Drug Admin-
istration, 1979c). Nonspecific symptoms such as anorexia, weakness, and constipation may occur. The major toxic effect is the deposition of calcium phosphate in the kidney, leading to progressive reduction of kidney function, scarring, and eventual renal insufficiency. Calcium deposition may also occur in other tissues, especially around joints, blood vessels, gastric mucosa, lungs, and heart. Death from hypervitaminosis D usually results from renal insufficiency.

The conventional laboratory tests to determine vitamin D deficiency have been serum alkaline phosphatase, serum calcium, and serum phosphorus (Sauberlich et al., 1974). Vitamin D therapy for rickets and osteomalacia causes a decrease in the elevated alkaline phosphatase levels and an increase in low serum calcium and phosphorus levels. The alkaline phosphatase and phosphorus levels in the reported cases of hypervitaminosis are usually within normal ranges and are of little diagnostic value. Hypervitaminosis D has been most commonly assessed from an elevated serum calcium and is suspected when this value exceeds 12 mg/dl (DiPalma and Ritchie, 1977). However, high calcium levels cannot be considered a unique indicator of excessive vitamin D because they may also occur in various bone and endocrine disorders and after excessive calcium intake (Kramer and Gribetz, 1971).

A more precise measure of the vitamin D status of an individual is the plasma level of 25-hydroxyvitamin D (25-OHD) which, within rather broad limits, varies directly with the intake of vitamin D (Stamp et al., 1977). The normal level has been reported to be 15–35 ng/ml (Arnaud et al., 1977; Stamp and Round, 1974). In most cases of overt vitamin D toxicity, the levels have exceeded 350 ng/ml (Hughes et al., 1976). The lowest reported level of 25-OHD in hypervitaminosis D is 130 ng/ml (Haddad and Stamp, 1974; Silver et al., 1978).

The determination of 25-OHD involves a competitive protein binding assay which is not yet routine in hospital laboratories. Consequently, the determination of serum calcium probably remains the simplest procedure for general screening of large numbers of persons for possible hypervitaminosis D. However, this parameter is rather nonspecific and subject to various vitamin and mineral interactions and antagonisms. Plasma from patients with calcium levels above 12 mg/dl should be referred to qualified laboratories for 25-OHD determination.

6. Selenium

Selenium was recognized as a toxic mineral element long before its essentiality for normal growth and development was established in animals. Because soils in certain areas contain high levels of selenium, ingestion of seleniferous forage by livestock can lead to alkali disease and "blind staggers", two related
forms of selenosis that can be fatal. In cattle, sheep, and swine, manifestations result from both acute and chronic ingestion of seleniferous forage but depend on the quantity, rate, and total dose consumed (Fishbein, 1977; Martin, 1978; National Research Council, 1976a). Both acute and chronic toxicity have been produced in several laboratory animal species (National Research Council, 1976a).

Despite extensive study of the occurrence, mechanisms of toxicity, prevention, and treatment of this disorder in animals, relatively little information is available on human toxicity. Epidemiological studies have shown that persons living in areas where soils have high selenium levels may exhibit loss of hair, brittleness of nails, a characteristic "garlic-like" breath due to respiratory excretion of dimethyl selenide, and indefinite responses including fatigue and irritability (National Research Council, 1976b).

Smith et al. (1936) and Smith and Westfall (1937) reported that persons living in Nebraska, South Dakota, and Wyoming, areas where selenosis in livestock was widespread, often had signs and symptoms suggesting selenium toxicity. These included nail sloughing, dermatitis, gastrointestinal disturbances, jaundice, arthritis, and dental caries. Reports of human toxicity from this or other geographical areas have not increased over the past 45 years despite considerable interest in the relationship between selenium blood levels and the level of the element in soils, as well as suggestions that selenium may have a role in the etiology of, and protection from, cancer (Carcinogenesis Testing Program/National Toxicology Program, 1980; Fishbein, 1977; National Research Council, 1976a). However, few case reports of human selenium toxicity are based on substantive evidence of a dose response from elevated levels of ingestion.

Human toxicity from industrial exposures is usually manifested by dermal or respiratory disorders and depends on the chemical form of selenium to which the individual is exposed. Burk (1976) noted that despite its extensive use in industry and the existence of several land areas worldwide with high levels of selenium in the soil, there was no firm evidence of a significant toxicity problem in man. Wells and Alfin-Slater (1979) state that the toxic level of selenium is about 100 times its essential level. However, Diskin et al. (1979) have cautioned that the consequences of chronic ingestion of selenium-containing dietary supplements are unknown.

Selenium content of whole blood or plasma is a relatively accurate indication of selenium nutriture. Urine concentrations may vary, probably because of fluctuations in selenium intake and urinary volume (Howe, 1979). Similarly, hair analysis may be confounded by use of selenium-containing shampoos (Howe, 1979).
There are several methods for analysis of selenium content of biological samples. However, concentrations of selenium in biological materials are minute, especially in comparison with iron, zinc, copper, and calcium. The most frequently employed technique involves a fluorometric procedure (National Research Council, 1976a). Neutron activation analysis and spark-source mass spectrometry are accurate methods, but require more than routine laboratory equipment. Atomic absorption spectroscopy can be used but sample preparation is a problem (National Research Council, 1976a). Sullivan et al. (1979) and McConnell et al. (1975) have used neutron activation analysis successfully, while McKenzie et al. (1978) and Howe (1979) have used enzyme analysis as a measure of selenium deficiency states. In animals, glutathione peroxidase levels are low on selenium-deficient diets and high when toxic levels are fed. The National Nutrition Consortium, Inc. (1978) suggested that if this relationship was confirmed in man, glutathione peroxidase analysis could be a sensitive and inexpensive measure of potential selenium toxicity.

The Committee on Medical and Biologic Effects of Environmental Pollutants (National Research Council, 1976a), after an exhaustive review of available data, concluded that although reliable analytical methods for selenium in biological samples are available, improvements were necessary before routine screening for selenium toxicity could be undertaken. The Committee also recommended a study of the effects of long-term exposure to low levels of selenium. Because blood levels of selenium are affected by the quantity present in soils and the foods in the diet, it is necessary to establish the normal range of blood selenium within discrete geographic areas as a component of a screening program. Such data have been collected in New Zealand (McKenzie et al., 1978; Robinson et al., 1979), South Dakota (Howe, 1979), and Nebraska (Sullivan et al., 1979).

7. Zinc

Zinc is widely distributed in all body tissues although about 90% is found in muscle and bone (National Research Council, 1978, 1980). Total body zinc for a 70 kg adult is estimated to be 2.3 g, and only a small proportion can be mobilized rapidly to compensate for a zinc-deficient diet. Mean serum zinc is approximately 100 µg/dl. A normal diurnal variation in serum zinc levels may contribute to a broad range, about 50-150 µg/dl. Precautions must be taken to avoid zinc contamination from anticoagulants or rubber stoppers used in conventional vacuum collection tubes. About 32% of serum zinc is bound to α2-macroglobulin and 66% is loosely bound to serum albumin. Approximately 30% of dietary zinc is absorbed, primarily in the duodenum; however, the degree of absorption varies widely (Prasad, 1978). Excretion by healthy subjects in zinc balance is primarily via the feces, with about 500-800 µg/day in the urine and 115 µg/dl in sweat. Many
factors affect zinc balance and status. Urinary excretion of zinc increases sharply during the rapid weight loss due to total starvation treatment of obesity; however, moderately overweight persons maintained on a low calorie diet for several months may show high zinc retention (Prasad, 1978). Absorption and fecal excretion are markedly influenced by dietary intake of phytate, fiber, calcium, and phosphorus (Prasad, 1978).

While acute toxicity from ingestion of doses of 6 g zinc or zinc sulfate has been reported (National Nutrition Consortium, Inc., 1978; National Research Council, 1978), the adverse health effects of chronic ingestion of zinc in excess of the RDA (15 mg/day) are uncertain but include potential adverse interactions with other trace minerals. The National Nutrition Consortium, Inc. (1978) has suggested that consumption of a toxic dose from normal dietary components is impossible. However, zinc concentrations in acidic foods and beverages held in galvanized containers for prolonged periods may be at least 40 ppm (National Research Council, 1978). Greaves and Skillen (1970) fed 18 patients with leg ulcerations 660 mg zinc sulfate per day for periods of 16–26 weeks and observed no toxic manifestations. However, Murphy (1970) reported lethargy, fatigue, and acute hemolytic anemia in children who were believed to have consumed an undetermined quantity of zinc from bath water, and in a 16-year-old male who had ingested 12 g of elemental zinc in 2 days. In all cases where toxicity was observed after excessive intake, serum and urinary zinc levels were elevated. However, data are sparse on serum zinc levels during prolonged consumption of high doses. It would be informative to study patients receiving zinc for treatment of sickle cell anemia or wounds to establish more clearly whether a relationship exists between increasing serum level and zinc dosage. It may be desirable to establish the validity of the relationship in normal individuals who for various reasons are consuming elevated zinc.

Colorimetric procedures for measuring zinc based on production of colored coordination compounds with dithizone are relatively precise but time consuming and require considerable sample processing (Sauberlich et al., 1974). Serum, urine, and other fluid samples can be analyzed rapidly, reliably, and accurately by atomic absorption spectrophotometry though emission spectroscopy, x-ray fluorescence, and other techniques have been employed (National Research Council, 1978). The average zinc level in hair of normal subjects is $193 \pm 18 \mu g/g$; however, acute changes in zinc content of the blood and other tissues due to nutritional alterations are not reflected by hair analysis (Prasad, 1978a). Presumably, hair analysis might indicate long-term, and possibly serial, changes in zinc nutrition although at present plasma and urine examination would be equally or more valuable for studying toxicity as a function of dietary intake.

