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Technical News From NBS

With this issue of the *NBS Journal of Research*, we begin a news department, Technical News Briefs, which will carry short items covering recent technical developments and new services offered by NBS. With this new feature, we expect to make the subscribers to the *Journal* aware of some of the exciting work that is going on at NBS. At the same time, we are quite interested in hearing from you, our readers, as to what you think of this new department. We invite your suggestions on how to make Technical News Briefs more useful to you.

Hans J. Oser
Chief Editor

Foreword

Symposium on Standardization of Collection and Preparation of Biomedical Samples for Trace Element Analysis

The past two decades have brought a significant improvement in the trace element analysis of biological samples. The development of new analytical techniques and advances in existing analytical methods were fostered by innovation and rapid development of new and improved instrumentation as well as by energetic research in analytical chemistry. Emphasis on the accuracy of the analytical data was strengthened by the development and issuance of biological reference materials, first by H. J. M. Bowen, and nowadays available from organizations such as the National Research Council of Canada, the Commission of the European Communities, the National Institute for Environmental Sciences of Japan, the International Atomic Energy Agency, the U.S. National Bureau of Standards, and others. The combined effects of this development resulted in more reproducible data on biomedical samples and often much lower detection limits in these materials, but not in an immediately recognizable improvement of accuracy.

However, the better reliability of the analytical procedures has made research possible into the cause of discrepant and biased data in biological trace element analysis. Previous efforts to develop improved data on trace minerals in human and biological media have included several workshops and reviews sponsored by the American Medical Association, the National Institutes of Health, and the International Atomic Energy Agency. We have learned that the wide spread of reported values for both organic and inorganic trace constituents in biological specimens can be attributed largely to the uncritical application of analytical techniques to a particular analytical subsample. Both the origin and the selection of a specific sample from a population and the subsampling from a sample for analysis must be considered as sources of error and, if unknown, may lead to significant bias in data. To preserve a valid subsample that is representative of the original bulk sample for subsequent analysis, a multilevel analytical approach is required. As with the analytical technique, certain strict precautions must be taken during selection, sampling, preparation, transportation, and storage of a biological sample that is to undergo trace analysis. As part of the analytical process, the preanalysis steps need to be critically evaluated; and formalized procedures should be adopted that will aid in assuring the quality of the analytical data. Publications on those activities will become available in the near future. The recommendations emerging from these studies necessarily focus on general issues such as the design of biomedical experiments, characterization and selection of meaningful samples, good laboratory practice, etc., and will not include specific procedures.

To follow up on the aforementioned initiatives and to complement the existing work, the special Sessions on the Standardization of Collection and Preparation of Biomedical Samples for Trace Element Analysis were held at the American Nuclear Society's 1984 Winter Meeting followed by a workshop at the National Bureau of Standards. Although this meeting was initiated by a group of analytical chemists who utilize nuclear techniques, the symposium brought together experts from the different fields of science involved in this type of work, thus creating a forum for input from all necessary experience and technical expertise. The symposium lectures and contributions published in this issue of the Journal of Research therefore are not limited to nuclear analytical techniques but applicable to all methods of trace element analysis of biomedical samples. Plenary lectures dealt with the topics of selection and representative sampling of human specimens, technical considerations and

presampling factors that influence the data, and specimen banking. Experiences with actual protocols and procedures which have been implemented for the accurate trace analysis of various biological media were presented in several lectures.

The formal presentations were followed by a workshop session of which a major part was devoted to a fact-finding discussion. Of main concern at this 1984 meeting was the notable absence of existing validated procedures for the collection and preparation of biomedical samples. It appeared to be of advantage to develop standardized procedures which are applicable to specific situations or which can be adapted to a particular research problem. There was general consensus among the participants that "cookbook" type procedures should be developed. This should be the task of follow-up meetings which again will include the much-needed multidisciplinary approach. The participants expressed their optimism that the contributions made at this meeting and the future work will further enhance our ability to provide more accurate data on the important trace constituents in biological media.

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Representative Sampling of Human Tissue

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In the chemical analyses of tissues for trace elements, quality control of the tissue sample for its *anatomic* composition is a critically important step that is frequently overlooked. This is because the analyst often assumes a degree of homogeneity that does not exist. The means of attaining a representative sample vary greatly depending on the organ or tissue involved, and also on the level of resolution chosen, i.e., the size of the sample.

Key words: homogeneity of tissue samples; quality control tissue samples; representative tissue samples; tissue analysis; tissue sample size; tissue trace element data base; trace element analysis.

1. Introduction

Dr. Kemper has stressed the importance of an *environmental* tissue specimen banking program—and this is certainly an important perspective. However, our knowledge of the nutritional requirements of man is quite deficient, particularly in relation to essential trace elements, thus there is also an urgent requirement to address this *nutritional* aspect of the program. We need much more information about the normal range of concentrations of essential trace elements in various human tissues to answer such questions as: Which organs or tissues serve as principal reservoirs of the elements in question? and Which tissues are most important to sample in order to detect borderline deficiency? In addition to purely nutritional concerns, we also need to know much more about trace element composition of normal organs and tissues if we are to recognize abnormalities that may bear causal relationship to diseases or disorders presently of unknown etiology.

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Unfortunately, analyses of trace elements with respect to nutrition or specific diseases is a more complex problem than analyses to evaluate environmental pollution. As an example, cadmium levels in total kidney seem an adequate indicator of body burden, and if this is the sole objective, there seems to be no reason to determine concentrations in the renal cortex, medulla, and calyx. But if one is concerned about the role that cadmium plays in hypertensive disease, for example, the analysis takes on a different dimension. It becomes important to know precisely where in the kidney cadmium is to be found, as well as how much is present, if one is to determine how cadmium may be affecting renal function to produce hypertension.

Hopps and O'Dell [1][1] state: "Data on the concentrations of most elements in human tissues are quite limited. Many of the analyses are not reliable for a variety of reasons ...." Of the six reasons stated, the first two are: 1) the status of the donor was not well characterized; and 2) the precise site of the tissue sample was not specified. These two aspects of the total analytical procedure are discussed in some detail by Koirtyohann and

[1] Figures in brackets indicate literature references.

Hopps [2]. But regardless of whether one approaches the problem because of environmental or nutritional concerns, or as a means of understanding more fully the etiology and pathogenesis of disease, the fact remains that the samples of organs and tissues must be precisely characterized if their chemical analysis, no matter how carefully performed, is to be truly meaningful. The sample is as important as the analysis. This is because of the great variation among cellular structures within most organs and tissues. The importance of such variation is well recognized and dealt with for the tissue, blood. Samples are carefully selected and characterized as whole blood, or plasma, or serum, or the cellular components, and even this latter heterogeneous sample is now amenable to precise characterization. Modern techniques of flow cytometry make it possible to provide samples of (only) erythrocytes, or platelets, or lymphocytes or, if desired, only B or T type lymphocytes, for example. Some solid organs can also be manipulated to yield relatively pure cellular components of one type, but this is the exception rather than the rule, and the hazards of contamination are great. In general, achieving a truly representative sample of most solid organs (excepting liver and striated muscle) is a much more difficult task, and one that may not even be addressed, because the lack of homogeneity of these tissues is not generally recognized. In fact, however, organs are composed of a *mixture* of tissues, and are far from homogeneous. Moreover, solid tissues that are considered normal because they appear normal grossly may be recognized as very abnormal if sections of them are made and examined microscopically.

Very careful selection and characterization of the *tissue donor* is also critically important. This includes not only age, sex and certain important physiologic states such as adolescence, pregnancy, and lactation, but much more: body size and nutritional status, occupation, race, nationality and ethnic group, habitat (geographically), socio-economic status, social habits, dietary habits, medical history, also data regarding use of non-prescription drugs such as antacids, laxatives, antifertility pills, and the like. But a detailed discussion of this aspect of quality control would require a separate presentation.

2. Dishomogeneity of Organs and Tissues

Analytical chemists tend to focus their efforts of quality control on the analytical methodology. The attention to tissue specimens usually concentrates on minimizing additions to and losses of substances that would significantly affect the analytical result. But quality control must also be extended to the *anatomic* characteristics of the tissue sample in order for the chemist to know that his analysis truly represents what it was intended to. For example, "kidney" varies considerably depending on whether it is cortex or medulla or calyx, also whether or not it contains pelvis, subpelvic adipose tissue, large blood vessels, and the like. Because of this, analyses of similar kidneys by highly qualified analytical laboratories, each using similar analytical procedures, often produced results that vary considerably. Although the kidneys were similar, the samples were not. Obviously, this important aspect of this problem, proper selection and characterization of the tissue specimen, is not addressed by the use of standard reference materials to test analytical capabilities.

3. Factors Affecting Characteristics of a Tissue Specimen

There are two principal factors that affect the characteristics of a tissue sample from the chemical analysts' viewpoint: 1) the size of the sample; and 2) the particular organ or tissue being sampled.

Large variations in sample size—which can span a range of volumes from 0.1 M^3 to 1 μM^3, i.e., from a whole body to an intracellular component—are reflected by great variations in anatomical resolution of organs or tissues. This results in very different sorts of problems with respect to homogeneity of the sample. We shall focus on the more common range of sample sizes, however, those that represent a whole organ or a selected portion of an organ. But before we go further, some definition and general statements are in order.

Organs are discrete, (usually) localized collections of (predominantly) parenchymal tissues that are dedicated to performance of a collection of related functions. Because of the multiple functions, various parts of the same organ often vary considerably in their structure. In the kidney for example, the glomeruli, the proximal convoluted tubules, the loops of Henley, the distal convoluted tubules and the collecting tubules each serve quite different junctions and have different anatomical and chemical characteristics.

Another important cause of dishomogeneity is the large variation in the proportion of parenchymal and stromal components among different organs—even within different portions of the same organ. *Parenchyma* comprises those cells whose functions are directly related to those ascribed to the organ. *Stroma* consists of the *supporting* cellular and fibrous elements—blood and lymph vessels, excretory ducts, adipose tissue, mesothelial cells, collagen, elastic and reticular fibers, and the like.

Tissue, as defined in Dorland's Medical Dictionary (25th edition), is "an aggregation of similarly specialized

out the body, e.g., bone marrow and smooth muscle. Furthermore, although the same kind of stromal cells may occur in essentially pure aggregates of considerable size, e.g., subcutaneous adipose tissue, blood vessels such as the aorta, and collagen, as in the form of a ligament, these same kinds of tissue are also to be found inexorably mixed with organ aggregates of mainly parenchymal cells. Thus, even with such a homogeneous organ as the liver (assuming a sample size of several milligrams or more), unless analysis is of a specimen of separated, pure parenchymal cells, i.e., hepatocytes, it will reflect, *in addition to hepatocytes*, the sample's content of blood, lymph and bile, as well as blood, lymph and bile vessels (each of which may, in turn, contain multiple tissues), nerves, adipose, fibrous and lymphoid tissues, histiocytes and, perhaps, mesothelium. Most other organs are much less homogeneous than the liver. Unfortunately, there have been relatively few critical studies to measure variations in chemical composition in small foci within specific organs, foci which represent specific parenchymal components. One of the reasons has been the requirement of relatively large samples for conventional analyses, samples of a size that have precluded selection of predominately one type of parenchymal component from such an organ as the kidney (e.g., the proximal tubules) or of a functionally defined focus in highly complex structures such as a brain (e.g., the hypothalmus). It is for this reason, in part at least, that the most careful, comprehensive analyses of organs and tissues have been made on large, relatively homogeneous ones such as liver, skeletal muscle, and heart. Generally, in these cases, when analyses from different (large) portions have been compared, these differences have been (understandably) small. As an example, studies carried out by the NBS in an analysis of human liver specimens showed but small variation among *lobes* of human liver (which are histologically quite similar). Thus representation at this level of resolution is quite adequate in the liver. As mentioned above, few organs are as homogeneous as the liver. Upon examining the hepatic *lobule*, however, which is the functional unit (on the order of several mm^3) one finds significant functional differences among peripheral, mid, and central portions. It would be remarkable if chemical analyses of these lobular portions did not vary significantly as well. This, of course, represents quite a different order of resolution.

4. Characterizing the Tissue Sample

I have described many complex problems that stand in the way of getting truly representative tissue samples, but have provided few solutions. Often there are no ideal solutions, only reasonable compromises. In the case of the kidney, for example, unless one uses highly sophisticated micro-analytical methods that allow inspection and selection of the specific tissue elements to be analyzed, it is not feasible to analyze *only* glomeruli or proximal convoluted tubules, or the like. However, if one wishes to analyze glomeruli *and* convoluted tubules, he can select the renal cortex as a sample; the renal medulla will provide a high concentration of Henle's loops; and the calyces, mainly collecting tubules.

If one cannot solve the sampling problem to complete satisfaction, one should at least be aware of the sample's limitations, and should characterize the sample in such a way that others, as well as oneself, will know what has been analyzed. Among other things, this allows interpretative evaluation of the result, also production of *comparable data* in subsequent experiments. This very important aspect has been addressed in detail in [3].

5. Selection of the Tissue Sample and Its Precise Characterization

The selection and collection of a "representative sample" first requires that the chemical analyst determine precisely what it is that he wants the sample to represent. This decision, as well as the subsequent selection process, usually requires specialized knowledge of an extent and degree comparable to that required for the chemical analyses itself. Thus the selection and characterization of most tissues specimens warrants consultation and close collaboration with an anatomist, or pathologist, or surgeon, or other appropriate biomedical scientist. The precise characterization of the sample should include its exact location in the organ, in the case of a non-organ tissue, precisely what region the tissue was collected from. For example, in the kidney—the upper pole of the left kidney, cortex only, excluding the capsule. In the case of a lymph node—one, apparently free from adipose tissue, from the left mid auxillary group. In the case of bone—cortex only, freed from periosteum, at the juncture of middle and upper thirds of the left femur.

Characterization of every tissue specimen should include histopathologic evaluation of sections taken from the margins or, in the case of samples such as a lymph node, a section from the middle of the specimen itself or, second best, sections of adjacent, similar lymph nodes. Such evaluation not only allows one to determine precisely the tissue elements one is analyzing, but whether or not the tissue is normal or represents the specific disorder or disease being studied. The tissue sections also provide the means to estimate the amounts and types of "contaminating" stromal tissues—assuming that the primary objective is analysis of parenchyma. If

warranted, the estimate of stromal substances can be made at least semiquantitative through planimetric measurements which, when gathered from several sections taken at appropriate intervals, can be extrapolated into reasonably accurate volumetric data.

In summary, regardless of whether or not the specimen represents precisely what is desired, it is very important to determine precisely what it *does* represent. This characterization is best accomplished by a well planned, multidisciplinary effort which considers variations in concentration and distribution of functional parenchymal units as well as the types and amounts of stromal elements.

6. Conclusions

No matter how much quality control goes into the analytical chemical procedures, if the tissue donor is not well characterized in the context of the experimental design, and the tissue sample doesn't correspond with what the analysis is supposed to represent, the result will not be meaningful. The data base of trace element composition of most human tissues (blood, liver, and muscle are exceptions) suffers greatly from this defect in quality control.