While the absorption, metabolism, biological activity, and excretion of zinc are relatively well known, the toxic range from dietary intake has not been defined for man. The toxic dose
for humans would be influenced by levels of intake, absorption, and other metabolic and dietary factors such as the amount of phytate, fibrous material, and other trace elements in the diet.

8. Copper

While copper in its ionic forms is chemically reactive, its potential chronic toxicity for man appears relatively low except for individuals with Wilson's disease (Hill, 1977). Wilson's disease is an inherited autosomal recessive trait characterized by progressive accumulation of copper in erythrocytes, kidney, liver, and brain, having a prevalence of about 1 in 200,000 persons (National Research Council, 1977a). However, acute copper poisoning does occur occasionally, usually from accidental or intentional ingestion of cupric sulfate or consumption of acidic beverages after prolonged exposure to metallic copper. Chuttani et al. (1965) estimated that 48 patients admitted to a hospital in India with acute copper poisoning had ingested doses of copper sulfate larger than 1 g. During the past two decades, instances of copper poisoning have occurred following ingestion of various beverages that supplied as little as 10 mg or less of copper (National Research Council, 1977a; Select Committee on GRAS Substances, 1979a). Acute copper intoxication is a recognized complication in hemodialysis where copper tubing or valves are used (Manzler and Schreiner, 1970).

The acute effects of excess copper consumption may include nausea, hemolysis, hepatic necrosis, gastrointestinal bleeding, oliguria, azotemia, hemoglobinemia, hematuria, proteinuria, hypotension, tachycardia, convulsions, coma, or death (Chuttani et al., 1965; National Research Council, 1977a). However, vomiting and diarrhea resulting from such ingestion usually protect the individual from serious and life-threatening consequences (National Research Council, 1977a).

Human copper toxicosis may occur in patients with Wilson's disease from use of copper-containing intrauterine devices, consumption of meats such as pig liver from animals receiving copper-supplemented feeds, and from exposure to copper sulfate used as an algicide, fungicide, or molluscicide (Prasad, 1978). No recent reviews refer to chronic toxicity in man from prolonged or excessive intakes of copper-containing foods or dietary supplements (Cartwright, 1950; Hill, 1977; National Research Council, 1977a; Prasad, 1978; Underwood, 1977; Venugopal and Luckey, 1978).

The copper intake of man is primarily from food although drinking water may contribute significant amounts in some cases (National Research Council, 1977a). The average daily intake from all sources is about 2-4 mg, and on occasion as high as 10 mg (Underwood, 1977). However, recent data suggest that many diets provide lesser amounts of copper and that the average daily copper
intake is probably less than 2 mg (Holden et al., 1979; Klevay et al., 1979; National Research Council, 1980). Copper is present in foods as metallo-organic complexes. In healthy adults, about 95% of the absorbed copper is bound in ceruloplasmin and the remainder is loosely bound to albumin. The copper is widely distributed to body tissues (Hill, 1977), all of which contain minute amounts bound in enzymes such as cytochrome c oxidase, monoamine oxidase, and superoxide dismutase (cytocuprein). Copper transported to the liver is apparently stored or incorporated as cytocuprein; however, the exact nature of the storage form is still being investigated (Prasad, 1978). Excretion occurs primarily through the feces which contain both unabsorbed copper and that secreted in the bile.

Ceruloplasmin concentration in healthy adults is about 20-45 mg/dl plasma or 80-140 μg copper/dl. Cartwright (1950) measured higher total plasma copper in females (114.0 ± 4.67 μg/dl) than in males (105 ± 5.03 μg/dl) and Turner et al. (1978) demonstrated that sexual differences in copper levels are age dependent. The normal range for total serum copper is relatively large, 100-200 μg/dl (Scully, 1978). Both ceruloplasmin and total plasma copper afford convenient measures of copper nutrure except in persons with Wilson's disease who have little or no ceruloplasmin, but may exhibit elevated plasma levels (National Research Council, 1977a). Plasma copper levels are not markedly affected by fasting or ingestion of food and the slight diurnal variations are not considered significant (Prasad, 1978). Deviations from normal ranges of plasma copper concentrations are used as indicators for monitoring persons with acute leukemia and other lymphomas (National Research Council, 1977a). Total plasma copper can be measured colorimetrically (Cooper, 1961); however, atomic absorption spectrophotometry is the method of choice. While atomic absorption spectrophotometry of serum copper levels is accurate and reliable, a major difficulty is minimizing contamination of the specimen during collection.

Except in the case of persons with Wilson's disease, there is no evidence of chronic toxicosis from prolonged excessive dietary intake of copper; there is also little evidence to delineate the effects of chronic exposure. Pimental and Menezes (1975) have reported malignancies in livers and lungs of laborers chronically exposed to fungicides containing copper sulfate. However, Scheinberg and Sternlieb (1969) reported little or no elevation of serum or hepatic copper levels in miners chronically exposed to an environment containing 1% copper, primarily copper sulfide and oxide dusts.

In summary, chronic toxicity from dietary ingestion of copper is a remote possibility except in persons with Wilson's disease. Plasma levels or fecal excretion afford safe and reliable, but not clinically routine, measures to evaluate copper nutrure in man. Finally, little evidence exists that currently available methodology will be able to detect possible copper toxicosis in healthy persons chronically ingesting elevated levels of dietary copper.
9. **Cobalt and vitamin B<sub>12</sub>**

Cobalt is unique in that its only known function in human nutrition is as a component of vitamin B<sub>12</sub>, cyanocobalamin. The vitamin is a required coenzyme in a wide diversity of metabolic reactions. The trace element is present in both plant and animal tissues.

Herbert et al. (1980) have stated that doses (30 mg/day) of vitamin B<sub>12</sub> that are 10,000 times the RDA (3.0 μg/day) are not toxic. A recent review of the effects of vitamin B<sub>12</sub> concluded that there are no known toxic effects from ingestion of single oral doses of vitamin B<sub>12</sub> as high as 100 mg, or weekly doses of 1 mg for periods of 3-5 years (Food and Drug Administration, 1979b). However, cobalt per se is known to be toxic. Coates and Watson (1971) suggested that interstitial pneumonia in tungsten carbide workers was related to inhaled cobalt. Schirrmacher (1967), Kriss et al. (1955), and Gardner (1953) reported adverse health effects in patients treated with cobaltous chloride as a hematinic. Manifestations reported by these and other authors include nausea, emesis, thyroid hyperplasia, myxedema, skin hypersensitivity, elevated erythropoiesis, and eighth nerve deafness following doses of 25-100 mg/day for periods of 4-44 weeks, or doses of about 3.0 mg/kg body weight/day for 12 or more weeks (Sullivan and Burch, 1978). Interstitial pulmonary fibrosis and contact dermatitis associated with cobalt ingestion have also been reported.

Cobalt in beer at 1.1-1.2 ppm has been related to a syndrome known as cobalt-beer drinkers' cardiomyopathy. A number of fatalities from this disorder were reported in North America and Europe during 1964-1966 (Sullivan et al., 1969). One distinct feature of the disorder was increased deposition of cobalt in myocardial tissues with inflammatory cellular reactions and myocardial necrosis (Sullivan and Burch, 1978). A second feature of the disorder was its disappearance with the cessation of the use of foam-stabilizing agents containing cobalt in beer production. Li and Vallee (1980) noted that the myocardial manifestations are not unique to cobalt. Thus, as pointed out by Sullivan et al. (1969), the role of cobalt in a cause-effect relationship is only chronological.

There are few data on chronic toxicity of ingested cobalt beyond those associated with its use as a hematinic. However, intravenous injection of cobaltous chloride elevates blood sugar, glucagon, and lipid levels, and induces hypothyroidism and thyroid hyperplasia. Intravenous administration also stimulates erythropoiesis, and intramuscular doses have been reported to induce rhabdomyosarcomas (Sullivan and Burch, 1978).
Waslien (1976), in a review of dietary intakes of various trace elements, cites levels of cobalt intake that ranged from 16-920 μg/day for adults on institutional diets in the United States, U.S.S.R., and Germany. Li and Vallee (1980) indicated that the cobalt concentration in human plasma is about 60-80 pg/ml and that of whole blood 80-300 pg/ml as estimated from bioassay of vitamin B₁₂ concentration. Because vitamin B₁₂ is water-soluble, it is readily excreted in the urine, and urinary excretion is the primary route of loss in man (Sullivan and Burch, 1978). In the case of cobalt, there is some evidence for binding of a limited fraction of ingested cobalt by the liver and kidneys even though the major portion is excreted in the stool.

Estimation of total body burden of cobalt is difficult. Blood levels of cobalt are minute because absorption in the gastrointestinal tract is limited. Li and Vallee (1980) indicate indirect measure of blood and plasma levels by vitamin B₁₂ bioassay is possible. Sullivan et al. (1969) noted that urinary excretion in normal subjects was too low to be measured by atomic absorption spectrophotometry. There is a broad spectrum of acute manifestations from ingestion of excessive doses of cobalt; however, the possibility of such ingestion is remote. Methodology to assess possible toxicity of vitamin B₁₂ or cobalt requires further development before it can be applied to surveying large populations.