Because of the nonhomogeneity of most organs and many tissues it may be impossible to get the ideal organ or tissue specimen. With proper care, however, this problem can be minimized. In any event, it is possible to characterize the sample in terms of what it truly represents so that, at least, one knows precisely what has been measured.

7. References

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Technical Considerations for Sampling and Sample Preparation of Biomedical Samples for Trace Element Analysis

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Sampling and sample preparation procedures are to a large extent determined by the analytical method used since different methods vary in the amount of material required for analysis and in how this is pre-treated before being introduced into the measuring instrument. Judging from intercomparison studies conducted by the International Atomic Energy Agency (IAEA), the most widely applicable methods now in use are Neutron Activation Analysis, Atomic Absorption Spectrometry, and Inductively Coupled Plasma Atomic Emission Spectrometry, though the latter still seems to have insufficient sensitivity for many trace elements of biomedical interest. Common to all these methods is the problem of contaminating the sample before or during analysis. For many elements (e.g., As, Cd, Co, Cr, Mn, Mo, Ni, Pb, Sb, and V) sufficient control over contamination can only be achieved by the use of special tools and reagents, and by working in a controlled (dust-free) environment. Several important elements (e.g., As, Hg, Sb, and I) are subject to losses on drying or ashing, but can be recovered reliably if wet-ashed in a closed container such as a PTFE "bomb." For representative sampling it is almost always necessary to start with several grams of material, and to homogenize this, if the effects of sample heterogeneity are to be reduced to an acceptable level. Quality assurance procedures covering all these aspects are difficult both to define and to apply. However, much can be learned from the statistical evaluation of results for duplicate samples, and from a determination of the limit of quantitation of the analytical procedure.

Key words: analysis; analyte losses; biomedical samples; contamination; quality assurance; sample handling; sampling; trace elements.

1. Introduction

This paper discusses some of the technical considerations underlying sampling and sample preparation of biomedical materials for trace element analysis, leaving aside medical, legal, ethical and financial aspects. The two main classes of problems to be considered are 1) the avoidance of contamination (and its inverse, the loss of the element of interest from the specimen), and 2) the requirement to obtain an analytical sub-sample that is representative of the specimen to be analyzed. Quality assurance aspects are also discussed.

Technical solutions for many of the problems that arise have been available for several years, but unfortunately still appear to be ignored by many analysts working in this field judging by the poor agreement between the results reported by different laboratories for the same or similar materials [1,2].[1] Recent efforts to improve this situation are exemplified by the actions of official bodies such as the International Union of Pure and Applied Chemistry [3], the American Chemical So-

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[1] Figures in brackets indicate literature references.

ciety [4,5], and the International Atomic Energy Agency (IAEA) [6], which have developed relevant definitions and guidelines for data acquisition and quality assurance. If followed conscientiously, these would do much to help analysts identify and minimize some of the errors affecting their work.

What follows is the author's own personal assessment of some of the more important factors that analysts should consider when sampling and preparing biomedical materials for trace element analysis. The references quoted are not intended to be an exhaustive list but rather to be illustrative of the problems being discussed.

2. Choice of Analytical Method

Procedures for sampling and sample preparation are to a large extent determined by the analytical method used since different methods vary in the amount of material required for analysis and in how this is pre-treated before being introduced into the measuring instrument. The main problem, that of contamination, can generally be reduced by keeping the number and complexity of sample handling operations to a minimum. In this respect, Neutron Activation Analysis (NAA) is regarded by its proponents as an ideal method since sample preparation may involve nothing more than placing the specimen in an irradiation vial, and exposing it to neutrons; after this, contamination (unless with radionuclides) has no effect.

Other common trace analysis methods such as Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) may also sometimes be used directly without sample preparation, particularly for the analysis of biological fluids. In general, however, other steps are also involved, each of which should be considered as a potential source of error, particularly of contamination. Examples include drying, ashing, grinding, dissolution, mixing with internal standards or spikes, and/or addition of reagents to effect a chemical separation or to reduce interferences. Each step involves its own special problems. Of fundamental importance for the analyst is that he should have a thorough understanding of the whole procedure and an awareness of the analytical errors that may be introduced at each step.

Although the number and complexity of sample handling operations is an important factor in evaluating an analytical technique, the most important criterion has to do with whether or not it is appropriate for the element and matrix of interest, i.e., whether it can deliver results of acceptable accuracy and precision. Most analysts prefer to draw their own conclusions on this point, though useful guidance is offered in the literature [7–10]. Table 1 identifies methods recommended by a working group sponsored by the American Institute of Nutrition [9]. Similar guidance (table 2) has been offered by an Advisory Group of the IAEA [10]. Experimental confirmation of the latter's predictions is provided by the results of several recent intercomparisons organized by the IAEA [11]. However, the following exceptions should be noted. In practice, NAA is rarely, if ever, used for the determination of F, Ni, or P; AAS is rarely used for Sb, Se, or Si; ICP-AES is rarely used for Si; and mass spectrometry is rarely used for Cd, P, or Sb.

Summing up, it would appear that the most widely applicable method for trace element research is NAA, which is commonly used for 23 of the 26 elements listed in table 2. Then follows AAS, which is widely used for 19 of these elements. ICP-AES is applicable to only nine elements, most of which, in biomedical materials, occur at "minor" rather than at "trace" levels. Mass spec-

Table 1. Methods recommended for various elements [9].

Instrumental neutron activation analysis (INAA)	Radiochemical neutron activation analysis (RNAA)	Flame spectrophotometric emission and absorption	Electrothermal atomization atomic absorption spectrometry (ETAAS)	Inductively coupled plasma emission (ICP)	Hydride generation-atomic absorption	Electrochemical analysis
Fe	Cr	Ca	Cd	Cu	Se	Cd
Se	I	Cu*	Cr*	Cd*	As*	Cu
Co	Mn	Fe*	Cu	Fe	Hg	Pb
Ca	Mo	K	Fe	K		
Cl	V	Mg	Mn*	Mg		
	As	Na	Ni	Mn*		
	Fe	Zn	Pb	Na		
		Li	Al	Ni*		
		Al		P		
				Pb*		

* May not be acceptable for those elements by that technique in all types of samples.

Table 2. Analytical methods applicable to four biological materials (blood serum, muscle, liver, and a plant material, kale) with precisions better than 10% relative standard deviation (ref. [10], p. 367).

	Analytical method *										
Element	A-1	A-2	A-3	C-1	C-2	E	M	N-1	N-2	N-3	X
As		x					x		x	x	
Be											
Ca	x	x		x		x	x	x	x	x	
Cd		x					x		x		
Cl				x	x			x	x		
Co		x							x	x	
Cr	x		x						x		
Cu	x	x	x		x	x	x		x	x	x
F									x		
Fe	x	x	x	x	x	x	x	x	x	x	x
Hg		x						x	x	x	
I									x		
K	x			x		x			x		
Mg	x			x		x	x		x	x	
Mn	x	x	x			x	x		x	x	
Mo		x	x				x		x	x	
Na	x			x		x		x	x		
Ni		x	x						x		
P		x		x		x	x		x		
Pb		x	x		x						
Sb		x					x		x	x	
Se		x	x					x	x	x	
Si		x	x	x		x	x		x		
Sn		x							x		
Tl		x							x		
U									x		
V							x		x		
Zn	x	x	x	x	x	x	x	x	x	x	x
	9	18	10	9	5	10	13	7	26	12	3
28		22			11	10	13		26		3

* A-1: atomic absorption spectroscopy (AAS)—flame atomization with direct nebulization of aqueous sample solutions
A-2: AAS—flame atomization using special sample techniques, or after preconcentration and separation of the analyte
A-3: AAS—electrothermal atomization in graphite furnace
C-1: chemical methods
C-2: electrochemical methods
E : emission spectroscopy—inductively coupled plasma source
M : mass spectrometry—spark source
N-1: neutron activation analysis (NAA)—instrumental methods
N-2: NAA with single-element separation
N-3: NAA with group separation (simple scheme involving removal of alkali metals, halogens and phosphorus)
X : X-ray analysis—PIXE

trometry is also applicable to a fairly wide range of trace elements, but only a few research centres appear to have access to it.

A word of warning. Not only is it necessary to choose an appropriate analytical method, but also to use an appropriate *version* of the method. For example, with NAA, some analyses can only be performed reliably if the radionuclide of interest is separated radiochemically before the activity measurement. Similarly, with AAS, a chemical separation may be necessary to overcome matrix effects reliably.

3. Reduction and Control of Contamination

For most biomedical materials, significant contamination can be introduced right from the very moment of taking the sample. This is not usually a problem for elements occurring at concentrations of a few parts per million or more, such as Cu, Fe, and Zn in liver. However, many trace elements of biomedical interest, such as As, Cd, Co, Cr, Mn, Mo, Ni, Pb, Sb, and V, often occur at much lower levels, and their values are therefore more easily disturbed by contamination. For such elements it may be essential to work in dust-free conditions and with ultra-pure reagents, and to use non-contaminating tools and containers such as titanium knives, and PE, PTFE, or quartz specimen vials, which should be rigorously cleaned before use. All of these aspects of the subject have been discussed extensively in the scientific literature [12–27].

The use of class 100 laminar flow clean air benches, or rooms, is now becoming the norm in trace element work [23]. Whether or not such conditions are essential is, however, open to question. Lievens et al. [28] measured dust fallout in a normal laboratory and found values for many elements below 1 ng cm^{-2} day^{-1} (for As, Co, Cu, I, Mn, Sb, and V even below 0.1 ng cm^{-2} day^{-1}). At such low levels, contamination from dust can hardly be a significant risk factor except possibly for the determination of Mn in blood serum [29] and Pb in many biomedical materials [13]. Even for such difficult analyses, normal clean conditions with a filtered air supply (but not necessarily class 100) and an overpressure of 2–4 mm water column are almost certainly adequate [13].

The availability of class 100 conditions may even lull the analyst into a feeling of false security if the analyst is not aware that the placement of equipment, and activities of laboratory personnel, can cause turbulences which drastically affect the performance of clean air installations. Dust carried into or created in the laboratory by personnel may be a much more potent source of contamination than the background levels in the laboratory itself [19]. An important feature of good laboratory design is therefore not just that clean air be provided but also that the pattern of air flows in the laboratory be arranged so as to protect the sample from cross contamination arising from other samples or from the laboratory personnel [23].

Most biomedical specimens are collected, not in a clean laboratory, but in a normal room for treating patients or conducting autopsies. Obviously, such rooms should be of a reasonable standard of cleanliness. However, there is no real need to demand class 100 or similar conditions, particularly if a sufficient amount of material is taken to permit removal of the possibly-contaminated surfaces at a later stage.

Whether tissue specimens should be treated before analysis to remove residual blood is a question that has so far not received much attention. Most workers consider it is sufficient just to remove *excess* blood, for example by blotting with ashless filter paper [30].

The contamination problems encountered in collecting tissue specimens or biological fluids using stainless steel tools such as surgical blades and blood collection needles are now well recognized [24,27,31] particularly for elements such as Cr, Co, Mn, Mo, and Ni. This is the principal reason why analysts have been recommended to use alternative materials such as titanium for knives and polypropylene catheters instead of metal needles. For some kinds of sample, however, conventional tools appear to present no particular hazard [32], and may even be acceptable for "problem analyses" such as chromium in blood serum if they are appropriately treated before use such as by siliconing [33].

In any case the careful analyst does not permit himself the luxury of assuming contamination to be insignificant unless good evidence exists for this. If necessary, exhaustive tests should be made of every sample handling operation, and of every tool and reagent used. Such investigations are all the more necessary if complex sample preparation procedures are used, as for example in biochemical fractionation studies [31,34].

4. Representative Sampling

Biological aspects of this problem are discussed in detail in the companion paper by Hopps [35]. Here the author would only like to draw attention to the fact that the problem of obtaining a representative sub-sample is also dependent on the analytical method chosen since different methods vary in their requirements regarding sample size. This may typically be 0.5–2 g (dry matter) for ICP-AES, 0.1–1 g for flame AAS, 0.1–0.5 g for NAA and 0.01–0.05 g for electrothermal AAS. X-ray methods such as PIXE may only require a few mg or less. Very few biological materials, however, are homogeneous for sample sizes as small as these.

The mathematical basis of sampling theory is discussed in many publications [5,36–38]. An important concept is that of the sampling constant, K_s, which is the mass (in grams) of material needed to reduce sample heterogeneity to 1%. This is related to R, the relative standard deviation (%) of the measurement, and m, the mass (in grams) of sample taken for the measurement, by the relation $m = K_s/R^2$.

Very little information is available from the literature on sampling constants for different tissues. According to Heydorn [8] it is around 200 g for trace elements such as Fe, Se, and Zn in human liver and kidney medulla, though much less than this (<10 g) for heart muscle. For Na in human kidney it is about 35 g [37]. The implication of these figures is that, taking $K_s = 200$ g as an example, one would need a sample size of 8 g to reduce sample heterogeneity to 5% RSD, and much more if one wanted the heterogeneity to be better than this. Obviously, these are much larger amounts than are normally used for a single analysis. It therefore follows that, for representative sampling, the analyst should start with a large amount of material, such as 8 g, and homogenize this to produce a suitable sub-sample for analysis.

Fortunately, suitable methods are available for performing such homogenization without at the same time introducing significant contamination. For small amounts of material (up to a few g), the brittle fracture technique applied at LN_2 temperature is effective even for difficult-to-homogenize materials such as hair and bone [39]. For larger amounts (up to a few 100 g) the same principle can be applied using a PTFE disc mill [40].

5. Analyte Losses

Much of the foregoing is concerned with inadvertent addition of elements to the sample. The inverse of this problem is the loss of analytes due to inappropriate storage or sample handling operations. Sample storage before analysis presents special problems as discussed in the companion paper by Kemper [41]. Among the operations most likely to result in a loss of the analyte are ashing and drying.

This subject has been reviewed extensively by Iyengar and Sansoni [16], and more recent data are available in the work of Pietra et al. [42]. Many of the investigations to test for losses on drying or ashing have, however, been done with radioisotopes and are therefore of somewhat uncertain value since there is no guarantee that the tracer will behave in exactly the same way as the non-radioactive forms of the element, which may be quite different chemical species. Nevertheless, there is good evidence that naturally occurring forms of elements such as As, Sb, Hg, and I may be lost in significant amounts from some matrices not only when the sample is ashed at a high temperature ($\geqslant$400 °C) but also under the milder conditions of oven-drying or even freeze-drying.

The latter method, however, has in recent years emerged as the way preferred by most analysts to preconcentrate their samples before analysis, and is probably safe for most elements. Even for "problem elements" such as mercury, it may be safe for many kinds of specimen [43]. Prudence requires, however, that if there is any possibility of losing an element during a drying or ashing operation, then these steps should either be avoided or should be carried out in a closed system that prevents any loss from occurring. A solution to this problem can be found in the use of PTFE "bombs" which have come into widespread use in recent years. These appear to offer a very convenient and reliable means for wet ashing biological materials without losses of trace elements [42]. Mercury, however, may still be a problem due to adsorption on PTFE surfaces, but can be reliably recovered if a suitable closed quartz digestion vessel is used instead [44].

6. Quality Assurance

Quality assurance procedures for sampling and sample preparation are difficult both to define and to apply. The main requirement is simply to follow the rules of good laboratory practice, which can be summarized as: 1) work in a clean environment, 2) use specially purified reagents, 3) select tools and containers with the lowest possible impurities, and 4) be constantly aware of your own effect on the blank.

As regards quantitative measures of analytical quality, much can be learned by the statistical evaluation of results for duplicate samples together with a detailed investigation of the individual sources of error in the whole analytical procedure. This technique has been developed primarily by Heydorn, who defines a test statistic T, with a χ^2 distribution, which is derived from the results of duplicate analyses and their respective errors [8,45–46]. Results from successive duplicates can be accumulated. Departures from acceptability only show that something is wrong, and that some unknown source of variability is operating. However, Heydorn has shown that this is a sensitive test, which can be very helpful in identifying problems such as unexpected contamination [29,47].

Current rules of good laboratory practice [5] place emphasis on the study of the analytical blank, in particular its standard deviation, since this is the primary determinant of the limit of detection of the analytical procedure (defined as three times the standard deviation of the blank) and the limit of quantitation (10 times the standard deviation of the blank). If actual measured values in samples are less than the limit of quantitation, reliable results cannot be obtained until the analytical blank has been reduced further. The determination of the limit of quantitation (to the extent that this can be done realistically) is thus an important means for investigating whether sample preparation has been performed satisfactorily.

There are, however, some problems in applying this concept. For example, in NAA, the analyst normally just takes an empty vial, and defines the blank as the amount of the analyte that can subsequently be extracted from it. Alternatively, some analysts just report the spectrum blank, based on the background under the photopeak of interest. Both of these, however, are only components of the total blank. They ignore, for example, the contamination arising from other sample preparation steps such as drying and homogenization.

In principle, there is a simple way to test for such errors, namely be preparing and analyzing samples of different weights. If the blank is zero, then the regression line for the plot of measured amount of analyte versus weight of sample should go through the origin [48]. Alternatively, if the blank is different from zero, it can be estimated from the intercept of the regression line at zero sample weight.

7. Conclusions

Technical solutions are now available for most of the problems that arise during sampling and sample preparation of biological materials for trace element analysis. Perhaps better solutions can still be found. The lack of them, however, is certainly not the main reason for the large discrepancies that can still be found in the scientific literature between results reported by different analysts for the same or similar materials. Reliable data can only be obtained if the analyst is made to be aware that serious errors can be introduced at almost every step in the analytical procedure. Quality assurance is therefore, in the first place, mainly an attitude of mind. In helping to draw attention to these problems, and offer practical solutions to them, the organizers of this workshop have taken an important initiative, and it is hoped that their efforts will be rewarded with success.

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Environmental Specimen Banking

The Selection, Collection, Transport, and Storage or Biomedical Samples

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In order to adequately ensure the protection of human health and the environment from the thousands of presently suspected hazardous substances and the new compounds added to those by new industrial processes, sophisticated approaches to hazard assessment and monitoring are being established. Environmental specimen banking (ESB) is necessary, useful, and important for environmental monitoring currently, and in the future for monitoring the past. ESB has already proved a good tool for recording inorganic and/or organic pollution trends over the years. Moreover, ESB offers the possibilities and potentials for retrospective analysis of authentic samples from the past by improved future analytical procedures, including the detection of presently unnoticed environmental chemicals of biological interest. Among the specimens representing the environment, specimens of human origin play a key role. The selection criteria for human specimens include ethical and legal considerations together with the appropriate scientific approaches and epidemiological criteria. Technical considerations for sampling, preparation, transportation, and storage of the specimens include the selection and development of specific materials and implements, cold storage, and clean room technology in order not to compromise the original composition of the sample.

Key words: biomonitoring; environmental monitoring; environmental specimen banking; human specimens; monitoring human exposure.

1. Introduction

Ever since the industrial revolution, man has been subjecting the earth's biosphere to an increasing variety of chemical insults. Some, such as naturally-occurring toxic elements and compounds, are reentering the environment via industrial processes at rates much greater than their natural degradation or removal from the biosphere; other hazardous pollutants are being synthesized that have molecular structures never before encountered by living organisms. During the last decade, however, increasing attention has been focused on the occurrence and effects of pollutants in the environment. Refinements in analytical techniques have resulted in the discovery that many substances are geographically more widely distributed than previously assumed. Serious accidents with exposure to and accumulation of toxic materials have lead to a public demand for more effective governmental control.

A list of all presently suspected environmental hazardous substances would contain thousands of chemical compounds; and industry is adding new compounds to that list every year. Up to now we are not in the position to analyze environmental samples for all 70,000 or so man-made chemicals and their metabolites circulating in the environment today. In order to adequately ensure the protection of human health and the

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environment from the often subtle effects of these materials, it is necessary to perform several major activities. These include toxicological research, control technology development, the promulgation of regulatory guidelines and standards and monitoring of environmental materials and specimen banking. There are at least three vital services which a biological specimen bank can render:

1) Provide up-to-date information on the spread of man-made chemicals in the environment, including man;
2) Allow extrapolation of concentration trends with respect to chemicals considered to present threats to man and his environment; and
3) "Preserve the presence" by "fossilizing" selected environmental specimens in a repository so as to be able to analyze them for specific chemicals scavenged in past years.

These major activities are extremely important because without an effective monitoring of environmental materials and specimen banking the detection of serious environmental contamination from pollutants may occur after critical damage has been done.

Numerous environmental pollutants must be classified as health hazards for man; e.g., domestic poisons (detergents, pigments, propellants), naturally occurring poisons (plant or animal toxins), food components (additives, bacterial toxins, nitrosamines), stimulants (nicotine, ethanol, drugs). Human populations are increasingly exposed to substances that are potentially detrimental to health. Many of these substances are already found in human tissues, fluids, secreta, and excreta, and some tend to concentrate in specific parts of the human body.

Biological monitoring programs can provide direct evidence of exposure and must therefore be implemented to supplement physical and chemical monitoring programs in areas where these are already in existence. Specimen banking will be of great importance in analyzing trends in exposure to previously unrecognized harmful pollutants or pollutants for which current measurement techniques are inadequate. Biological monitoring and analysis of stored specimens can provide a warning for the initiation of remedial measures before critical damage occurs.

From the toxicologist's and pharmacologist's point of view, it is of interest in connection with xenobiotics to look for the target organs, the possibility of accumulation, and the hazards of exposure. The results of analytical work in real time monitoring as well as long-term trend analysis are the basis for medical estimation of the ecotoxicological risk concerning man's life.

Moreover, possibilities of conversions of pollutants in different systems must be taken into consideration; these conversions may result in substances with increased or lowered toxic qualities, e.g., for mercury and organohalogenated hydrocarbons.

2. Goals and Objectives of Environmental Specimen Banking

At present the number and variety of environmental chemicals are such that a systematic and all-encompassing determination of their ecotoxic behavior and discernible effects is all but impossible. Neither is it feasible to account for their circulation and accumulation in the environment. Furthermore, it seems self-evident that the same must hold for their decomposition products and metabolites.

In order to obtain more realistic information about the threats posed to man and his environment by environmental chemicals, however, it seems logical and highly desirable to consider the systematic and repetitive collection of such environmental specimens which are known to scavenge and accumulate hazardous man-made compounds from their respective environment. Systematic and repetitive analyses over time of comparable samples will yield three most important types of information with respect to environmental chemicals already recognized or believed to be harmful:

1) Real time information as to the distribution of manmade chemicals and perhaps some of their decomposition products in the environment;
2) Trends with respect to increasing threats posed by certain environmental chemicals believed to be deleterious to the environment, including man; and
3) The long-term preservation of aliquots of such samples which were originally analyzed for mapping out the present-day distribution of known harmful chemicals and interpreted to determine any trend as which may exist. The possibility of analyzing well preserved samples ("fossils of the past") when need arises in the future for the clarification of specific problems will help greatly to develop action plans as they will then be required and also to improve public policies.

Having recognized the potential importance of environmental specimen banking, the Government of the Federal Republic of Germany began developing plans for exploratory research late in 1974. An important impetus

was provided by several ongoing R&D activities in the U.S. which were initiated in 1971 and by German research proposals based upon these. Under U.S.-German Environmental Agreement of 1974, detailed plans began to take shape in 1975 for a joint R&D effort. The German decision for funding nine selected projects was reached in January 1976. In its official Report on the Environment, dated 14 July 1976, the Government of the Federal Republic of Germany has specifically stated its intention to support the preparatory work toward establishing an environmental specimen bank (ESB). The German research program concentrates at present on the intake and metabolism of selected groups of environmental chemicals recognized to be harmful (e.g., group-fingerprint analyses with multiple apparatus link-ups) and on basic research regarding sampling techniques, container materials, and storage techniques. The expansion of the German research efforts is currently being considered. The German environmental specimen bank pilotprogram is coordinated, developed, and evaluated by the Federal Environmental Agency. Funds are provided by the Federal Ministry for Research and Technology through an interagency agreement. Findings from a considerable number of R&D projects from the UFOPLAN (Environmental Research Plan), administered by the Federal Environmental Agency (some 500 current projects), as well as from pertinent R&D activities sponsored by the Federal Ministry for Research and Technology, are also utilized.

The Environmental Specimen Bank and the parallel Data Bank, both situated at Muenster, started operation in mid-1980; a further bank was inaugurated in 1981 at Nuclear Research Center (KFA) Juelich.

3. Selection of Specimens

The scientific, logistical, and technical problems associated with the development of selection criteria, biological sampling, and specimen banking programs are generally recognized as being exceedingly profound and complex. Systems ecologists, toxicologists, physiologists, pathologists, molecular biologists, analytical chemists, and other participating scientists all have differing perspectives to bring to bear. Thus, the essential multidisciplinary nature of environmental monitoring and specimen banking will require maximum understanding, considerable forbearance, and some compromise among contributing disciplines.

Among the specimens representing the environment, specimens of human origin play a key role. Hence, the analytical results from these could be used for a critical (eco-)toxicological evaluation. Thus, the selection of human specimens should be made very carefully and should be ecologically meaningful with regard to the area of collecting samples.

According to the above-mentioned suggestions and in regard to the selection and characterization of a "best" human sample for monitoring and banking, the following summarized factors, some more obvious than others, should be considered in attempting to meet objectives:

- sex
- age
- ethnic origin
- social and economic level
- past and acute health status (including available informations on children's diseases, operations, clinical chemistry etc.)
- past and acute medication (kind and frequency)
- past and acute nutritional status
- smoking habits (kind and frequency, also former habits)
- drinking habits (kind and frequency)
- history of employment (including if possible, the nature and concentrations of the pollutants to which the donor has been exposed)
- use of cosmetics
- hair color and skin status
- available further data

There must be a differentiation between (parts of) human organs to be obtained in real-time monitoring (RTM) and those for ESB. In RTM, whole blood (serum), urine, saliva, hair of different body regions, and nail material can be obtained, and if necessary and advisable, stored under appropriate conditions. In addition, for ESB, human organs are wanted that represent not only a momentary exposition but also long-term body burden of the analyzed chemicals. Materials for those specimens are available from autopsies and, incidentally, from surgical operations.

Joint working groups of the U.S. Environmental Protection Agency and the Federal (Republic of Germany) Environmental Agency have decided that suitable human organs of first priority for ESB are liver, adipose tissue, and whole blood. In addition, the storage of kidney, brain, and placenta was proposed. Within the pilot phase of ESB in the FRG, we collected and stored 19 different organs.

Generally it must be indicated that considerable thought must be given to establishing criteria for selection of biological specimens for biological specimen banking in aquatic and terrestrial ecosystems. Major considerations in selecting or recommending sensitive species or tissue sampling for biological specimen banking revolve around a set of broad biological problems including but not limited to the following points:

- importance to man and environment
- position in the food chain
- availability and collection costs

An important fact for the choice of different kinds of mussels is their worldwide availability and the extensive experiences regarding bivalves as an indicator of water quality. The carp and the buttfish can be used as a predicator for the constitution of sediments and water. This is important as long as sediments are not tested directly also. The macroalgae are interesting because of their ability to accumulate heavy metals. The sludge was chosen because it is a very important indicator for industrial and urban pollution. A German basic research study found no great differences in the composition of sludges taken from various areas.

In the area of food chain, the combination wheat/soil is of the same priority as cow milk. Here it should be seen that wheat is spread all over the world as important corn and that the concurrent investigation of the soil can yield important information. Carabids and earthworms were selected as known indicators for the quality of soils and the front soil-atmosphere. In the area of air quality, the grass cultivation should get the first priority. For many years extensive experiences have been gained in Nordrhein-Westfalen about grass cultures as indicators of air pollution.

Because of the importance and the heterogeneity of samples of human origin, it was recommended that more than the average number of samples be taken from liver, blood, and fatty tissue. On the other hand, the number of samples taken from sludge can be limited because of the similar composition which is nearly independent of origin.

- distribution and abundance
- mobility and action radius
- accumulation of environmental pollutants

These factors and perhaps some further similar ecological considerations must be addressed as criteria along with the physical-chemical factors of collection methods, transportation, analysis, storage, and cost considerations.

In regard to these suggestions and the environmental specimen pilotbank the following bioindicators were discussed and taken in priority:

1) human tissues
   - liver, blood, fatty tissue
   - kidney, brain, placenta
2) aquatic ecosystem
   marine
   - macroalgae
   - Mytilus spec. Ostrea edulis, Pleuronectes fresh
   - Dreissena polymorpha, Cyprinus carpio, sludge
3) terrestrial ecosystem
   - wheat/soil, cow milk, Carabids, earthworm, grass culture.

The following considerations influenced these decisions: The liver has to be prefered to the kidney because of the size of organ and because of its homogeneity as shown by trace element distribution studies. The fatty tissue and the brain are interesting for the lipophilic compounds. Because of ethical and legal considerations the fatty tissue should be preferred. For the "Real-Time-Monitoring-Program," milk, whole blood, hair, skin, urine, are suggested because they are more available.

4. Technical Considerations

In the sampling, preparation, transportation, and storage of environmental materials and specimens for monitoring and bank programs, certain strict precautions must be taken to obtain adequate information relative to the purpose of the study and the substance to be measured. Analytical chemistry begins when a sample is taken; the analyst should be fully involved from this point. Contamination of samples and loss of volatile pollutants during collection and following steps may be more of a problem in some cases than others. Specific risks of contamination and loss are described in the following.

The wide spread of reported values for both organic and inorganic substances (especially in the ng/g level) can be derived from uncritical application of analytical techniques as well as from "positive" or "negative" contamination.

Contamination and loss problems start with collection of environmental materials and biological specimens. Sampling in areas remote from laboratories requires certain strict precautions. Samples that have been subjected to prolonged transportation under adverse conditions may be useless and collection should therefore be restricted to those samples that can be adequately preserved in the field. Speed is of the essence if one wants to minimize the errors in estimates of pollutants within organisms that are obtained in the field. No chemicals may be used to collect environmental materials and specimens. Samples should be collected in an adequate, noncontaminating manner (e.g., shocking, trawling, and trapping).