B. TOXIC MANIFESTATIONS LESS FIRMLY ESTABLISHED

1. Calcium

Homeostatic mechanisms limit serum calcium levels within a narrow, normal range of about 8.5-10.5 mg/dl (Scully, 1978). Within this range, values for children are somewhat higher than those for adults. Serum calcium values outside the normal range suggest pathological problems rather than dietary excess or deficiency (Sauberlich et al., 1974). Healthy individuals achieve balance with diets providing widely differing levels of calcium because the efficiency of absorption depends on nutritional needs or dietary levels (Avioli, 1980). Alteration of intestinal absorption as well as kidney reabsorption and fecal excretion are the main features of adaptation. Thus, urinary calcium excretion (150-175 mg/day) as well as serum calcium values have limited utility as an indicator of potential adverse effects from excessive consumption of calcium (Scully, 1978; Venugopal and Luckey, 1978). The net intestinal absorption of calcium depends upon plasma 1,25-(OH)₂D in normal subjects and in vitamin D-replete patients, including, among others, anephric and kidney stone patients (Wilz et al., 1979). Ample evidence exists showing vitamin D supplements, high dietary protein, lactose, and the amino acids lysine and arginine affect calcium absorption; however, the importance of these dietary factors at high dietary calcium levels has not been sufficiently examined.
Venugopal and Luckey (1978) consider most orally ingested calcium salts other than the arsenate and molybdate to be "practically nontoxic" and that hypercalcemia is unlikely to result from an excess of dietary calcium in human beings. However, some data indicate that certain animal species may develop lesions from excess dietary calcium, e.g. ultimobranchial tumors and osteopetrosis in the bull (Krook et al., 1969). The absorption of zinc, magnesium, iron, manganese, and copper is decreased when dietary calcium levels are high. Given marginal intakes of one or more of these trace minerals, a clinically observable effect might be attributable to long-term calcium supplementation of the diet. It is unlikely that screening for such deficiencies would provide a reliable indicator of excessive calcium consumption.

Clinical investigations of calcium requirements and the adequacy of calcium intakes frequently employ balance studies which are not amenable to clinical survey and routine clinical methodology. There is a need for additional criteria to estimate the adequacy of calcium nutrure, particularly when large amounts of calcium are ingested either as a natural component of the diet or as a supplement (Avioli, 1980). Irwin and Kienholz (1973) conducted a careful review of the scientific literature and found that "the upper limit of calcium intake in all age groups has had little research".

2. Phosphorus

Discussion of the potential adverse effects of excess dietary phosphorus can be restricted to the effects of phosphorus in the form of polyphosphates and orthophosphates. Phosphorus contributes to many metabolic processes and plays an important role in mineralization of bone. Phosphate-dependent enzyme processes are resistant to fluctuation of dietary phosphorus in part because of the presence of about 80% of the body phosphorus in the skeleton (Draper and Bell, 1978).

Animal studies suggest that chronic ingestion of excess dietary phosphorus may have adverse effects on the homeostasis of calcium and bone metabolism leading to secondary hyperparathyroidism, bone resorption, and calcification of soft tissues, particularly kidney and heart tissue. Depending on the phosphate content of the diet, additional dietary calcium and magnesium may have a favorable influence. The significance of the animal data to the human situation has been difficult to assess and the long-term implications remain unanswered (Draper and Bell, 1978; Ellinger, 1972; Select Committee on GRAS Substances, 1975). Draper and Bell (1978) reviewed the evidence which supports the possibility that diets relatively high in phosphate content and low in calcium might have a protracted adverse effect on human subjects. Some evidence exists from acute studies in human subjects for the following generalized sequence of reactions: orally
ingested phosphates depress serum calcium; this stimulates parathyroid function resulting in parathyroid-induced bone resorption and subsequent normocalcemia. The long-term effects of repeated stress to this normal homeostatic mechanism may contribute to "aging bone loss" and osteoporosis.

The importance of renal excretion of excess phosphate is seen in cases with limited renal function. In human subjects as in experimental animals, it is clear that hyperphosphatemia, by facilitating the entry of excess calcium into soft tissue cells, may contribute to pathological calcification. Patients with renal insufficiency risk hyperphosphatemia, hypocalcemia, hypomagnesemia, and tetany after receiving laxatives or enemas containing sodium phosphate (Chesney and Haughton, 1974). Hypocalcemic tetany observed in newborn infants fed bovine milk has been attributed to the high phosphorus content of the milk (Oppé and Redstone, 1968). Mazess and Mather (1974) provided epidemiological support from their study of an Eskimo population that subsists on a high phosphorus, low calcium diet and shows a rapid rate of bone loss with age. Bell et al. (1977) have shown in a controlled study that subjects consuming a diet containing added phosphates had lowered serum calcium levels, evidence of bone resorption, and elevated blood concentrations of parathyroid hormone.

Accurate estimates of the level of phosphorus and the calcium/phosphorus ratio of the U.S. diet as well as their variability for diets of individuals and subsets of the population are not currently available. The natural occurrence of phosphorus and the common addition of phosphate-containing ingredients to foods severely limit the value of dietary recall for estimates of phosphorus ingestion (Draper and Bell, 1978). Urinary excretion of phosphates in the absence of phosphorus-calcium balance studies may be useful in validating compliance in prospective studies. The immunoassay of blood parathyroid hormone is an investigative technique of clinical value but has not been applied in large-scale surveys (Bell et al., 1977). Additional experience may permit its use as an early indicator of the adverse effects of excess phosphate consumption or for the identification of those at increased risk of age-associated bone demineralization.

3. Magnesium

Lipsitz (1978) noted that "the only humans with magnesium toxicity reported thus far are patients with renal failure, an occasional toxemic mother receiving parenteral magnesium sulfate, and the newborn of such a mother." A common nonfood source of magnesium is magnesium-containing antacids and laxatives. Only about a third of orally ingested magnesium is absorbed from the gastrointestinal tract. An intake of 0.5 meq/kg/day for adults, 0.18-1.6 meq/kg/day for infants, and about 1 meq/kg/day for young children provides adequate amounts of magnesium (Seelig, 1971). In a clinical setting, patients have been maintained in balance
with less magnesium, providing evidence of a lower requirement (Flink, 1976). The Select Committee on GRAS Substances (1976) reviewed consumption and toxicity data and concluded that the usual adult dietary intake of magnesium is about 300 mg or less per day.

Normal kidney function conserves magnesium when intake is low and excretes it when excessive amounts are absorbed (Flink, 1976). Hormonal control, probably by the parathyroid glands, maintains serum magnesium levels within a normal range of 1.5-1.8 meq/l with a mean value of about 1.77 meq/l (Flink, 1976; Jackson and Meier, 1968). Uremic patients have slightly elevated serum magnesium levels, but these are considered of no clinical significance (Lipsitz, 1978). However, in patients with renal failure, hypotension has been observed at serum magnesium concentrations of 4-6 meq/l; difficulty in urination at levels greater than 5.4; central nervous system depression at 6-8; loss of deep tendon reflexes, drowsiness, ataxia, slurred speech, and ECG changes at 8-12 meq/l. Above 12 meq magnesium/l of serum, respiratory depression, coma, and cardiac arrest in diastole may occur. In an early study on nephritic patients, Hirschfelder and Haury (1934) measured the increase in plasma magnesium after oral administration of 20-30 g of magnesium sulfate (330-500 mg/kg body weight). This dosage in nephritic patients caused an increase of 2.9-3.7 meq/l, while in normal individuals it produced a maximum increase in plasma magnesium of less than 0.2 meq/l.

The most precise method available for determining magnesium is atomic absorption spectrophotometry, but other methods including flame emission spectrophotometry, ethylenediaminetetraacetic acid titration, and colorimetric and fluorometric methods also give excellent results (Flink, 1976). The biochemistry and physiology of magnesium metabolism are closely associated with those of calcium; possibly both share a common absorption site. The skeleton contains about 55-60% of the body's magnesium, and soft tissues contain 40-45%, with extracellular fluids containing less than 1%. Serum concentrations can indicate the satisfactory functioning of homeostatic control mechanisms, but provide little evidence of prolonged intakes of high levels of magnesium from dietary or supplemental sources. Thus, in the absence of additional long-term studies in humans, the current data indicate that magnesium is of relatively low toxicity and effectively maintained in homeostatic balance given wide ranges of dietary intake.

4. Sodium, potassium, and chloride

The following discussions have been drawn largely from the recent reviews by Tobian (1979), the Select Committee on GRAS Substances (1979b), and Battarbee and Meneely (1978), which integrate data from many decades of epidemiological and clinical studies on sodium toxicity. Similarly, Kaunitz (1978) has reviewed
the toxic effects of chloride and Ettinger (1978) those of potassium. Average U.S. diets provide a wide variation in the amounts and in the ratios of sodium, potassium, and chloride ingested daily. Fortunately, given adequate water and normal kidney function, homeostatic mechanisms rapidly adjust and maintain the electrolyte balance necessary for numerous physiological actions including the osmotic pressure of body fluids, nerve transmission, and acid-base balance. However, limited kidney capacity increases the susceptibility of infants to toxic effects of imbalances of these electrolytes (Janovský et al., 1967).