Special cleaning procedures for materials and instruments used for sampling are necessary, as well as special sampling techniques to avoid contamination of the

sample by pollutants from the environment during sampling environmental materials and specimens intended to be examined for inorganic pollutants. Similar considerations may be applied to knives, scissors, and other instruments necessary for organ dissection. Of course there are plastic or glass knives and scissors, but no guarantee is given that they do not contaminate the sample perhaps more than the metallic instruments. The recommended procedures for obtaining samples free from contamination include the use of quartz or borosilicate glass instruments, or those made of titanium, titanium carbide, boron carbide, carbon steel, or aluminium.

Titanium knives have been used with the result of no contamination except for titanium. They may be easier to use than the other types of instrument. Contamination by copper, manganese, chromium, nickel, iron, cobalt, silver, tin, antimony, and tantalum have been demonstrated by several authors when using stainless steel instruments. Recently the use of lasers has been proposed for cutting biological tissues, but laser cutting might produce excessive heat at the cut surface and potential losses of volatile organic and inorganic compounds or pyrolytic changes.

For organic compounds, instruments and other materials made of organic compounds that come into contact with the sample must be avoided (plastic gloves should be used with caution). Studies requiring data on the distribution of a substance within a tissue would, of course, involve the preservation of the intact tissue or whole organ. Homogenization of the specimen may be employed in instances where a substance is inhomogeneously distributed or where replicate analyses are to be carried out by different labs. However, great care must be exercised with the homogenization procedures to avoid contamination.

All specimens should be transferred to permanent storage containers as soon as possible and handled as few times as possible bearing in mind that each additional handling enhances the possibility of introducing contamination.

For transport and storage, the container materials are also of great importance, e.g., to avoid migration from the specimen into the wall of the container or contamination from the container. There have been numerous studies performed in the past to find out the most suitable materials that will be inert and will neither lead to migration effects nor catalyze unwanted chemical reactions in the sample. It has been shown that glass (borosilicate glass) gives the needed safety. However, problems may arise due to a lack of reliable methods of sealing. For the estimation of inorganic compounds, plastic material may also be useful, but should be cleaned very carefully, e.g., with diluted acids prior to use. This is necessary because of the observation that inorganic residues of this material can be leached during this type of cleaning.

To preserve the integrity of samples, all possible types of storage were carefully investigated, resulting in the following conclusions:

1) Chemical preservation is not possible as most organic compounds are affected.
2) The possibilities of using irradiation are under investigation but seem not to be advantageous.
3) Lyophilization can obviously be used for those samples destined for the estimation of inorganic trace elements compounds, though the loss of highly volatile elements like mercury seems to be possible.
4) The storage of extracts may be an advantage only in atmospheric samples; further studies are necessary.
5) Ashing at low temperatures could probably be used for trace metal pollutants but will be limited to certain elements.
6) Rapid deep-freezing is the most acceptable procedure for maintaining sample integrity and preservation of contaminants.

It has been shown in our own investigations that deep-freezing provides good stability of the sample regarding both inorganic and organic contents (contaminants). Storage temperatures should be at $-80\,^{\circ}C$ or lower. Thus, walk-in deep freezers, as well as liquid nitrogen freezers or containers, can be used and have been proven to be preferable for long-term storage. Storage under these conditions makes it necessary to limit the sample size in order to avoid "transport" effects within the sample. Also, the thawing process must be the subject of strict precautions.

Appropriate and uniform sampling and storage protocols must be used by all participants in the program. Moreover, all techniques used in the storage of the specimens must be uniform. The technique of cryogenic brittle-fracture appears to be most suitable for the pulverization of frozen tissue.

5. Legal and Ethical Aspects

Most of the scientific community seldom thinks about the legal aspects of the conduct or communication of science. Aside from issues of patent or publication rights, only the occasional scientist, even in a regulatory agency, becomes familiar with water law, conservation law, administrative law, or courtroom procedures pertaining to the uses and limits of science in formulating

public policy. Yet, all humanity and human endeavor exist and function subject to governance by the law.

The law is a creature of its community, a creature of man-made protocols, and is itself quite admittedly unable to reach conclusions in any area other than law per se. Therefore, in the interaction of science and the law it is of paramount importance to understand that at any time and under any circumstances, science remains science—with all of its own community standards of self evaluation and judgment—and the law too remains a system into itself. Thus law cannot make good science bad, nor bad science good. Therefore, the scientist need not fear it and, in fact, need only look to the law for governance in nonscientific aspects of scientific work.

We in science from time to time have had to consider the legal aspects of field and laboratory studies as they have been used for regulatory purposes. The information that we offer here represents not legal counsel but rather relates to the experiences of scientists helping to formulate public policy or to enforce existing law, and offers some afterthoughts about them.

There are differences among various countries and sometimes between states within the same country, with respect to the regulations, customs, and restrictions that must be considered in planning and implementing programs for the collection, storage, and analysis of biological specimens. One must especially distinguish between human and other biological specimens.

Experience with Western European and North American countries participating in the studies of the Organization for Economic Cooperation and Development has indicated that, with respect to data obtained from wildlife sampling and analysis could be limited by laws protecting wildlife, but difficulties arising from such laws are unlikely in view of the need to choose species which are abundant. However, there may be restrictions (e.g., closed seasons) on the capture of some bird or fish species.

Storage of specimens within the country of origin should not present difficulties, but any requirements to transport specimens across frontiers for analysis or specimen banking may infringe on laws of import and export or on disease control enforcement.

A broad review of the regulations, customs, and restrictions that must be considered in planning and implementing programs for the collection, storage, and analysis of nonhuman biological specimens is given in the proceedings of the International Workshop on Monitoring Environmental Materials and Specimen Banking, held in Berlin in 1978. During the International Workshop in the Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants, held in Luxembourg in 1977, a summary of legal and ethical aspects in respect to human biological specimens was given.

As mentioned above there are differences among various countries in this special field. For example in some countries regulations require a formal institutional committee review to ensure proper safeguards in the health of subjects, informed consent, and confidentiality of data. Each responsible investigator must be cognizant of and proceed according to the customs and legal restrictions applicable to his country and/or any collaborating nation. The investigator may find it desirable to seek legal advice before starting the program. It is generally accepted and should be recalled, above all, that the Nuremberg Code of 1947 precludes any experiment on a human subject without his "voluntary consent" and this principle has remained absolutely unchallenged. More recently the term "informed consent" has become widely used. Such consent for biological monitoring may be given, in particular, when there is a suspected exposure to environmental pollutants which may be detrimental to the health of the individual.

While the format of the "informed consent" to be used may vary from country to country, with the circumstances and the types of indicator specimens to be collected, the following basic elements of information should be considered for inclusion in "informed consent":

- procedure to be followed for taking the biological specimen
- description of any attendant discomforts and risks which might reasonably be expected for the individual and the community
- an assurance that the data and results will be kept confidential but that the individual (and/or his physician) will be informed, if he/she wishes, of the results with comments regarding their significance.
- in the case of a study requiring repeated sampling, an instruction that the person is free to withdraw his consent and to discontinue participation at any time without prejudice to the subject.

In the case of collection of post mortem specimens, the concept of "informed consent" should be replaced by "permission of next-of-kin." This permission must be requested whenever appropriate and some information regarding the purpose of the study should be provided if requested. Usually permission to acquire post mortem specimens is more difficult to obtain than ante mortem specimens because of legal reasons, as well as local customs or mores. The need for collaborating pathologists to perform autopsies in the accordance with the laws of the country is emphasized. In conclusion, both science and law are evolving independent systems unto them-

selves. They do not change each other in their practice, but only in their evolution of thought. Thus, the sampling, archiving, and utilization of biological tissues never shall affect the practice of law, but may influence the development of present and future legislation concerning environmental protection, natural resource management, conservation, preservation, and other human benefits. Similary, passage of human law cannot affect the laws of nature, but it can influence the activities of science and scientists by providing positive and negative incentives to investigate selected subjects.

6. Conclusions and Recommendations

Environmental specimen banking (ESB) is necessary, useful and important for environmental monitoring currently, and in the future for representing the past. ESB has already been proven to be a good tool for recording inorganic and/or organic pollution trends over the years. Moreover, ESB offers the possibilities and potentials for retrospective analysis of authentic samples from the past by improved future analytical procedures, including the detection of presently unnoticed environmental chemicals of biological interest. Besides this, ESB is also of interest in supervising the effectiveness of restrictions, regulations, etc., because pollution problems are worldwide and include terrestrial, aquatic, and atmospheric contamination. The operation of ESB should be harmonized on an international basis.

In order to ensure the efficient and economical operation of specimen banks, sample collection, storage, analysis and quality control of data should be centrally located or at least coordinated for each program and the number of storage sites and analytical laboratories kept to a minimum.

The above activities should be harmonized internationally as much as possible. They should utilize the resources and experiences of closely related programs which already exist, e.g., the U.S.–German Environmental Tissue Bank Program, the U.S. Pesticide Monitoring Program and the Mussel Watch Program. All highly industrialized countries, and increasingly developing nations, too, have yet to come to grips with the complex problems of providing facts and figures for establishing ecological criteria as baselines for environmental impact assessments. This is as true for human installations and activities already impacting upon the ecosphere as it is for the prejudgment of the future ecological consequences of plans as yet to be implemented.

Major activities are necessary to adequately ensure the protection of human health and the environment from the adverse effects of environmental pollutants. These activities include toxicological and ecological research, control technology development, the promulgation of regulatory guidelines and standards, and the monitoring of environmental materials and specimen banking. In the absence of effective monitoring of environmental materials and specimen banking, the detection of serious environmental contamination from pollutants may occur only after critical damage has been done. The link between legal wants and scientific necessities must be found. Regulations must cover a maximum of safety and a minimum of scientific demands. Only in this way can a practicable "environmental protection" be established that considers both: man against environment and environment against man.

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Presampling Factors

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Choosing the right kind of samples from human subjects for trace element studies poses many difficult problems. First of all, due to practical considerations, specimens with clinical relevance are restricted to a few such as whole blood, hair, nail, urine, and faeces. Although autopsies provide access to collect various organs, their usefulness is restricted to monitoring type of activities and not for clinical diagnosis. Besides these basic differences one is also confronted with procuring "valid" samples for analysis. Validity refers to both analytical and biological aspects and the material collected should satisfy both the demands to make the specimen meaningful. In practice this is not a simple task because a number of presampling factors need to be taken into account. Significant situations among these are the biological variations, post mortem changes, intrinsic errors resulting from internal contaminations, etc. The impact of these factors alters the status of the sample and calls for adequate discription of the specimen. In the absence of a well defined sample protocol accurate characterization of the material will not be possible and renders the analytical effort worthless. Solutions to these problems should be sought at interdisciplinary level and effective team work is mandatory to make any meaningful progress in our endeavours to answer public health questions.

Key words: biological systems; biological variations; biomedical; human tissues and body fluids; internal contamination; intrinsic errors; precision and accuracy; presampling factors; post mortem changes; reference values; sampling; trace element analysis; valid samples.

1. Introduction

There is mounting concern among trace element researchers dealing with biological systems that the elemental composition data, especially for trace elements at lower concentration levels, are inaccurate [1-8][1]. Major efforts are therefore necessary to generate reproducible and reliable results to build up a reference data base for human tissues and body fluids. Unless this is achieved, true variations in elemental concentrations arising from physiological changes, pathological influences, and occupational and environmental exposures remain submerged in the wide ranges of the largely uncontrolled analytical data. This being the case, very little progress can be achieved in applying the knowledge gained from trace element studies to practical problems in human health.

Several literature surveys on the elemental composition of biological systems have exposed the fact that reported data vary over a very wide range [9-10]. This feature is very effectively illustrated when analytical results for the same test material are pooled from various laboratories [11]. Generally speaking, the sources responsible for this kind of a situation in the reported information can be broadly classified into two groups as illustrated in figure 1: presampling factors and analytical and data handling errors. Identification of anal-

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[1] Figures in brackets indicate literature references.

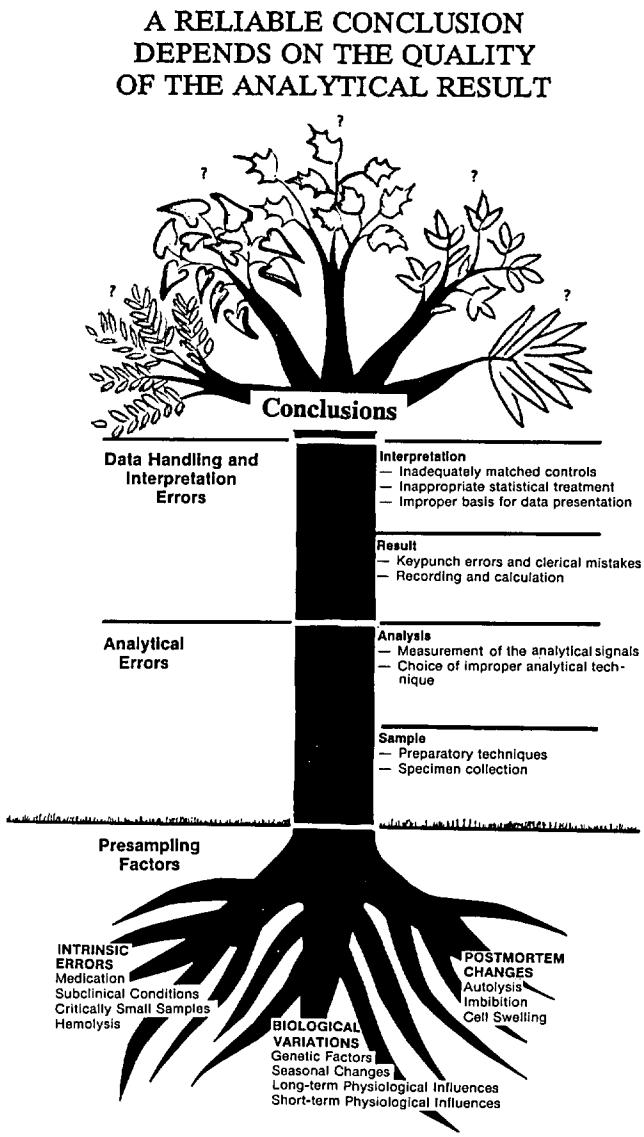

Figure 1–The elemental analysis of biological systems.

ytical errors mainly arising from choosing an inappropriate methodology or paucities due to sampling and sample handling have received considerable attention by the analytical community especially in the past few years [12]. However, an understanding of the impact of presampling factors on the entire analytical sequence is sadly lacking and awaits immediate recognition since the "validity of the sample" itself becomes questionable if the influence of presampling factors is neglected.

Procurement of normal human tissues for elemental analysis is complicated by some inherent difficulties. Only a few samples, such as blood, excretory products, and occasional biopsy samples are obtainable from living subjects; most other samples must be sought at autopsy. Irrespective of the mode of collection, the samples are subject to the influence of presampling factors. The purpose of this report is to identify the special difficulties associated with biomedical systems destined for elemental composition studies. The main emphasis will be on the quality of the sample by elaborating the role of presampling factors, which have great effect on the overall accuracy of the analytical result but are yet to be fully appreciated.

2. Definition of Presampling Factors

Essentially, presampling factors may be defined as events associated with biological specimens in situ and before the arrival of the samples at the laboratory for analysis. A host of circumstances such as biological variations, post mortem changes, intrinsic and inadvertent errors arising from internal contaminations for specific elements, and situations such as preferential accumulation of elements in selected organs or even within an organ, etc., fall in this group. As illustrated in figure 1, a number of components are involved in the process of acquiring a biological specimen to study its elemental composition and emphasize the complexity involved in contrast to the situation dealing with static inorganic systems. Thus, there exist numerous pitfalls and shortcomings associated with presampling factors leading to erroneous result and conclusions in acquiring and intepreting elemental analysis data.

3. Biological Variations

These may be divided into genetic factors, long and short term physiological influences, and seasonal changes.

Genetic predisposition of human subjects belonging to different racial groups is an example in this context. However, because of the overriding influences of environmental and other factors, changes of elemental concentrations in body components directly attributable to genetic predisposition are somewhat difficult to identify. This has been illustrated in a recent survey involving Zn in serum covering black and white populations. The results showed that blacks generally tended to have lower serum Zn values than did whites but there were inconsistencies [13]. On the other hand, in animals, changes arising from differences in breed are well recognized. Commonly encountered examples include variations in the composition of I in cow milk [14] and Cu in the blood of sheep [15].

Long term physiological influences include age, sex, habit geographical and environmental factors, diet, pregnancy and lactation. The definition of long and short term influences is somewhat arbitrary since factors such as diet and smoking habits overlap. For example, habitual diet may be regarded as a long term effect,

while a single meal may be looked upon as a short term effect.

The age of the examined individuals is an important factor in elemental analysis studies since shifts in concentrations occur as a function of age. Zn in serum is a good example in this context. It has been shown that as a function of age, lowest values in serum are found in babies under one year, while the concentration reaches a peak around 20 to 40 years and starts to fall at the age of 50 and above [16]. Similar tendencies have been observed in the second National Health and Nutrition Examination Survey (NHANES II) in the U.S.A., involving 14,770 subjects and completed recently [13].

An example of differences due to sex is that of Fe in liver [17]. It has been shown that men have higher concentrations of this element than women. The recorded concentrations on dry weight basis are 913 and 700 micro g/g, respectively. It has also been shown that serum Zn levels in males is significantly higher than in females in several age groups with the difference being greatest in the 20-44 year olds [13].

The influence of geographical and environmental factors has been established in several cases. Elevated blood Pb levels in several urban areas of the world is an ideal example with gasoline considered as one of the main sources where unleaded fuel is not mandatory [18]. Other known examples in this context include a dramatic accumulation of various trace elements, in particular Sc, found in lung tissue from people living in Duisburg in Germany due to the environmental pollution from the steel furnaces operating in this area [19]. Populations living near Cu smelting areas have had greatly elevated As concentrations in urine than those living far away [20]. In a recent investigation dealing with human milk distinct geographical differences were shown for As, Mn, Se and Zn, among others, between Guatemala, Hungary, Nigeria, Phillipines, Sweden, and Zaire [18,21].

Several examples have been cited to record the influence of diet on the elemental composition profile of tissues and body fluids [22]. Some elements show profound effects as exemplified by I. When elemental and foods rich in I are consumed, the concentration of this element in blood and other tissues is elevated to very high levels and remains so for several weeks [23,24]. Recently it has been reported that consumption of dietary algae by nursing women in Japan elevated the concentration of I in milk ranging from 80 to 7000 micro g per L with the highest frequency at 150 micro g per L [25].

Smoking tobacco is a good example of the impact of habit on trace element picture. It induces variations in Cd level as seen from a number of studies; its level in blood [18,26], kidney [27] and placenta [28] is elevated.

Pregnancy induces several physiological and biochemical changes of which an increase of plasma volume by as much as 50% and a decrease of plasma protein up to 30%, are of great consequence from a trace element point of view. These changes partly account for the shifts observed in electrolytes and Zn, all of which are consistently low during pregnancy. The reverse tendency of elevated Cu in serum in pregnant women is well documented [29].

Concerning lactation, it is well established that both humans and animals show a large decline in the concentration levels of several elements with the progress of lactation and therefore, it is essential to define precisely the stage of lactation if comparison of results between different milk samples is desired. These aspects have been exclusively discussed by Iyengar in a separate report [30].

Short term physiological influences can be regarded as those which affect the system over short periods. Thus, circadian rhythm, recent food, fasting, posture and stress during or just before sampling can be grouped under this.

Circadian rhythm or the diurnal variation has been examined for electrolytes and for certain trace elements such as Zn, Fe, and F. In healthy adults on normal diet, the excretion rate for Ca and Mg is usually greater during the day than during the night. P and Na reach maximum in the evening, while K follows the opposite trend. A rapid rise also occurs in the excretion of Ca and Mg after a meal [31].

Concerning trace elements, although there is some disagreement with respect to the exact time of the day at which peak concentrations are reached, there is considerable information for a few elements. For example, there is clear evidence that Zn concentration of serum reaches maximum concentration in the mornings [13,32] and that Fe in plasma reaches high levels during the night and early morning [33]. A short term Fe rhythm in children showing variations up to 40% in two consecutive estimations within 30 minutes has also been identified [34]. F does not undergo any cyclic variation. The effect of circadian rhythm has also been observed for Cd in urine. On five of the seven days Cd was measured in seven subjects it was found that the concentration was highest between 10–11 hours [35].

The influence of recent and specific foods is also an important factor. Amino acids present in the foods may chelate some elements in the blood and remove them from the stream. This appears to be the case with Zn in serum which drops to less than 70% of its initial facting level within three to six hours following a large meal [36]. A recent comprehensive survey also reported the same tendency but to a lesser degree in a large group of subjects [13].

Recent dietary intakes can act as internal contaminants for certain elements. Ingestion of a specific food, namely fillet of plaice, elevated As levels in blood serum by more than an order of magnitude. Even after 12 hours its level did not return to fasting value [37]. This is important in the context of accepting overnight fasting values as normal levels. Another good example of the short and intermediate term influences from food is seen for the case of Hg in hair. In four groups of subjects consuming fish once a month, every week and every day, the concentration of Hg in hair was found to be 1.4, 1.9 and 11.6 micro g/g, respectively, thus distinctly reflecting the effect of the frequency of fish consumption [38].

Fasting before sampling and posture and stress during sampling also contribute to significant shifts in concentration levels of fluids such as serum. There is evidence to show that fasting values for Zn in serum are elevated [39]. A set of examples is shown in table 1 to illustrate the influence of "status at sampling" on the concentration of selected elements in serum and blood. Basically, this table reveals that samples collected under a given condition may not necessarily be suitable for carrying out multielement determinations and great care is needed in interpreting the variations observed for different elements in such samples. Furthermore, it is known that even changes from an upright to a recumbent postion affect the concentrations of plasma proteins inducing alterations in the concentrations of protein bound elements such as Zn and Se [40]. Stress, imparted through venous acclusion applied prior to venepuncture has been shown to increase plasma protein up to 30%

Table 1. Influence of "status at sampling" on the concentration of selected elements in human subjects: some examples.

Sample	Element	Condition	Influence	Remarks
Serum	Zn	Fasting	Elevated	Even overnight fasting
		After normal food	Lowered	During first few hours
		After high Zn food,	Elevated	e.g., Oysters, liver, etc.
		Low Zn intake	Lowered	Rather sudden effect
		Stress	Elevated	e.g., Standing posture
		Non-stress	Lowered	e.g., Recumbent position
		Pregnancy	Lowered	Progressive decline
	F	Fasting	Normal	Overnight fasting
		After normal food	Variable	If sampled immediately
		Tea consumption	Elevated	If sampled immediately
		Low F intake	Lowered	Over a few days
	I	High intake, e.g., sea foods, iodinated salts etc.	Elevated	Uptake is rapid but decline is slow since biological half life is 2.5 weeks
		Low I intake	Lowered	Over a few days
	As	Fish intake	Elevated	Well absorbed and slowly excreted
Blood	Cd	Tobaccao smoking	Elevated	Remains chronically high in smokers
	Pb	Alcohol consumption	Elevated	Especially wine drinkers

with concomitant increase in Zn value [41]. Such a situation might call for controlling the pressure used on the arm and using standard conditions in serial samples.

Seasonal changes can be grouped under two categories: physiologic and climatic. The combined influence of these two factors has been previously illustrated through various examples [22]. In humans, changes brought about by summer may be envisaged through recreational activities, differences in diets due to the availability of fresh fruits and vegetables, and changes in the excretory patterns becausee of excessive sweating.

Seasonal changes due purely to climatic conditions are very well illustrated in animals that are confined to stalls in winter and graze out in the fields in summer. This leads to dietary and other changes that lead to altered elemental composition of tissues and fluids. Depending upon the soil composition of the fields, concentrations of Cu, F, I, Mn, Mo and Se in milk are affected [18]. For example, I content in cow's milk fluctuates throughout the year, reaching minimum levels in summer. These variations are demonstrated in figure 2 by plotting data from [14].

In a number of species such as birds, reptiles, amphibia, and fish, different physiological states such as moulting, wintering, laying, or spawning bring about specific changes in the metabolism of certain elements, e.g., Ca. There are also great internal shifts in tissue composition of fat and protein in organs such as liver, resulting in extreme variations of elemental concentrations. Therefore, any intersample comparison has little relevance. For example, physiologicallyinduced seasonal variations in the concentrations of Zn, Cu, Fe, Cd, and Hg in starling liver has been identified [42]. Therefore, it follows that if the object of an investigation is to make an assessment of the extent to which an animal might be polluted, then it is necessary to take the samples at more than one time of the year.

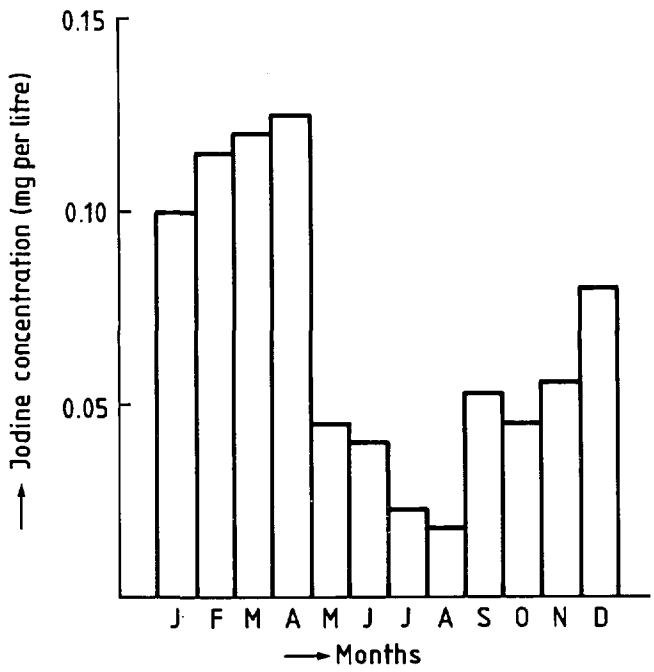

Figure 2–Variation in the iodine content of cow's milk.

4. Post Mortem Changes

Literature reveals that elemental concentrations based on autopsy samples generally show great variations in contrast to blood or serum which are obtained from living subjects (fig. 3). It should be recognized that autopsy sampling from humans involves a certain time lapse between death and sample collection and that, immediately after the death of an organism several post mortem changes set in with varying rapidity depending upon environmental temperature, humidity, body temperature at the time of death, insulating effect provided by the fat layers in the body, time elapsed before the body was put under cooling, and the storage time. Of the many changes that occur, cell swelling, tissue dehydration, imbibition, putrefaction, and autolysis are of particular significance to the analyst since they influence the "tissue status" as long as the organs remain inside the body. By implication, post mortem changes (which are inevitable to a certain extent in human situations) could have important bearings on the elemental composition profiles of individual organs.

Rapidly metabolizing organs such as liver, spleen, kidney and heart are severely affected by cell swelling, imbibition, and autolysis. The former two events produce changes in organ volume due to fluid influx and expulsion while the latter accounts for the actual tissue degeneration. Recently an animal model study illustrated the effects of post mortem changes on the elemental concentrations in liver [43].

According to the above mentioned study retention of rat liver inside intact carcasses for prolonged periods of time at ambient temperature induced significant changes in its weight due to post mortem tissue degeneration. Livers from animals that were frozen at -15 °C also showed significant decrease in weight when they were thawed on the third day. The effect of these changes on the elemental concentrations of various elements depended on the association of the elements with extracellular fluid and intracellular components. For example, concentration of $K+$ was affected more by the lysis of the cell and sustained losses up to 30% in relation to the control values, while the total content was reduced by more than 40% as a result of both lysis and tissue liquefaction. For $Na+$, differences ranging from $+10$ to

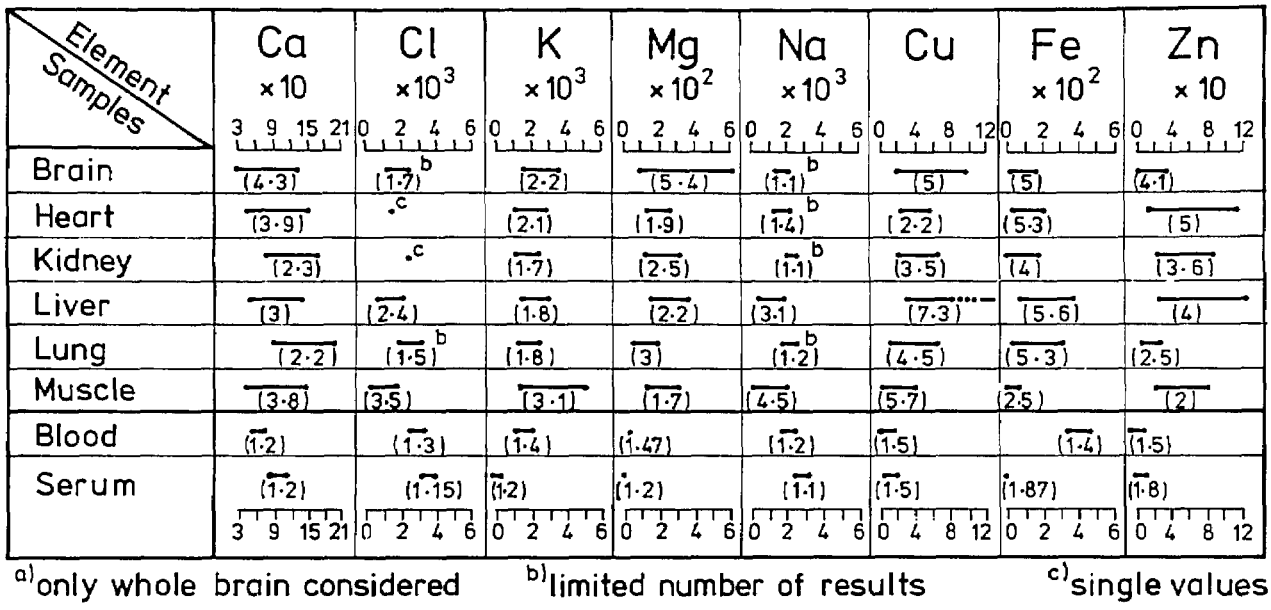

Figure 3–Variations in the elemental concentrations (mg/kg fresh tissue and mg/liter fluid) of normal adult human tissues reported in the literature (Iyengar, Kollmer, Bowen 1978). Figures in parentheses represent the ratio maximum/minimum.