In healthy subjects, absorption of excess potassium ions results in increased urinary excretion of potassium. This process is rapid, and blood potassium concentrations remain within narrow limits, 3.5-5.0 meq/l (Ettinger, 1978; Scully, 1978). Urinary excretion of potassium depends on the availability of sufficient sodium to permit the exchange of potassium for sodium ions in the distal portion of the renal tubule. A low sodium diet or sodium depletion due to vomiting or diarrhea could induce hyperkalemia in cases of greatly reduced renal function.

Ingesting large doses of potassium-containing salts usually induces nausea and vomiting in healthy human adults (Ettinger, 1978; Select Committee on GRAS Substances, 1979b). Thus, in the absence of disease, it is difficult to exceed the body's capacity to regulate potassium by storage and urinary excretion. Gastric irritation, ulcers, and stenosis of the small intestine occasionally occur in patients ingesting concentrated potassium chloride solutions or enteric-coated tablets (Baker et al., 1964; Bockus, 1974; Jacobs and Pringot, 1973). Keith et al. (1942) observed the effects of large single doses of potassium salts on several normal human volunteers, and concluded that a dose of 80-100 mg of potassium/kg body weight may have toxic action on the kidney. These investigators also observed predictable effects on the electrocardiogram (ECG) in two normal subjects and a patient with hypertensive cardiovascular disease given single oral doses of 12.5 g potassium chloride. The concentration of potassium in the serum and the amplitude of the T-waves rose steadily in all three subjects until a maximum was reached in approximately 2 hours. In the normal volunteers, there was a gradual decline in serum potassium and T-waves to control values in 4-6 hours. In the patient, however, the fall in serum potassium was much slower, and the T-waves had not returned to control levels after 12 hours.

The potential for toxic effects from chronic ingestion of excess potassium appears to be low, but controlled long-term studies have not been reported for humans. Studies in rats support the idea that high dietary potassium has a protective effect against the hypertensive effect of dietary sodium. However, this protective effect is limited; hypertension results if sodium intake is sufficiently high even in the presence of elevated dietary potassium. In addition to the influence of potassium, several
additional parameters observed in recent short-term clinical studies are reviewed here because they may provide clues to useful methodology for prospective studies on sodium and potassium.

Serum sodium and chloride concentrations are homeostatically controlled within narrow ranges, 135-145 meq/l and 100-106 meq/l, respectively. In theory, urinary excretion of sodium, potassium, and chloride ions can be used to verify dietary intake levels; however, in practice, urinary excretion values have limitations. In a free-living population, the variability of daily intake levels for a given subject can be rather large, and urinary values will reflect only very recent dietary intakes, making random urine samples of little value for measuring average daily electrolyte intake. Even overnight and 24-hour samples are of limited value in this regard. For instance, Langford and Watson (1974) found that neither overnight nor 24-hour urinary excretion of these electrolytes predicted the 6-day excretion of electrolytes in a study of 108 black females (age 19-21 years). The overnight excretion of sodium and potassium correlated with the 24-hour excretion. Analyses of overnight urine specimens for sodium, potassium, and chloride content provide information of clinical utility and may be useful in verifying actual consumption (Pietinen et al., 1979). Battarbee and Meneely (1978) pointed out that subjects consuming constant daily amounts of sodium excrete varying amounts in 24-hour urine samples. Few studies have recorded success in obtaining multi-day specimens, much less 6-day samples which the data of Langford and Watson (1974) suggest may be required to accurately predict intake even under steady state conditions (Battarbee and Meneely, 1978).

Battarbee and Meneely (1978) describe the toxicity of sodium and potassium as interdependent, with an intake of 35-40 g of sodium chloride producing visible edema in healthy male adults on an average diet containing 3.7-7.4 g of potassium chloride daily. Water consumption and renal efficiency affect the toxicity. A high sodium intake has an osmotic effect resulting in diarrhea (Herman, 1979). Luft et al. (1979) reported that sufficient water intake eliminated the tendency to develop diarrhea in subjects consuming 800 meq of sodium daily (equivalent to a daily intake of 46.8 g sodium chloride). Radioimmunoassay permits study of the change in human plasma prostaglandin A (PGA) concentration in response to dietary sodium levels. Payakkapan et al. (1975) reported that the PGA in 65 normotensive subjects increased from about 1.76 to 3.06 ng/ml when dietary sodium, expressed as 24-hour urinary sodium, decreased from 300 to 50 meq/day. A small rise in plasma renin and a comparable rise in urinary aldosterone also occurred. In nine short-term tests of 14 normal male subjects given six levels of sodium intake from 10 to 1500 meq/day, urinary sodium excretion was inversely correlated with urinary norepinephrine excretion (Luft et al., 1979). Dietary sodium was limited to 600 to 800 meq daily with normal saline given intravenously to
achieve higher exposures. Over the range of sodium intake, daily excretion of norepinephrine ranged from $54.3 \pm 3.4$ to $23.4 \pm 2.9 \mu g$ while a decrease from $467 \pm 63$ to $67 \pm 24 \mu g/ml$ occurred for venous plasma norepinephrine with subjects in the upright position.

The problems encountered in the study of the long-term toxicity of sodium particularly illustrate limitations which exist for methodologies designed to study the possible protracted toxicities associated with excessive intakes of nutrients. Epidemiological and clinical investigations attempting to establish a causal relationship have demonstrated an association of hypertension with excessive sodium intake and an imbalance of sodium: potassium intake. Indeed, the sodium requirement of a healthy adult can probably be met by 0.5 g of sodium chloride although many people consume 10 g daily in the United States, and consumption of over 40 g daily is common in certain areas of Japan (Battarbee and Meneely, 1978). This degree of excess consumption of sodium produces no known acute toxic effects for healthy adults adapted to these intakes. A consensus now exists that chronic excessive consumption of sodium ions is associated with development of essential hypertension. Other factors known to influence the development of hypertension include dietary potassium, genetic predisposition to hypertension, degree of obesity, life-style, race, and individual characteristics of endocrine and kidney function. Establishing a causal, rather than a permissive, relationship of dietary sodium and hypertension requires additional data on the biochemical and physiological mechanisms of the pathology. The enormous problem of acquiring the database necessary to establish the association between essential hypertension and dietary sodium illustrates the difficulties and limitations which should be anticipated in any studies of nutrient toxicity involving toxic effects of a protracted nature.

Clearly, a subset of the population exists that would benefit from a methodology capable of early detection of their susceptibility to essential hypertension (Tobian, 1979). Estimates range from about 10-30% of the U.S. population as the proportion that develops hypertension. Tobian (1979) has noted that there is no certain methodology capable of identifying that subset of the population or individuals genetically predisposed to the development of hypertension.

5. Thiamin, riboflavin, and niacin

As noted earlier, experts consider thiamin, riboflavin, and niacin to be of low toxicity (National Nutrition Consortium, Inc., 1978). Because these vitamins are water-soluble, they and their metabolites are excreted readily in the urine. At normal dietary intakes, urinary levels of riboflavin and thiamin provide reliable indices of nutrient status. Normally, little nicotinic acid occurs in urine, but the urinary excretion of two metabolites, N1-methyl nicotinamide and N1-methyl-2-pyridone-5-carboxylamide, has proven useful (Sauberlich, 1975a). The utility
of these urinary parameters for studies with individuals who are ingesting relatively large doses of these vitamins remains to be established.

Itokawa (1978) views the physiological action of thiamin as a cofactor in intermediary carbohydrate metabolism distinct from its pharmacological and toxicological actions related to nervous system function. In animal models, a margin of safety exceeding 1000-fold exists between a therapeutic dose of thiamin, related to its role in carbohydrate metabolism, and adverse effects, related to its action in nerve excitation. Oral administration of 2-3 g/kg body weight as a single dose is lethal for mice, rats, and rabbits; death due to respiratory paralysis of central origin is preceded by restlessness, labored respiration, vasodilation, cyanosis, muscular twitching, and clonic convulsions. Maintained with artificial respiration, dogs tolerate blood thiamin levels up to 120 mg/dl; otherwise levels of 7-10 mg/dl are lethal (Smith et al., 1948). When thiamin is given as a feed component, experimental animals have ingested high levels without adverse effects on growth or reproductive performance (Select Committee on GRAS Substances, 1978a). High dietary thiamin elevates tissue thiamin concentrations, particularly in heart, kidney, liver, and testes. There is little evidence of significant interactions with other B vitamins even when excessive levels of thiamin are imposed on animal diets deficient in riboflavin, pyridoxine, nicotinamide, or pantothenic acid (Morrison and Sarett, 1959a, b; Unna and Clark, 1942).

In attempts to effect clinical improvements in various disorders, oral doses greatly in excess of physiological requirements have been administered to man, with few reports of adverse effects. The most frequently reported adverse effect is the development of hypersensitivity, which in several cases resulted in anaphylactic shock after injection of thiamin. The Select Committee on GRAS Substances (1978a) has reviewed several case reports of apparent hypersensitivity-type reactions after oral ingestion of thiamin (17 mg-10 g daily) and approximately 200 case reports of adverse reaction to parenterally administered doses of thiamin (5-100 mg), including five instances of sudden death. In view of these reports, screening of subjects for hypersensitivity to thiamin should be a prerequisite to their receipt of thiamin-containing supplements.