−20% in concentrations and +20 to −40% in total content were observed which could be explained by the movement of fluid in and out of the organ. Among trace elements, variations observed for Fe ranged from −20 to +40% while both Cu and Zn were found to fluctuate between −20 and +20%. The loss observed in the total content in the liver for the five trace elements studied (Cu, Fe, Mn, Rb and Zn) was found to be about 20 to 40% [43,44]. Similar differences were observed also for Co, Cs and Se [45].

This study indicates the need for standardizing the sampling time in order to obtain reliable results for elemental analysis in human autopsies. This step would reduce fluctuations due to volume changes. Once the organ is removed from the body, careful freezing, or freeze drying permits prolonged storage.

5. Internal Contamination, Intrinsic and Inadvertent Errors

Internal contamination of tissues and body fluids from elements may arise due to a number of reasons governing many aspects of presampling factors and lead to intrinsic errors.

Intrinsic errors reflect the factors inherently present in the sample that may falsify the results. These errors, as the definition itself suggests, are difficult to detect and the analyst has little or no control over them. Good examples in this context are medication, haemolysis, prevalence of subclinical conditions and certain inescapable medical restrictions.

Certain types of medication, e.g., chelation therapy, are recognized for their role in upsetting the balance of trace elements in various body pools. Also, there are certain baffling situations such as prior exposure to I containing drugs or x-ray contrast media which generally elevate the tissue I levels with varying retention times in body compartments. Widespread use of I containing drugs (some as prophylaxis) and x-ray contrast media signal a formidable source of internal contamination for this element. Careful evaluation of case history is necessary to minimize such errors. Antinausea drugs and sleeping pills are additional examples.

Haemolysis is another source of intrinsic errors. Normal plasma contains much less hemoglobin in relation to serum which may contain 10 to 20 mg/mL, an equivalent of 350 to 700 ng of Fe per mL. Since the naked eye cannot distinguish hemolysis in serum below a certain degree, errors of this kind virtually go unnoticed at sampling stage. Depending upon the methodology used (measuring total Fe or exclusively transferrin Fe) the analytical values differ. These and other situations of intrinsic errors are discussed elsewhere [22].

Besides intrinsic errors sometimes errors may also be introduced inadvertently.These may happen while dealing with critically small samples (e.g., needle biopsies) due to changes in humidity in the sample environment thereby presenting formidable difficulties in assessing the correct weight of the sample material. The need for retaining viability of cells (e.g., platelets) is yet another example. Several such examples are discussed [22].

6. Differences Between Different Segments of an Organ

Significant differences in trace element concentrations between specific segments of an organ such as kidney, brain, and bone are well known. For example, sectioning of kidney into pure cortex and medulla is necessary since metal levels (e.g., Cd and Zn) differ significantly between tissue sections [46]. Similarly, great regional variations have been reported for Cu, Mn, and Zn between epidermis and dermis, an important factor while dealing with skin samples. The differences cover a wide range and may be of the order of 2 and 7 micro g/g for Cu, 0.1 and 1 micro g for Mn and 24 and 132 micro g/g for Zn, all on dry weight basis for dermis and epidermis, respectively [47]. Careful sectioning of hair to account for the distance from the scalp is another example of site dependency of elemental composition.

7. Conclusions

It is obvious from the foregoing discussions that presampling factors contribute their share to the overall variability of an analytical finding in biological systems.

The specimen to be sampled should be chosen with regard to the aim of the investigation and biological implications. Most often, the significance of the data can be strengthened if the sample characterization is thorough and if samples can be obtained at the same time from different body fluids and tissues.

There is a need for standardizing sampling and storage conditions for autopsy samples destined for elemental composition studies. It is important to incorporate the various aspects discussed in this report so that the true biological variations of the elemental composition of tissues may be easier to reveal.

Finally, it is imperative that analysts be associated with trace element investigations in biological systems at the planning stage and not just from the moment a sample arrives at the laboratory.

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The Sampling and Analysis of Human Livers

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A comprehensive approach to the analysis of human livers has been developed in a pilot program for a National Environmental Specimen Bank (NESB). Since 1980, the pilot NESB program has examined the collection, processing, storage and analysis of human livers. Sampling protocols, handling procedures and analytical methods have been developed and implemented considering the requirements for valid analytical results. Sampling and handling included the use of cleanroom technology, specific clean implements and packing materials made from titanium and Teflon and flash-freezing and preservation at liquid nitrogen temperature. Neutron activation analysis played a major role in the implemented analytical scheme. The scheme combined up to four analytical techniques to determine the distribution of 29 trace elements in 66 human livers.

Key words: analysis; human liver; low temperature preservation; neutron activation analysis; sampling; specimen banking; trace elements.

1. Introduction

1.1 Biomonitoring for Environmental Health

Monitoring is used to determine the present state of the environment with respect to concentrations of a variety of hazardous materials. Focusing on the biosphere, biomonitoring is the observation of symptoms, including both the registration of biological effects and the determination of trends in residual or accumulated hazardous substances. In this context, the monitoring of trends in pollutant loadings may provide the most comprehensive insight into interrelations between pollutant burden and environmental health.

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Biomonitoring of pollutant loading is an extremely complex task and requires a systematic approach to all aspects of a monitoring program. This includes the scientific evaluation of suitable biological specimens, their appropriate collection and sampling for analysis, the analytical methodology capable of obtaining the desired information, the dissemination of data, and the long-term storage of specimens for retrospective analysis. The requirements, experiences, and developments in monitoring environmental materials and specimen banking have been discussed in three recent international workshops [1–3][1] and have resulted in recommendations for the implementation of specimen banking programs related to biomonitoring. In concurrence with these efforts, two pilot programs for the implementation of an environmental specimen bank have been initiated [4,5]. The pilot programs provide the basic information needed for a more formalistic approach to the complex task of biomonitoring and specimen banking.

[1] Figures in brackets indicate literature references.

1.2 U.S. Pilot National Environmental Specimen Bank Program

The historical development in the United States during the 1970's of the concept of a National Environmental Specimen Bank (NESB) has been reviewed by Goldstein [6,7]. The purpose of such a national system is 1) to detect changes in the environment on a real-time basis (i.e., monitoring) using bioaccumulators as indicators, 2) to distinguish such changes from natural inputs, and 3) to provide a "bank" of well preserved and documented environmental samples for retrospective analyses in future years as analytical techniques improve or as new pollutants are identified.

Since 1975, the National Bureau of Standards (NBS), in conjunction with the U.S. Environmental Protection Agency (EPA), has been involved in research relating to the establishment of a National Environmental Specimen Bank. The initial plans and results of the EPA/NBS effort for the NESB in research and methodology evaluation have been described [8–13].

In 1979, a special "clean" laboratory/storage facility [14] was completed at NBS to initiate a *Pilot* National Environmental Specimen Bank Program. This pilot program is designed to evaluate the feasibility of a national program by providing actual working experience in all aspects of specimen banking, i.e., specimen collection, processing, storage, analysis, and data management. The major goals of this pilot study are: 1) to develop analytical protocols for sampling, processing, and storing four types of environmental accumulators and biomonitors; 2) to evaluate and improve analytical methods for the determination of both trace element and organic pollutants in biological matrices; 3) to evaluate the feasibility of long-term storage under various conditions; and 4) to provide a "bank" of samples for retrospective analyses in future years. The experience gained during the pilot study will be the basis for evaluating the feasibility of establishing a National (or International) Environmental Specimen Bank.

Four types of accumulators and biomonitors were selected for inclusion in the NBS pilot program as a result of an EPA/NBS Workshop on "Recommendations and Conclusions on the National Environmental Specimen Bank" held in 1976 [8]. These accumulators are: 1) human soft tissue—liver, 2) aquatic accumulator—marine bivalues and sediments, 3) food monitor—total diet composite, 4) air pollutant accumulator. Human liver was selected as the first sample type for the inclusion in the pilot specimen banking program. Sample types 2 and 3 are currently incorporated into the program with continued collection, storage, and analysis of the previous sample type. Consequently, the major efforts in the NESB have been concentrated on the development of the human liver as a monitor tissue. This contribution summarizes this experience with specific emphasis on sampling and analysis by nuclear methods.

2. Development of the Human Monitor Tissue

2.1 Criteria Determining the Selection of Monitor Tissues

For the selection and development of a monitor tissue the following aspects have to be considered: 1) tissues are selected because they are known either to respond to pollutant exposure or to accumulate pollutants; 2) a choice has to be made between biopsy and autopsy samples; 3) the quantity of material must be sufficient for baseline determination of constituents and storage for future analysis; 4) it must be possible to collect biologically or anatomically well described samples; and 5) analytical methods must be available to determine the pollutants. Table 1 lists a selection of possible monitor tissues and their evaluation according to the above considerations.

The general advantage of monitor tissue taken by biopsy versus autopsy is the possibility to monitor pollutant trends in the very same individual. The individual would reflect his own habitat over an extended time period. However, a wide variety of tissues is not available for sequential collection throughout the lifetime of an individual. Most of the biopsy tissue samples are collected in connection with surgical incisions, which usually provide only small samples, or one time events (e.g., placenta) where ethical barriers restrict the degree of sampling. Some monitor tissues can be collected on a routine basis without the above restrictions. Non-intrusive sampling includes hair, nail clippings, and various excretions. Requiring only minimal intrusion, blood has been demonstrated to be useful as an effective monitor that responds directly to the environment. In the U.S., the large-scale screening of blood from children helped to verify the accumulation of lead in blood and consequently resulted in the initiatives to lower the risk of exposure, e.g., by banning the use of lead based paints and reducing the lead content of gasoline [15,16]. The disadvantage of blood as a monitor is the fact that it reflects more acute exposure rather than being an accumulator. The natural dilution effect resulting from the blood's function as a distributor means that the concentrations of most pollutants are very low and difficult to measure. The danger of contamination during collection is very high (because of the low concentrations), although this problem can be minimized [17]. A more serious problem is the preservation of the blood sample and its long term storage without change, e.g., coagulation.

Table 1. Criteria for the selection of human monitor tissue.

Key: (1) highly favorable/no or little difficulties; (2) less favorable/limitations and problems; (?) questionable; and (0) no information.

	Sampling	Pollutant Accumulator	Sample Size for Banking	Biological Characterization	Sampling Characteristics	Inorganic Analysis	Organic Analysis
	Blood	?	1	2	1	2	1
	Milk	2	1	2	1	1	1
	Hair	1	2	1	?	2	0
	Nail	1	2	1	2	1	0
In Vivo							
	Teeth	1	2	1	?	2	0
	Placenta	2	1	1	2	1	2
	Feces	?	1	2	2	1	2
	Urine	?	1	2	1	1	1
	Brain	1	1	1	2	1	2
	Liver	1	1	1	2	1	2
	Lung	1	1	1	2	1	2
	Kidney	1	1	1	2	1	2
Post Mortem							
	Adipose	1	1	1	2	2	1
	Bone	1	1	1	2	1	0
	Muscle	1	1	1	2	1	2

2.2 Human Liver as Monitor

2.2.1 Biological and Anatomical Aspects. The liver exhibits several features which are advantageous to its use as a human monitor tissue. The liver is among the few organs which are significantly exposed to most of the pollutants that enter the body. Practically all substances which are assimilated by the human body are transported through the blood stream and consequently pass through the liver. The liver's function is to detoxify, store, and regulate trace substances in the body. Comparative studies on biological half-times [18] of trace substances in tissues support the storage function of the liver. Though some toxic elements have shown more affinity to calcareous tissue, biological half-times of trace substances in liver are so long that liver can be regarded as a general accumulator. In addition, the liver accumulates lipophilic organic trace substances because of its fat content. Therefore the liver is possibly the most universal accumulator tissue.

For the purpose of sample collection and specimen banking, the liver offers a biologically and anatomically well defined tissue, which also can be obtained in large quantities. The collection of the left lobes of the liver alone provides 200–400 g per sample depending on the physical stature of the subject. The liver is homogeneous in its function and in its macroscopic structure. During an autopsy, only a few cuts of ligaments and blood vessels are necessary to remove the liver from the corpse. The integrity of the sample can thus be preserved and subsequent sampling can be carried out under controlled conditions.

2.2.2 Sampling Protocol Development. Because of the extremely low concentrations of trace element and organic pollutants found in most environmental samples, extreme caution must be exercised during sample collection and sample processing to avoid contamination. A detailed sampling protocol, designed to provide samples suitable for both trace element and organic analysis, has been developed and implemented for the collection of human liver samples. For the complete detailed protocol see Harrison et al., [5,19]. This liver sampling protocol was developed in conjunction with those individuals performing the autopsies, and its implementation within the bounds of practicality required periods of education and close cooperation. The need for careful communication was evident from perceived meanings of such terms as "clean," i.e., interpreted as "sterile" by autopsy personnel vs. "non-contaminated chemically" by analytical chemists.

The sampling protocol was designed to avoid possible contamination of the sample by either inorganic or organic constituents. Teflon supplies (e.g., sheets, bags, and storage jars) were selected as the most suitable materials to prevent contamination of the sample from inorganic and organic constituents and from the diffusion of water [7,15]. The protocol specifies the use of such non-contaminating items as non-talced vinyl gloves, pre-cleaned dust-free Teflon FEP sheets and bags, high-purity water, and a titanium/Teflon TFE knife. These

items are provided by NBS at each collection site to insure uniformity in sampling materials. A special knife with a titanium blade and a Teflon TFE handle was designed and constructed at NBS for use during dissecting of the specimen. This special knife is used to avoid trace element contamination by various constituents associated with a regular stainless-steel scalpel/knife (e.g., Ni and Cr) and to limit the possible contamination by an element of little environmental interest, namely titanium. The liver samples are sealed in Teflon FEP bags, frozen in liquid nitrogen (LN_2), and shipped to NBS in a special biological shipper at LN_2 vapor temperature.

To eliminate potentially infectious liver samples from the specimen bank, a blood sample is removed from the donor at the time of the autopsy to be used for hepatitis B screening. In addition, liver specimens from the right lobe are removed for preparation of histological slides. These slides are examined by a pathologist to verify the absence of infectious diseases and then stored at NBS. They are also intended to serve for possible future reference.

A data form, sent to NBS with each liver sample, contains information about the donor, e.g., date of birth, sex, residence, ethnic group, height, weight, smoking history, occupation (if known), date and time of death and autopsy, diagnosis of autopsy, and liver specimen weight (see ref. [18,19]).

During the first 18 months, 300 liver samples from three geographical locations (Baltimore, MD; Minneapolis, MN; and Seattle, WA) were collected using this protocol. During the remaining years of the pilot study, liver samples were received predominately from one location (Seattle) at a rate of approximately 50 per year, bringing the number of banked specimens to about 500. After the first year of human liver collection, the sample collection protocol was evaluated with respect to such items as: initial set-up costs for each site, sample procurement costs, transport time, time required for receipt of hepatitis results and histological slides, and suitability of the donor selection criteria [20].

A conclusion from the experience of the first year of collection was that a technician was needed at the collection site whose primary responsibility was to work closely with autopsy personnel to select, remove, and prepare the liver samples. Since these technicians were responsible for implementing the sampling protocol, NBS personnel worked closely with them to stress the importance of following the protocol precisely. NBS must have confidence that the samples stored in the specimen bank are collected exactly as prescribed in the protocol.