Subcutaneously administered thiamin (5-100 mg) decreased blood riboflavin and increased urinary riboflavin in patients with liver disease, tuberculosis, or anemia, but did not affect blood or urine riboflavin levels in normal subjects (Fujiwara, 1954). These effects have not been reported following oral administration of thiamin; however, Itokawa (1978) advises physicians to monitor the nutritional status of the other B vitamins in patients receiving thiamin. The active absorption of thiamin depends upon saturable sites and, at high lumen concentrations of thiamin, passive
diffusion may contribute to net absorption. The amount of thiamin in the adult body is approximately 25 mg; there is an apparent limit on the increase in body stores (as thiamin pyrophosphate) in response to large doses of thiamin (Select Committee on GRAS Substances, 1978a; Williams and Bissell, 1944). In general, doses exceeding 10 mg daily of thiamin are excreted unmetabolized by normal subjects (Itokawa, 1978).

Studies with animal models suggest a potential for orally administered thiamin to affect thyroid function and drug metabolism via hepatic microsomal enzymes. Grosse and Wade (1971) fed thiamin to rats (10 mg/kg) for a period of 3 weeks and reported decreased activity of hepatic microsomal cytochrome c reductase. Slingerland and Sullivan (1968) fed rats a high thiamin diet (100 mg/kg body weight for 4-15 days). The formation of organic radiiodine compounds was inhibited, but the results were not consistent. No enlargement of the thyroid occurred. It is emphasized that while these effects of thiamin are demonstrated in experimental animals, their significance to human dietary thiamin supplementation remains speculative.

In a literature review, Rivlin (1978) cited no instances of toxicity from orally ingested riboflavin in human subjects. The most common biochemical measurement of riboflavin status in nutrition surveys is its urinary excretion. The capacity of the gastrointestinal tract to absorb orally administered riboflavin may be less than 20 mg (Mayersohn et al., 1969; Stripp, 1965). The suggested daily oral dose for treatment of riboflavin deficiency is 10-15 mg (Goldsmith, 1975). Large doses are associated with high fecal loss and increased excretion in the urine and bile. No further increase in urinary excretion occurs if doses exceed 50 mg of riboflavin. In animals, riboflavin and its coenzymes, flavin mononucleotide and flavin adenine dinucleotide, do not increase appreciably beyond normal levels in tissues after high dietary riboflavin intakes. Thus, clinically verified methodologies are of limited value for characterizing levels of riboflavin supplementation exceeding 20-50 mg daily.

Persons ingesting excessive quantities of niacin from its inappropriate addition to meat and meat products (0.5-3.7 g/kg meat) have experienced acute itching, sensations of warmth, and cutaneous flushing of the face, neck, extremities, and trunk (Lyman et al., 1957; Press and Yeager, 1962). Generally symptoms from acute exposure dissipate within a few hours. Nicotinamide has no vasomotor or hypolipemic effects in man; the most detailed description of adverse effects relates to pharmacologic doses of nicotinic acid. Doses of 200 mg to 10 g daily of nicotinic acid or nicotinamide have been administered for periods of a few days to 10 years or more in the treatment of patients with various circulatory deficiencies, hyperlipoproteinemia, sprue, and schizophrenia (Charman et al., 1972; Mosher, 1970). Cutaneous flushing, pruritis, nausea, vomiting, and diarrhea have been commonly reported, but often improve with continued therapy. Varying
degrees of hyperpigmentation and acanthosis nigricans occurred in rare cases (Waterman, 1978). Additional effects are abnormal glucose tolerance, hyperuricemia, peptic ulcer, hepatomegaly, jaundice, increased serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, lactate dehydrogenase, serum alkaline phosphatase, serum creatine phosphokinase, blood glucose levels, and among patients with diabetes mellitus, a moderate increase in glycicosuria (Charman et al., 1972; The Coronary Drug Project Research Group, 1975). The most common abnormal hepatic function test is increased sulfobromophthalein retention seen in about 50% of patients receiving nicotinic acid in pharmacologic doses (Parsons, 1964).

The pharmacological effects of nicotinic acid in man, namely, reductions in plasma fatty acids and cholesterol and transitory vasomotor effects, offer possible clinical utility; however, further definition of the mechanism of action of nicotinic acid is required (Waterman, 1978). The reported adverse effects associated with long-term ingestion of pharmacological doses of nicotinic acid are variable, reversible, and disappear rapidly after therapy is discontinued. Large doses of nicotinic acid can be expected to lead to urinary excretion of greater amounts of unaltered nicotinic acid and its metabolites. While measurements of the urinary parameters are feasible, these appear of limited clinical applicability in assessing adverse effects of niacin.

6. Vitamin B₆

Haskell's (1978) review of the literature revealed no evidence of adverse effects in patients treated with daily oral doses of 20–1000 mg (10–500 times the RDA) of vitamin B₆ for periods up to 3–4 years. Sauberlich and Canham (1980) point out that much attention has been given to the effects of vitamin B₆ deficiency because pyridoxal 5-phosphate is required for function of more than 60 enzymes, including amino acid decarboxylases, transaminases, racemases, and enzymes of tryptophan and cysteine metabolism. In addition, they observed transient abnormal EEG's in human volunteers who were on 100–200 mg of vitamin B₆/day for serveral weeks and then suddenly stopped taking these amounts. Sauberlich (1975a) suggested that determination of urinary vitamin B₆ is the most feasible method of measuring nutrient status under survey conditions; total erythrocyte and serum vitamin B₆ concentrations can also be determined. Because microbiological analysis is used for vitamin B₆ determinations and the facilities for this type of analysis may not be readily available, alternative systems such as erythrocyte transaminase activity have been developed (Sauberlich, 1975a). In a clinical setting, measurement of urinary levels of vitamin B₆ or one of its major metabolites, 4-pyridoxic acid, may be useful for quantitation of dietary vitamin B₆ intake.
Children with certain central nervous system (CNS) disorders (especially hyperkinetic children and those with Down's syndrome) have received vitamin B₆ therapy in doses of 10–90 mg/kg/day without evidence of toxicity (Bhagavan et al., 1975; Coleman et al., 1979; Rimland et al., 1978). Liver biopsies were not performed on these patients; however, liver toxicity has been indicated in rats fed high pyridoxine diets (Cohen et al., 1973). In one study, vitamin B₆ (10–30 mg/kg/day) elevated whole-blood serotonin levels in patients with low pretreatment levels, and the investigators suggested that the serotonin blood level is a useful criterion for determining the administration of large doses of vitamin B₆ (Coleman et al., 1979). In these studies and similar studies with thiamin and riboflavin, it has not been customary to include a control group, thus making it difficult to extrapolate results to a normal population.

7. Ascorbic acid

The body pool size of ascorbic acid is between about 1500 and 2800 mg for normal adults; estimates of the daily intakes of ascorbic acid which satisfactorily maintain these pools vary between about 30 and 250 mg (Harper, 1975; King, 1975; National Research Council, 1980). Based on analysis of all available data, the RDA's for ascorbic acid have been set at 35, 45, 50, and 60 mg, for infants, children, adolescents, and adults, respectively (National Research Council, 1980). Additional allowances of 30 mg and 20 mg of ascorbic acid are made for pregnant and lactating women.

Supplementation of the normal dietary intake of vitamin C, ascorbic acid, is a common practice (Herbert, 1978; National Nutrition Consortium, Inc., 1978). This fact has led to extensive efforts to document potential adverse effects that may result from long-term, high-level ingestion of ascorbic acid including the possibility of an adaptive dependence of metabolic and physiologic systems (Cochrane, 1965). Methodologies employed in these research efforts may have clinical application for verifying level of daily intake, identifying patients showing unusual reactions or increased sensitivity, and identifying biochemical parameters that may be useful early indicators of adverse effects in prospective studies.

Ascorbic acid enhances iron absorption in human subjects (Cook and Monsen, 1977; Lee et al., 1967). However, there are no reports of excessive iron absorption attributable to large intakes of ascorbic acid. Cook and Monsen (1977) caution that ascorbic acid supplements might result in excess iron absorption in persons with disturbed regulation of iron absorption: idiopathic hemochromatosis, thalassemia major, and sideroblastic anemia. An effect not studied in human subjects is the ability of ascorbic acid to inhibit absorption of dietary copper that has been demonstrated in young pigs (Gipp et al., 1974). The interaction of
ascorbic acid with iron and copper absorption requires further study if it is to serve as a clinically useful method for evaluating potential adverse effects of ascorbic acid supplements. The review of Körner and Weber (1972) of acid-base and electrolyte balance in relation to ascorbic acid ingestion indicates that normal adults can ingest 3–6 g of ascorbic acid daily without altering sodium balance, pH of the urine, or urine titratable acidity. While electrolyte balance could be affected by long-term ingestion of gram quantities of ascorbates, little significance can be ascribed to this possibility in the absence of clinical effects. Instances of abdominal cramps, nausea, and diarrhea have been reported as acute responses to daily ascorbic acid dosage exceeding 1 or 2 g in adults or 1 g in children (Coulehan et al., 1974; Hoffer, 1971). Such nonspecific effects offer little to the clinical identification of potential long-term adverse consequences attributable to ascorbic acid supplements.

Several other areas of investigation suggest that there are clinically measurable parameters that change, possibly in direct relationship to ascorbic acid dose. Orally ingested ascorbic acid increased the sensitivity of erythrocytes to in vitro lysis by hydrogen peroxide. Mengel and Greene (1976) measured an increase in the percentage of lysis from 3.4 ± 1.8 to 8.7 ± 1.3 after 2 days of administering 5 g of ascorbic acid daily in divided doses. Effects of shortened blood coagulation time, thrombocytosis, and blocking of the action of dicumarol anticoagulants have been suggested indicators of adverse effects which should be monitored in certain patients (Owen et al., 1970).

In several studies, urinary oxalate excretion increased markedly when the daily ascorbate dosage exceeded several grams (Takenouchi et al., 1966). Briggs et al. (1973) reported oral administration of 4 g of ascorbic acid in divided doses to one male subject for 7 days increased his daily oxalate excretion from a normal level of 58 mg to a level of 622 mg. It has been suggested that the risk of oxalate stone formation, renal calculi, or gouty arthritis might accompany long-term use of ascorbic acid in excess of 8 g/day (National Nutrition Consortium, Inc., 1978). However, Stein et al. (1976) have reported that 8 g of ascorbic acid daily for 3–7 days decreased the serum uric acid of three subjects.

The effect of oral doses of vitamin C on immunological reactivity is currently an area of intense investigation with both beneficial and adverse effects being reported for human beings (Gross and Newberne, 1980). Increased serum IgA, IgM, and C3 complement levels resulted from 1 g of ascorbic acid daily for 10 weeks (Prinz et al., 1977); 200 mg or 2 g daily correlated with increased resting hexose monophosphate shunt activity and increased shunt activity during phagocytosis (Shilotri and Bhat, 1977). Shilotri and Bhat (1977) observed a 100-fold increase in the number of viable bacteria after incubation with leukocytes.
from subjects ingesting 2 g of ascorbic acid daily compared with results of incubation tests with leukocytes from subjects ingesting 200 mg of ascorbic acid daily. The effect was reversible within 2 weeks after ascorbic acid supplementation was stopped.

The vitamin C status in man is normally determined by serum or plasma ascorbate levels which, for a normal intake, approximate 0.6 mg/dl. This parameter correlates well with dietary intake at low levels (Sauberlich, 1975b). When vitamin C supplements are taken, plasma levels may rise to approximately 1.4 mg/dl, at which point renal clearance increases because of body pool saturation (Sauberlich, 1975b). For investigative purposes, a reasonable indication of tissue stores of vitamin C can be obtained from leukocyte ascorbate concentrations although this parameter is not routinely employed in clinical studies and nutrition surveys. Urinary ascorbic acid measurements are less reliable indicators of nutrient status or dietary intake (Sauberlich, 1975b) However, urinary excretion of ascorbic acid or its metabolites may be useful in verifying dosage compliance in prospective studies when gram quantities of ascorbic acid are administered daily. For example, further characterization of the metabolism of ascorbic acid to ascorbate-2-sulfate and its subsequent urinary excretion may provide a useful indicator in these subjects.

C. STATUS OF METHODOLOGY AND TOXICITY OF OTHER VITAMINS AND ESSENTIAL MINERALS

Among the vitamins and essential minerals that were not included in the previous sections there are additional examples of lack of adequate methodology, insufficient evidence to judge the probability of toxicity or adverse effects resulting from high dosage, and lack of knowledge concerning pool size, tissue distribution, and flux. For example, the trace elements nickel, vanadium, silicon, tin, arsenic, and cadmium are considered essential in human diets at very low levels largely on the basis of animal experimentation (National Research Council, 1980). However, because the adequacy of the animal data has not been demonstrated and insufficient human experiential data are available, controlled prospective studies in human subjects may not be advisable at present. For example, the margin of safety between the level that meets essential requirements and that of toxicity is often quite narrow for trace elements in comparison to B-complex vitamins. Also, the ability to cope with excess levels of minerals appears more limited when compared to these vitamins.

Other minerals not included in the previous section, manganese, chromium, and molybdenum, provide illustrations of nutrient interactions or toxicity that require additional care in protocol design. Oral administration of trivalent chromium has not resulted in toxic effects in either animals or man; however, Mertz (National Nutrition Consortium, Inc., 1978) has cautioned
that excessive intakes of chromium may cause imbalances of other trace elements. Similarly, Davis (National Nutrition Consortium, Inc., 1978) cautioned against excessive intakes of manganese because of its influence on other trace elements and calcium nutrition. Also, persons exposed to manganese ores in an industrial setting have developed neurological disabilities. The significance of these effects to ingestion of manganese requires careful investigation prior to initiation of a clinical protocol designed to study potential toxic effects. Extreme caution is also suggested in the case of molybdenum ingestion, for which human toxicity has been documented and recently reviewed (National Research Council, 1980). In the case of each of these minerals, the overriding consideration must be maintenance of an adequate margin of safety for elements on which there is very limited data concerning dose response curves that range into levels that might be toxic.

Pantothenate, biotin, and folacin are vitamins for which there is little confirmed evidence of human toxicity. In the case of pantothenate, 4-7 mg/day is considered adequate to maintain health of an adult human, while levels as high as 10-12 g/day have been administered with only occasional diarrhea and water retention reported as ill effects (National Research Council, 1980). Preuss (1978) reviewed the literature on folacin toxicity and found that a report of gastrointestinal disturbances in subjects receiving 15 mg of folic acid daily was not confirmed by two separate studies in which similar doses were administered. Reports of administration of up to 80 mg/day in divided doses to patients with angina pectoris could not be evaluated because complete clinical studies were not published (Carr et al., 1975). There have been a number of reports that patients whose epilepsy was completely controlled by phenytoin will start having seizures again if given folic acid in doses substantially larger than the RDA. The convulsant effects of folate administration have been reviewed by Colman and Herbert (1980).

Fat-soluble vitamin E and vitamin K have been considered to be of relatively low toxicity (Briggs, 1978; National Research Council, 1980). High levels of vitamin E may be antagonistic to the normal function of vitamin K (Corrigan and Marcus, 1974). Water-soluble substitutes for vitamin K have produced adverse effects in infants, including brain damage, and these effects have been attributed to immature liver function (Hayes and Hegsted, 1973; Owen, 1971). Briggs (1978) has reviewed adverse effects of large doses of vitamin E, noting muscle weakness and fatigue accompanied by biochemical signs of muscle damage and increased concentrations of serum lipids and cholesterol (Dahl, 1974; Farrell and Bieri, 1975). A complicating factor in evaluating body burden of vitamin E is the limited response of plasma levels to dietary vitamin E intake. Normal adults ingesting 200 IU of vitamin E daily exhibit a doubling of plasma vitamin E concentration, and little additional increase occurs after ingesting 800 IU of vitamin E daily (Farrell and Bieri, 1975). Although questions have
been raised concerning the potential toxicity of vitamin E, the data have not been cause for concern (National Nutrition Consortium, Inc., 1978; National Research Council, 1980).

In summary, a successful protocol for the study of adverse health effects of vitamins and essential minerals requires the existence or development of two types of analytical methodology, one for determining the quantity of nutrients ingested and the second for detecting adverse health effects. For many vitamins and essential minerals, methods for assessing nutritional status have been developed that can approximate the level of chronic ingestion in terms of the RDA. However, few methods provide a basis for accurate estimates in regard to higher levels of ingestion or for determining range and variability of chronic levels of intake. These methodologies are required for correlating nutrient ingestion, suspected adverse health effects, and nutrient concentrations in various tissues. The low levels of many nutrients, particularly trace elements, and the specific target sites of potential toxicity often dictate the use of specialized techniques and instrumentation generally considered as research tools rather than for routine clinical application in large scale surveys.
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V. FEASIBILITY OF STUDY PROTOCOLS

Few adequate data are available on the toxicity of essential nutrients in either animals or man. This and ethical considerations make it difficult to study the potential adverse health effects of vitamins and essential minerals either in the subjects of controlled prospective studies or in the general population. Nevertheless, participants in the ad hoc group meetings were of the general opinion that relatively few subjects closely studied in a metabolic ward would yield more significant information than a much larger number followed on an outpatient basis. This opinion is based on the recognition that many of the specialized techniques useful in studying nutrient toxicities must be considered research tools rather than routine, clinical methodology. In instances where available data are sufficient to ensure that unwarranted toxicities will not be encountered, a carefully monitored trial with a small number of subjects, in the model of a Phase I drug study, could provide valuable information on metabolic response to high doses of a particular nutrient and permit study of pharmacokinetic, biochemical, physiological, and other parameters which should provide clues toward target sites of potential toxicity. While such studies are extremely unlikely to elucidate the adverse health effects of chronic excessive intake of a particular essential nutrient or combination of nutrients, they might provide knowledge useful in studying individuals who, independently and without medical advice, consume nutrients in excess of the quantities demonstrated beneficial and safe. Members of the ad hoc groups expressed the opinion that it was imperative to the public health to define potential nutrient toxicities in appropriate animal models and to establish that such toxicities were not extant in individuals and subsets of the population at highest risk.