2.2.3 Analytical Aspects. The U.S. Pilot NESB Program, and possibly any biomonitoring program, has to operate under constraints regarding the amount of available material from which the desired set of data will be obtained. In addition, modern analytical techniques require only small subsamples for analysis. Since significant trace element inhomogeneities have been found within human livers [21,22], homogenization of the bulk sample is required before analysis. The necessary reduction of a bulk sample to a laboratory sample suitable for analytical techniques can introduce errors due to contamination and heterogeneity. These errors may become the limiting factors for achieving the goal of precise and accurate analysis [23]. As the analytical test portion becomes smaller, better homogeneity is required so that the sample is representative of the bulk. Consequently, a major research effort of the pilot program has focused on homogenization procedures to provide uncontaminated analytical increments which represent the sample and which are homogeneous for subsampling of the analytical test portions.

Though the technique of cryogenic homogenization (brittle fracture technique) was first introduced and evaluated by Iyengar [24], considerable upgrading and evaluation was required before it could be routinely applied in the pilot program. The initial sample capacity of about 20 g was too small for application to the NESB program. Sample sizes for the human liver specimens in the pilot program are 120–180 g; and sample sizes up to 1000 g are anticipated for future specimens. As part of the pilot program, NBS has developed and evaluated the required technology for homogenizing larger samples [25].

NBS has designed two larger Teflon ball mills with capacities of 60 g and 150 g. A performance evaluation of the ball mills, based on particle size distribution and mixing ability, led to the design of the disk mill which has been determined to give superior performance and which is adequate for our needs (sec. 3.1). These experimental designs and their features are listed in table 2. Extensive evaluation for liver tissue showed that the cryogenic homogenization procedure with Teflon disk mills can be readily applied to soft tissues. Applications to other tissues resulted in equally fine particulate homogenates, using sieves for quick assay of the results of the homogenization process (fig. 1). These results were confirmed with the determination of sampling constants (sec. 3.1).

An important criterion for a comprehensive biomonitoring program is the availability of analytical techniques which can detect and determine all substances of concern at reasonable cost. The human liver, owing to its function as an accumulator, has higher concentrations of many elements and compounds of interest than most other tissues and therefore has less need for ultratrace level techniques. For most of the elements of

Table 2. Cryogenic homogenization machines used in the Pilot NESB Program.

	Teflon Milling Chamber Volume		Capacity for Soft	Drive Unit	
	Total	Milling Body	Tissue	Type	Motor Power/Revolutions
Ball Mill A	920 cm^3	ball, 65 cm^3	60 g	NBS shaker	0.25 kW, variable
Ball Mill B	1000 cm^3	ball, 200 cm^3	150 g		
Disk Mill #1	1400 cm^3	disk and 1 ring, 700 cm^3	100 g	Shatter box by Spex Industries	0.25 kW, 850 rpm
Disk Mill #2	2500 cm^3	disk and 2 rings, 1300 cm^3	250 g	Disk Mill TS 250 by Siebetchnik	0.75 kw, 1000 rpm
Disk Mill #3	6500 cm^3	disk and 3 rings, 3100 cm^3	~1000 g		

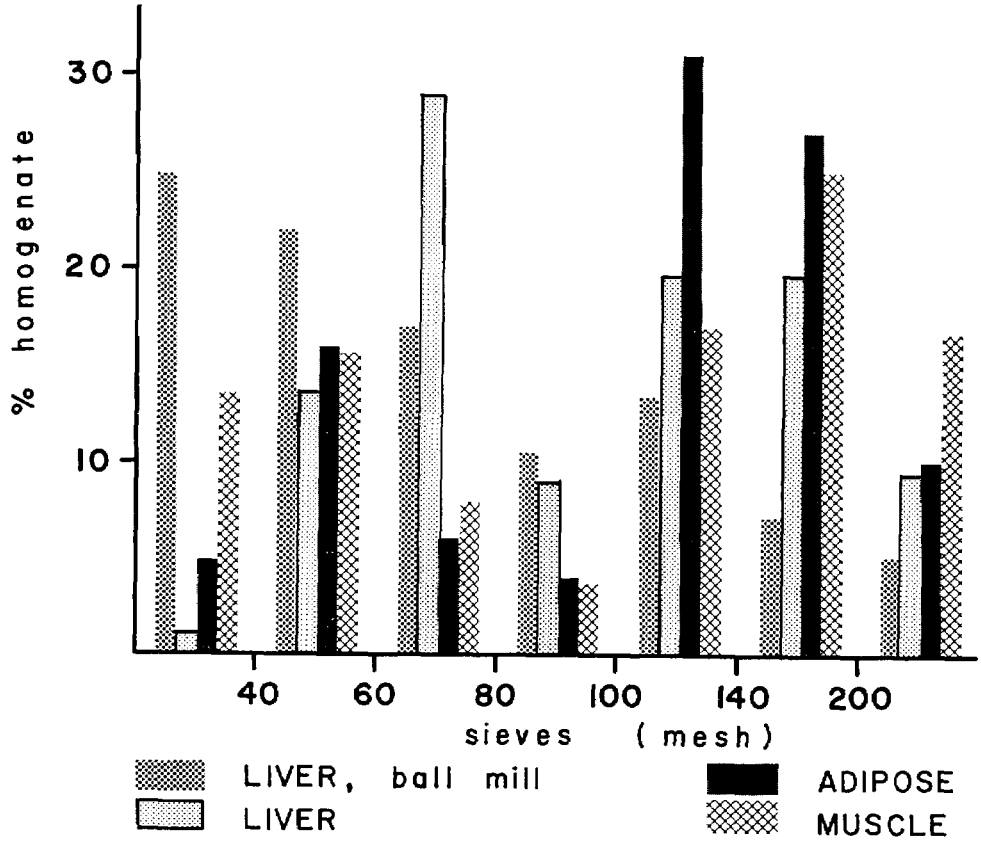

Figure 1–Particle size distribution of frozen tissue after homogenization in Teflon mills: approximately 150 g of tissue, grinding time 4 min. liver in ball mill B, and liver, adipose, and muscle tissue in disk mill no. 2.

interest [2,26], several routine techniques are available. Nuclear techniques can provide a broad data base on the liver (sec. 4) since no matrix interference is encountered. Furthermore, the liver tissue is easily ashed for techniques, including radiochemistry, that require wet chemistry before analysis.

In the U.S. Pilot NESB Program these basic conditions led to the implementation of a comprehensive analytical protocol, using four techniques for the determination of 31 trace elements of biological and toxicological importance [26]. This protocol is illustrated in figure 2. The quality of the analytical data is ensured by several measures. All analytical techniques used have demonstrated their capabilities during previous certification analyses of biological Standard Reference Materials (SRMs). Frequent parallel analyses of SRMs throughout the analytical program ensured high quality. Furthermore, it was possible to determine

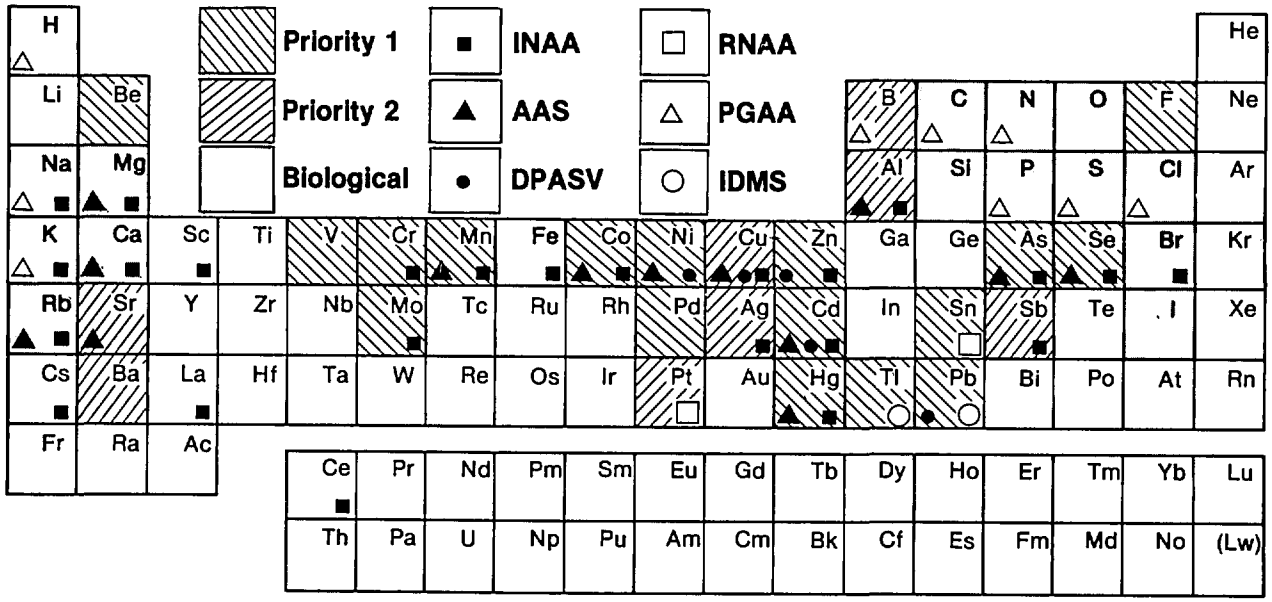

Figure 2–Elements of interest and analytical techniques used for the determination of trace elements in human liver specimens. Priority 1 elements are from ref. 2; priority 2 elements are additional elements of environmental concern; biological elements refers to elements of biological interest. (INAA—instrumental neutron activation analysis, AAS—atomic absorption spectroscopy, VOLT—voltammetry, PGAA prompt gamma activation analysis, RNAA—radiochemical neutron activation analysis, and IDMS—isotope dilution mass spectrometry.)

several elements by two or more independent techniques which helps to validate the accuracy of the results.

3. Use of Nuclear Techniques in the U.S. NESB Program

From the very beginning of the U.S. biomonitoring and specimen banking program, nuclear methods have been used. These methods have included radiotracer techniques, instrumental and radiochemical neutron activation analysis (INAA and RNAA, respectively), and prompt gamma activation analysis (PGAA). Evaluations of container materials [27] and ashing procedures necessary to provide small stable samples for storage [28] were investigated during the preliminary phase of this program. The subsampling procedures were evaluated during the implementation of the first phase of the program. For the determination of baseline data a comprehensive INAA scheme has been implemented and highly sensitive RNAA procedures for specific elements have been developed.

3.1 Evaluation of Cryogenic Homogenization

The sampling properties of particulate materials have been studied extensively [29], and a practical model to assess sampling has been developed by Ingamells [30]. Ingamell's sampling constant, K_s, can be used for direct comparison of the performance of the different brittle fracture systems. To experimentally determine K_s, a given particulate homogenate is sub-sampled and analyzed using different sample sizes. If the analytical error is sufficiently small, the sampling constant K_s can be determined using the equation $K_s = wR^2$, where R is the observed relative standard deviation, and w is the weight of the respective subsamples. The sampling constant corresponds to the weight of sample required to limit the sampling uncertainty to one percent with 68% confidence. A sampling diagram derived from this relation can be used to predict analytical errors and their dependence on sample size.

To evaluate the performance of the homogenization procedure, radiotracer experiments were carried out using ^{24}Na, i.e., a 5-g subsample was irradiated and homogenized with the bulk material. Subsequently, three different sized samples (nominally 0.1 g, 1 g, and 6 g) were subsampled from the homogenate and the activity of ^{24}Na was measured in each subsample. The high activity of ^{24}Na, its half-life of 15 h and standardized counting conditions ensured that the instrument error in this experiment was small compared to the observed error ($\simeq$1%). Therefore, from these experiments, sampling diagrams could be generated. Figure 3 shows the comparison of the ball mill with the disk mill #1 (100 g capacity). Using the ball mill, a sampling constant $K_s = 32$ g is obtained. Based on this result, it was concluded that the material was not sufficiently ground and mixed and that a typical 1-g analytical test portion could

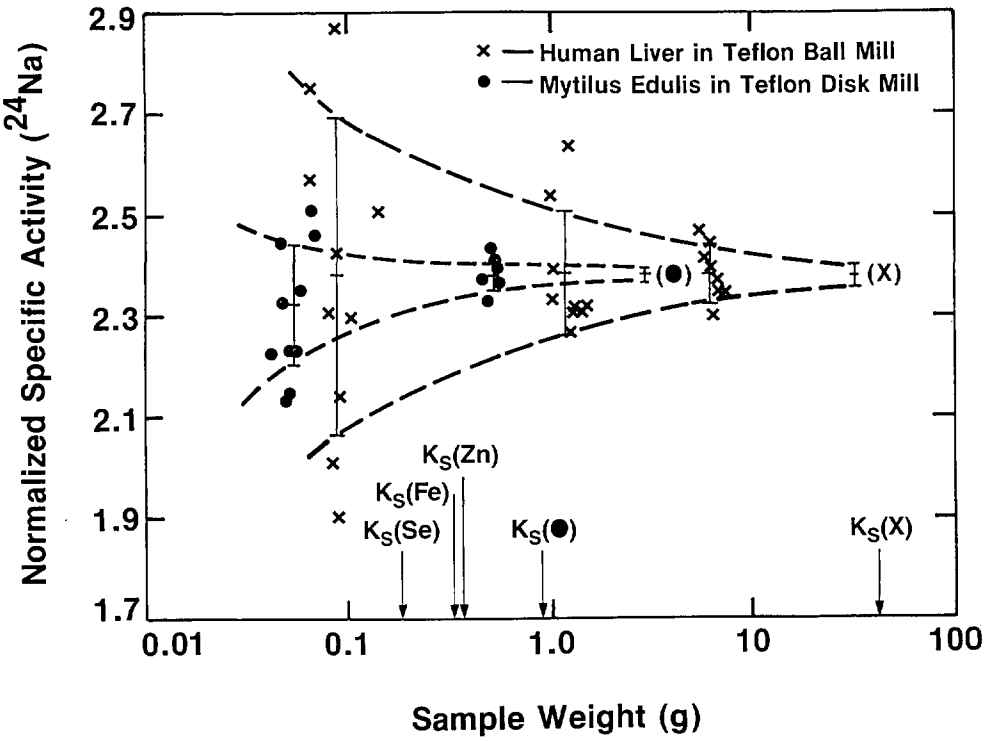

Figure 3–Sampling diagram of ^{24}Na in human liver and mussel homogenate. Specific activity (cts $g^{-1}s^{-1}\cdot10^{-2}$) scale is for the liver homogenate; values for mussel homogenate are normalized to the liver values for comparison.

be the cause of significant errors due to sample inhomogeneity. The inadequate performance of the ball mill resulted in the design of a disk mill which yielded a K_s of 0.95 g. Thus, using the disk mill, the analytical data obtained for a 1 g sample will depend on the precision of the analytical techniques rather than on sampling uncertainty.