From the review of the evidence for toxic effects produced by the ingestion of various vitamins and essential minerals in large, continuing doses, it is evident that each nutrient must be considered individually regarding potential adverse health effects. However, studying one nutrient in combination with others, such as are present in various readily available vitamin and mineral preparations, may be desirable in certain instances. In these instances there may be a lower probability for identifying causal relationships. Prior to selecting a suitable group of subjects for study with appropriate control groups, it becomes necessary to establish priorities in the selection of candidate substances. Criteria for this might include potential level of ingestion, suspected or demonstrated toxicities including evidence from animal studies, sensitivity of available methodology, and evolving knowledge of nutrient interactions. For example, some members of the ad hoc groups expressed concern about the potential toxicities of copper, cobalt, manganese, selenium, and molybdenum in comparison with other trace elements. Assignment of priorities will, in large measure, dictate the population sampled and greatly influence the feasibility of identifying potential adverse health effects.
The factors that influence the feasibility of studying the possible adverse effects of essential nutrients are apt to differ in each case; however, some considerations will be common to the study of many nutrients. One epidemiological method that is not too expensive and could be repeated periodically to determine if a particular nutrient toxicity represents a public health problem is the use of retrospective case-control studies (MacMahon and Pugh, 1970). Statistical and epidemiological expertise is necessary in planning, executing, and analyzing studies to ensure the validity of estimates of safety and the significance of the changes observed. This expertise will be useful in developing criteria for subject selection and assignment to specific studies and for validating the responses to any questionnaire employed. The examples chosen in the following sections exemplify a number of inherent problems.

A. POPULATION SAMPLE SELECTION

Several decisions are required in order to select an appropriate population from among those available for investigations of the potential adverse effects of nutrients. What, for example, should be the characteristics of the sample? Should subjects be otherwise healthy individuals representative of the average health of the entire population of users and nonusers, or should an effort be made to study individuals whose health may be compromised? Should individuals be chosen where the possibility of adverse health effects is greatest?

Companion decisions will also have to be made on which nutrients are the most appropriate for study, particularly with regard to the anticipated types of adverse health effects. Nutrients can be divided arbitrarily into two groups on the basis of our knowledge of adverse health effects. For some nutrients, the informed clinician knows the signs and symptoms of toxicity most likely to be presented and may be able to select the most sensitive indicators of nutrient toxicity. For these few nutrients, the incidence of toxicity might be measured. However, for most nutrients, toxic effects have not been reported or adequately described for animals or human beings, so the incidence of toxicity cannot be determined. Thus, it will be necessary to develop a larger database concerning pharmacokinetic, metabolic, biochemical, and other clinical parameters related to a very wide range of intakes in order to define potential toxicities. Recognizing limitations of case reports, investigators may obtain valuable experience and knowledge for judgment of anticipated toxicity and the biochemical and physiological changes peculiar to a given nutrient by exhaustive study of only a few subjects, e.g. effects of supplement withdrawal.

An opportunity to study the effects of large nutrient doses exists with three variously motivated groups. The first group is those individuals who consume supplements without medical supervision. Members of the group rarely present clinical symptoms
and are often described as self-selecting. In other words, a person who decides to take a specific nutrient supplement may, for example, experience ill-defined intestinal discomfort and decide to change to a different supplement or discontinue taking the supplement for a period of time. A study including this group could miss a major category of adverse effects. Also, this group is strongly convinced that the supplement is beneficial. Because of perceived benefit, the subjective recognition and reporting of symptoms may become less reliable. Attempts should be made to ensure that a study of this group does not reinforce the continued ingestion of nutrient supplements at high levels if adverse effects are observed. These factors make baseline data unavailable and documentation of actual dosage difficult, if not impossible. Moreover, the placebo effect may be real, yet unquantifiable. In spite of certain limitations (there are objections to all potential participants) this group provides a valuable source which could be utilized with special precautions. Initial observations here may provide clues for what to look for in other groups.

A second group includes subjects under experimental treatment with large doses of nutrients for certain medical conditions (Bhagavan et al., 1975; Rimland et al., 1978). The doses of vitamins or minerals administered are often several orders of magnitude greater than the amount provided by normal dietary intakes. Certain programs (e.g. the Orthomolecular Medicine Demonstration Project, Chapter 804, California Statutes of 1976, California Department of Health Services, Sacramento, California 95814) might offer opportunity for selection of appropriate controls and independent observation of patients receiving such doses for ill-defined complaints or diagnosed illnesses that would not be expected to mask normal responses to the nutrient under study. Follow-up could be single blind; the investigator would be kept unaware of the participant's status although the participant would, of course, know in the case of self-administration. The utility of data obtained is limited by the underlying medical condition for which the therapy was initiated. Such subjects are seldom representative of the general population because patients showing adverse effects may have been placed on other therapy. Data from these subjects thus do not provide evidence of safety of high doses for the general population but may provide data of value concerning potential adverse effects. This sample population might also be amenable to a better controlled, randomized, double-blind study; and this would also take care of the placebo effect (Calesnick, 1971). Ethics require that these trials be monitored by sequential analysis to ensure prompt and appropriate attention is given to observed effects (Armitage, 1975).

Subgroups of the general population consuming diets of unusual or restricted nutrient composition constitute a third group for study. For example, individuals consuming vegetarian, ovolactovegetarian, or macrobiotic diets may provide unique opportunities for studying nutrient interactions and nutrient imbalances.
such as the influence of low iron intakes or increased ingestion of fiber. Similarly, persons living in areas where selenium intake is high because of elevated selenium concentrations in soil or where intake of fluoride is high because of concentrations present in water supplies provide unique opportunities for obtaining baseline data on the effects of prolonged ingestion of elevated nutrient levels. Such subgroups provide an opportunity for developing methodologies and comparisons with other groups where certain environmental or nutritional factors are absent or of less significance. The type of elements distinguishing this third group of subjects may be superimposed on members of the first two groups, i.e. those who ingest supplemental vitamins or essential minerals for various reasons.

B. MEASUREMENT OF DOSE CONSUMED

A carefully constructed questionnaire should elicit useful information on the quantity of the test substance that has been and continues to be consumed. A suitable range of dosage must be represented in the study. Dosage span will be highly important; retrospective information should be considered tentative and of interest primarily to document long-term usage. Biochemical analyses of the status of each subject are more reliable, and blood and tissue concentrations of the nutrient, its bound forms, and metabolites are most useful for analysis of total body burden and pharmacokinetic parameters. The total consumption of the nutrient under study must be determined as accurately as possible and these values, which may require chemical analysis of diets, should be reassessed from time to time during the course of the study. Specific food preferences may be seasonal and contribute to greater intakes of one or more vitamins or minerals under consideration. For example, subjects selected from a location in which ingestion of seafoods, especially shellfish (i.e. oysters, clams) is more prevalent could have a significantly greater intake of copper and zinc than individuals with different food preferences. Sufficient data must be collected to permit assessment of the nutritional status of the subjects and to permit a reasonable opportunity for later analysis to detect contributions of nutrient interactions.

The exact nature of the preparation (dosage form) should be determined. Chemical analytical tests of the preparation might be required to fix the precise amount and form of the substance. The importance of this point for vitamin A was illustrated in an earlier section, and can be seen for most nutrients. For example, many minerals elicit acute but clinically undetectable effects on the cells of the proximal gut; however, this site is not very accessible in experimental studies of this type. Additionally, the acute effects such as emesis with copper, which is protective of overabsorption, might be bypassed if a chelated form were ingested.
C. CLINICAL HISTORY

The milieu for the history-taking and examinations must be one that encourages participation by the subjects. The safety and benefits of participation should be cited: potential health benefits, lack of future hazards by participation, minor use of invasive techniques (venipuncture), and contribution to medical knowledge. Comfortable surroundings, the reputation of the clinical setting, and the staff contribute to a successful history record and active cooperation by the subjects.

The subject's history must be developed carefully over a period of many sessions and after repeated examinations by experienced physicians and other health professionals interested in the study. A similar accurate history-taking regimen is required for all control subjects in those studies where such subjects can be identified. An accurate medical history is vital to the success of the study. The initial clinical evaluation and subsequent close rapport with the staff physician are most important, often contributing to the detecting of signs and symptoms before biochemical diagnostic tests confirm significant physiological changes. These observations may be early clues to toxicity and must be emphasized throughout the study.

D. FUNCTIONAL, BIOCHEMICAL, AND CLINICAL METHODS

The tests performed and the frequency of observations must be selected on the basis of both the specific substance(s) under study and the subject population available for study. In general, the usual tests performed in physical examinations will be required including cardiovascular, gastrointestinal, hepatic, metabolic, hematologic, and functional absorption and excretion tests. For nutrients that are known to cause anaphylaxis in sensitized individuals, appropriate testing may be desirable to determine if subjects have been sensitized to a specific nutrient, e.g. thiamin. Specialized techniques may be required for certain test measurements such as turnover rates and biochemical changes in body fluids of certain substances, e.g. atomic absorption spectrophotometry of serum copper levels. Analytical procedures used in surveys such as the Health and Nutrition Examination Surveys might be adapted for clinical trials, and quality control experiences can provide valuable insight into possible problem areas. Establishing comparability of results of trials and surveys might also allow generalization of trial results to the U.S. population in some cases.

It is important to note that while the nutritional status of the subject is significant, the major emphasis of these protocols is to obtain evidence of the safety of a given level of chronic intake and, if possible, to identify the upper safe limits of ingestion of these nutrients. Therefore, the early detection of causal changes in any objective test is most desirable because
they may be predictive of potential and latent toxicities. Preliminary animal data can be quite useful in the selection of methodology and development of protocols, particularly where such data include evidence on early indicators of potentially irreversible adverse health effects. It is important to assess the adequacy and appropriateness of conclusions drawn from animal studies. If sensitive techniques have not been developed in animals then human studies will be more apt to be limited on ethical grounds. Careful clinical appraisal by experienced physicians and other professionals is essential. The appearance and/or disappearance of behavioral and performance features in the individual may be useful criteria for diagnosis.

E. GENERAL PRINCIPLES

The general principles outlined for the conduct of clinical trials for drugs apply to the study of the potential hazards of high doses of nutrients (Food and Drug Administration, 1977). These are to:

- State clearly the objective(s) of the study.
- Define the selection criteria (including diagnostic criteria and reasons for exclusion) and show comparability of the population studied with the population likely to receive the test substance.
- Document the method of randomization and the analysis performed to verify how well the randomization procedure worked.
- Plan the suitable size of a clinical experiment. This will also depend upon the following appropriate decisions concerning the precision desired:
  (a) the degree of response one can or wishes to detect;
  (b) the desired assurance against a false positive finding; and
  (c) the acceptable risk of failure to demonstrate the response when it is, in fact, present in the population.
- Include, when appropriate, comparison group(s), usually simultaneously.
- Perform double-blind studies whenever feasible, as a means of avoiding patient and physician response bias and selection bias.
Use objective methods of observation where possible and appropriate.

Define response variables (parameters) rigorously, including description of methods of observation and quantification.

Maintain strict adherence to the protocol, or document any modifications that may be necessary or desirable.

Specify limitations imposed upon the study by failure to comply with the written protocol (withdrawals, failure of randomization to produce similar groups, etc.) with some idea of the effect the limitation might have on the result.

F. SUBJECT COMPLIANCE

A major consideration in detecting changes associated with long-term ingestion of a test substance is how faithfully the subject adheres to the dosage schedule. The feasibility of executing a clinical protocol is questionable for any nutrient or combination of nutrients for which available methodology fails to verify that the subject is taking the specified dosage. Monitoring the degree of compliance is less difficult for subjects controlled in a metabolic ward than for outpatients. For many nutrients, studies with inpatients may be required initially to develop methodologies applicable to high-dosage schedules. The biochemical estimation of blood or urine levels of the test substance or its metabolites at frequent intervals is the most satisfactory index of compliance. These data must be a part of the record for all subjects. Inclusion of data on complying and noncomplying subjects enhances the credibility of the study. Subjects refusing to continue in the study because they detect subjective effects provide significant data which should be documented. Most importantly, subjects must be free to stop participation at any time during the course of the study.

G. NUTRIENT INTERACTIONS

Nutrient interactions are difficult or impossible to control; thus it becomes necessary to consider the composition of the diet and the specific nutrient preparation consumed by the subjects. A complete dietary history should be maintained and the scope of the study limited when possible to a single nutrient and dosage form in order to minimize uncontrolled nutrient interactions. The metabolic and pharmacokinetic data accumulated during the study will not only be significant in determining target tissues but also in assessing absorption characteristics
of the specific nutrient under study. Enzyme induction may be a useful index of metabolic fate of a test substance and the biochemical tests suggested will assess this aspect of potential toxicity. Subjects under medication with therapeutic drugs are not likely candidates for admission to a study. However, drug therapy should be recorded and monitored and any medication, e.g. aspirin, consumed by the subjects should be documented. Alcohol consumption and tobacco use should also be recorded. A precise dietary history is particularly important and significant changes should be noted.

H. COSTS OF STUDIES

Studies of this character are expensive in terms of funding, professional time, and facilities. Major costs include establishing a biochemical laboratory, personnel, and data handling facilities. To facilitate such studies, it may be desirable to fund an established investigational group or research clinic to add a protocol for studying potential adverse health effects of nutrients to existing clinical investigations. Current programs in these centers and clinical nutrition research units that are designed to encompass shared facilities and research services may provide data already collected that could be examined to determine their value to study design (U.S. Department of Health, Education, and Welfare, 1979). These units include multiple specialities such as metabolic, endocrine, neurologic, and biochemical skills.

I. PERIODIC REVIEW OF FINDINGS

A review advisory group composed of representatives from clinical medicine including clinical chemistry, epidemiology and statistics, toxicology, biochemistry, nutrition, and the behavioral sciences should review the early progress obtained with a few subjects to modify the protocol as necessary. In addition to having the professional competence necessary, this review group should be required to assess legal issues, standards of professional conduct, and community attitudes toward studies of this nature. Because the issues of risks and benefits are emotionally charged they need to be as clearly understood as possible by everyone involved. Aspects of these issues have been and will continue to be debated in public, scientific, and legislative forums. Thus, the review advisory group should consider providing an objective overview of protocols and their impact.
VI. SUGGESTIONS FOR FUTURE INVESTIGATIONS

Additional research is needed to increase the sensitivity and reliability of tests for defining and studying nutrient toxicities. In the absence of underlying genetic and medical limitations, an individual's response to a high dose of a particular nutrient does in fact represent a "normal", albeit sometimes pharmacologic, and often predictable response. The responses are not necessarily adverse. They may be changes in certain metabolic, biochemical, blood chemistry, urine chemistry, or other clinically measurable values and appear "abnormal" compared with a control population consuming an average amount of a nutrient. What, for example, is the significance of altered levels of circulating hormones in the absence of other evidence of toxicity? In several instances, changes have been correlated with identifiable lesions and thus known to represent toxicity. However, in many instances additional research is required to develop and validate generally accepted indicators of nutrient toxicity. Functional tests of various cells such as lymphocytes, hepatocytes, erythrocytes, and induced enzyme systems, e.g. glutathione peroxidase and microsomal cytochrome c reductase, are among those methodologies that hold promise for elucidating potential mechanisms of toxicity.

A major problem still lies in the ability to obtain adequate data from individuals and from population subgroups consuming large excesses of essential nutrients. Additional expertise must be developed in this area before the prevalence of nutrient toxicities can be determined accurately. There is a need to pursue methodological studies and to obtain, through appropriate surveys, better quantitative information characterizing the use and composition of vitamin and mineral preparations. A concurrent analysis of data on dietary intake of nutrients would be necessary for relating findings to total nutrient exposure. Efforts to minimize the number of individuals at risk from potential nutrient toxicities would be enhanced if variables affecting consumer behavior were better understood and measured (Saegert and Saegert, 1976; Sparks and Tucker, 1973). This knowledge should enable epidemiologists and clinical investigators to obtain a more representative sample for the assessment of nutrient toxicities. The relationship of nutrition attitudes, nutrition knowledge, sociodemographic and psychological variables to the use of nutrition supplements should be defined through additional research.

Nutrient interactions, variability of human response to nutrients, and numerous other factors detract from the usefulness of nutrient intake data based on diet analyses. There is a need to develop new methodologies and improve existing methodologies for measuring current and long-term nutrient status, particularly for trace minerals. For example, hair analysis is one noninvasive method receiving increased attention for this purpose (Brown and Crounse, 1980). Many minerals, including those under homeostatic
control in blood, are concentrated in hair. Theoretically, mineral concentrations in hair could provide an acceptable indicator of long-term mineral nutrition. Several investigators have utilized hair analysis for measurement of the status of iron, zinc, copper, lead, calcium, magnesium, phosphorus, and manganese in humans; potential exists also for analysis of chromium, selenium, nickel, vanadium, cadmium, and possibly sodium and potassium. However, hair analysis has numerous difficulties, such as sample contamination, that at present severely restrict its usefulness. Improved analytical procedures, including specimen collection, must be developed and shown to be accurate, precise, and indicative of nutritional or medical status.
VII. CONCLUSIONS

The potential adverse health effects of vitamins and essential minerals are less well studied in man than are nutrient deficiency states. The prevalence of vitamin and mineral toxicities in the U.S. population is unknown. However, clinically significant conditions have been shown to be related to the chronic ingestion of excessive amounts of certain vitamins or essential minerals. Age, nutrient composition of the diet, pharmacokinetic phenomena, nutritional status, duration of elevated ingestion, and diseases are among the variables that affect the response of individuals to large doses of nutrients. To date no common or general indicators of susceptibility to nutrient toxicity have been identified. Thus, a nutrient-by-nutrient approach must be taken in the design of protocols for identifying and quantitating potential adverse health effects of vitamins and essential minerals.

Epidemiological principles suggest that studies with unrandomized self-selected treatment groups cannot be sufficiently definitive to establish a causal relationship between nutrient excesses and subtle, long-term adverse health effects. However, many individuals consume nutrients in excess of the quantities that have been demonstrated as beneficial and safe. Careful study of these individuals would contribute to protection of public health. Such studies should use epidemiologically acceptable protocols, give full consideration to animal toxicity data, and, in most cases, precede initiation of controlled, prospective, clinical trials (similar to Phase I drug studies) if and when such clinical trials are warranted.

Currently available data collected in national surveys on the normal consumption of vitamins and essential minerals by the general U.S. population, either as dietary components or as nutrient supplements, are of limited value for the design of clinical protocols. In the case of nutrient supplements, the levels of consumption, the identity of the micronutrient composition, and the nutrient usage schedules are not known with certainty. Data derived by survey techniques using probability samples of the population could provide evidence regarding changes in the pattern of nutrient consumption and one basis for establishing priorities for nutrients to be included in a clinical protocol. Knowledge of total nutrient intake is important.
Prospective clinical investigations of certain vitamins or essential minerals can help to provide a reliable and extensive database required for evaluating the competing risks of nutrient deficiency and toxicity. Successful design and execution of a protocol will require specialized knowledge and equipment. A prime objective of a clinical protocol should be the identification of early and sensitive indicators of toxicity associated with chronic ingestion of nutrient excesses. The study of the potential adverse effects of vitamins and essential minerals represents an essential and significant contribution to protection of public health.


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