This homogeneity evaluation was supplemented by the actual analysis of a considerable number of subsamples from a homogenized human liver specimen. Using PGAA and INAA for major and trace element composition, 10 randomly prepared subsamples (nominally 1 g) of the homogenate were analyzed. In all concentration ranges, from 10^5 μg/g for nitrogen to 10^{-4} μg/g for scandium, no sample inhomogeneity was observed; the sampling error being dominated by counting statistics. The elements Na, Br, Zn, an Fe, with small errors due to counting statistics, showed coefficients of variation $\leqslant 1\%$ for 1 g samples. This is in good agreement with the tracer experiment, though better data than those obtained for the bulk sample with its introduced gross inhomogeneity could be expected if the analytical error could be reduced.

The application of nuclear techniques to this problem has helped considerably to improve the cryogenic homogenization procedures. Though limited in their precision due to the radioactive decay, tracer techniques and instrumental multi-element determination provided useful information about the applied system.

3.2 Comprehensive Activation Analysis Scheme for Human Liver Analysis

An activation analysis scheme has been developed which is efficient in the use of sample material as well as in total cost and which provides a maximum amount of data on a routine basis. The procedure can be used to determine 33 elements in a specific subsample of the human liver. It consists of PGAA for the determination of H, B, C, N, Na, P, S, Cl, K and Cd and INAA for the determination of the elements Na, Mg, Al, Cl, K, V, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Cr, Rb, Mo, Ag, Cd, Sb, Cs, La, Ce, Sm, Au, and Hg, followed by a RNAA procedure for Sn.

The liver homogenates are freeze-dried before use for activation analysis. Approximately 250 g of dry material (corresponding to about 1 g fresh weight) is pressed into pellets and irradiated. An overnight count is used for the PGAA determination. Short (1 min) and long (4 h) irradiations, with two counts each after suitable decay intervals, are needed for the INAA. After obtaining all instrumental data, the sample is dissolved and ^{119}Sn is radiochemically separated using liquid/liquid extraction [31]. Activation analysis is very suitable for the types of analyses encountered in this biomonitoring program. Due to the variations in elemental concentrations encountered in human liver samples, a technique must cover a broad dynamic range without significant changes in precision and accuracy. Activa-

tion analysis is largely independent of changes in concentration unless the detection limit of the technique is approached. The analysis of the 66 human liver specimens that were collected during 1980 through 1982 showed that for several elements the detection limit of the applied instrumental procedure was approached when concentrations lower than previously reported [32] were found. The effects of this are demonstrated in figure 4, which shows that the precision of the cadmium analysis dependent on the concentration of the element. With the current activation analysis procedure, cadmium concentrations cannot be determined reliably below 1 μg/g. Similarly, the detection limit was reached in the majority of samples for the elements V, Cr, and As. A Compton Suppressor Spectrometer, recently installed at NBS, will be used for future analyses of the liver specimens. This new instrumentation will provide a considerable increase in sensitivity for the determination of these elements. In addition, a previously developed radiochemical separation procedure can be implemented to obtain accurate high precision data on Cr, Cu, As, Se, Mo, Ag, Cd, and Sb [3].

4. Value of Specimen Banking Program for Biomonitoring Environmental Health

4.1 Baseline Data

The process of obtaining reliable, high quality data requires the careful planning, investigation, and evaluation of every step in a chemical measurement system. To achieve this goal, the collaboration of scientists from the different disciplines involved is needed. This cooperation must start from the very beginning of a bio-

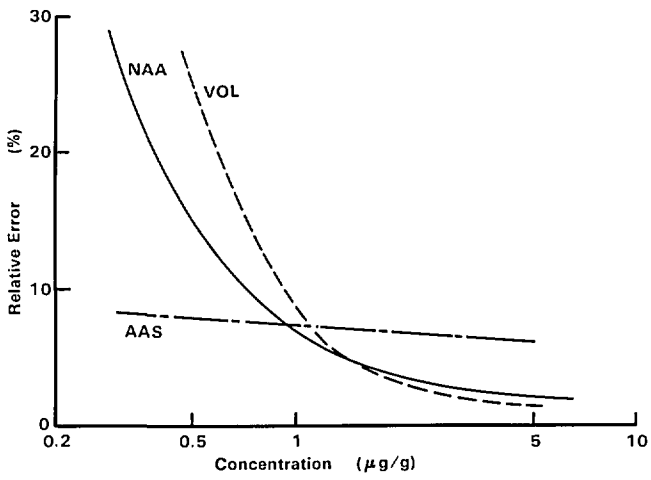

Figure 4–Imprecision of the analytical techniques under the constraints of the analytical protocol as observed for the determination of cadmium in human liver.

monitoring and specimen banking program. Just as the environmental ecologist must be aware of the measurement strategy and its benefits and limitations, the relationship between the analytical signal and the original question must be maintained. A formal specimen banking program for biomonitoring provides the necessary framework for such cooperation and consequently will achieve a credible data bank as a foundation for valid scientific conclusions. The current pilot programs have implemented this strategy and first results support this general approach.

The individual concentrations of 31 trace elements in 66 human liver specimens are summarized in figure 5. This figure is a plot of the more than 2,000 data points obtained in the first year's analysis of the human livers and also includes the concentration ranges of the elements reported in the literature and compiled by Iyengar for human livers [32].

The outstanding result of this study is the very narrow range of data obtained for many essential trace elements. There is only a factor of 1.8 difference between the lowest and highest value for selenium; other essential trace elements (Mg, Mn, Co, Cu, Zn) vary by a factor of 2 to 3.5. In sharp contrast to these findings for essential elements is the scatter of pollutant element concentrations (Al, As, Cd, Hg, Pb, Tl) over orders of magnitude. Since the biological variability of the individual specimens appears to be reasonably small, these broad ranges for the pollutant elements in the NESB data may point to sources of pollution to which the individuals were exposed.

When compared to the literature data [32], the elemental concentrations found in the liver samples, especially for pollutant trace elements, are at the lower side or even below the previously reported data. If these trends cannot be explained by the selection of the specimens and, for example, their low exposure to pollutant sources, then these results can be interpreted as a consequence of the implemented chemical measurement system. The precautions taken to preserve the integrity of the samples, especially the exclusion of possible sources of contamination during sampling and sample preparation, would naturally result in lower concentrations.

Further conclusions, especially on environmental impacts, are not readily available because the data base at this time is much too small. However, some interesting findings can be pointed out to illustrate the potential utility of a specimen banking program. The spread of the selenium data is much lower if individuals from the same geographical area are studied. When compared to the data obtained on samples from Seattle and Minneapolis, donors selenium levels in individuals from Baltimore are low. The average of the data from Baltimore is 4.4 μg/g,

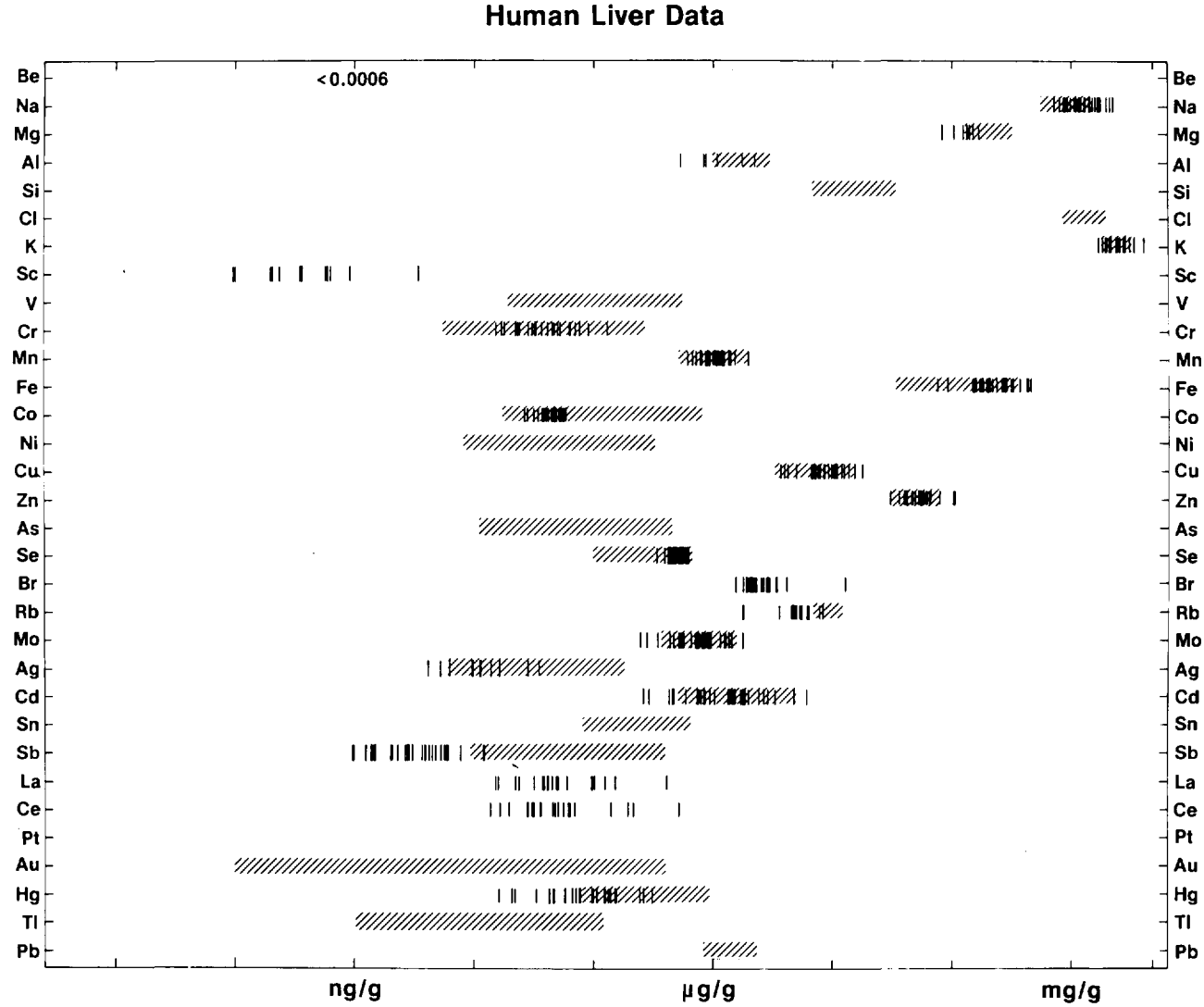

Figure 5–Elemental concentrations in 66 individual liver specimens and the concentration ranges reported in the literature [32]. Data from the pilot specimen bank program denoted by one line for each data point. Literature ranges denoted by the shaded areas.

the Seattle average is 5.6 µg/g, and the Minneapolis average is 6.1 µg/g. Because of concern about possible analytical error, we have also investigated some data points which exceeded the previously reported ranges. The highest iron value of 522 µg/g was from a 40-year steel worker, while the highest zinc value of 96.1 µg/g was found in the liver of a galvanizing plant worker.

4.2 Organized Assessment of Environmental Health

From the experience in the ongoing pilot programs, several advantages to formalized specimen banking and biomonitoring can be pointed out. A major aspect is that experience and expertise are easily transferable between programs which follow the same recommended criteria for their development. Duplication of effort has been avoided and by adopting common procedures, efficiency in implementation has been achieved. This principle is adaptable to a program having a few participants as to a program of global proportions.

Internationalization and/or inter-institutionalization of the banking and biomonitoring efforts is likely to provide the highest level of quality control and to make results comparable between institutes as well as nations. This is obviously essential for monitoring and assessing the true global burden from pollutants. Currently, samples are exchanged for quality assurance and analysis between the two pilot programs. In a completely transmissable system, specimens can be analyzed by the laboratory which is best qualifed for the specific task.

Although the feasibility of long-term specimen storage for retrospective analysis has not been established, preliminary analysis after one year of storage does not

indicate any changes in trace element concentrations for livers stored under the specific conditions of the U.S. Pilot NESB. It is without doubt necessary that biomonitoring programs need to comprise specimen storage for retrospective analysis. If specimens from previous lead analyses in Baltimore [36] had been collected and stored under the NESB protocol, it would be easy to determine if the decrease in concentrations by an order of magnitude is a realistic trend or a result of improved analytical methodology. If long-term stability can be guaranteed, banked specimens are far superior than data alone.

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The Collection and Preparation of Human Blood Plasma or Serum for Trace Element Analysis

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Trace element concentrations in blood plasma or serum have been assayed by numerous investigators using a variety of analytical techniques. For several elements, figures obtained in different centers are widely disparate. Impressive evidence has accumulated that a great deal of the inconsistencies should be ascribed to unsuspected contamination of the samples with exogenous material during their collection and preparation. In this paper, a number of potential sources of extraneous additions are indicated. Methods for controlling contamination are also briefly discussed.

Key words: blood collection devices; clean room conditions; high-purity reagents; sample containers; sample contamination.

1. Introduction

Circulating blood consists of formed elements (red blood cells, white blood cells, and platelets) suspended in a fluid (native plasma). Unless an anticoagulant is added, normal blood withdrawn from the circulation forms a clot due to the polymerization of fibrinogen to fibrin. On standing, the clot retracts, expressing serum which differs from plasma chiefly in that it contains no fibrinogen. When an anticoagulant (heparin, potassium oxalate, sodium citrate, or another) is added, clotting is delayed or prevented. So, plasma can be separated by centrifugation. In this way, different types of cells and platelets can also be isolated for investigation.

Blood and its constituents are frequently submitted to clinical and experimental laboratories for chemical analyses. Both because of their established importance to life and their ready accessibility, these matrices also occupied the attention of numerous trace element investigators. Particularly plasma and serum have been the subject of intensive search.

Many of the chemical substances in whole blood are present in unequal concentrations in the different fractions. Whole blood analyses give only overall values of potentially widely differing levels. Most trace elements occur at a higher concentration in packed blood cells (in ng or μg/g wet weight) than in plasma or serum (in ng or μg/mL): for selenium, the ratio is about 1.2, for cesium 6.5, for zinc 10.5, for rubidium 25.2, and for manganese 26.3. However, there are exceptions: for copper, the ratio is about 0.62 and for molybdenum 0.41 [1].[1]

A survey of published data on trace element levels in human blood plasma or serum shows that widely divergent values were measured in different laboratories. Certainly, a few sources of physiological variations are well documented [2]. However, solid experimental evidence has been accumulated that shows that much of the existing controversy should be ascribed to poor analysis or to inadequate sample collection and preparation [3].

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[1] Figures in brackets indicate literature references.

There are a number of factors which threaten sample integrity—undoubtedly, unsuspected contamination with exogenous material is the most important.

2. Methods

Different approaches have been employed to assess the impact of unwanted extraneous additions on the accuracy and precision of trace element measurements in human blood plasma or serum

Most published information is based on indirect measurements. Indeed, most investigators suspecting extraneous additions changed their sampling procedures until the lowest values were obtained and estimated the errors from the difference between their original high values and final low values.

At the University of Ghent, this approach was also used but, in addition, a method was developed to estimate the errors more directly. Using neutron activated instruments and other materials with which a sample may come into contact, several sample collecting and handling steps were reproduced in vitro with the underlying idea that, in these experimental conditions, additions from the devices to the samples should be reflected by traces of radioactivity in the samples. In the first place, this approach allows a rapid identification of potential sources of contamination. In addition, by using suitable irradiation conditions and post-irradiation measurements, it is possible to estimate the unwanted additions of a number of elements simultaneously. Indeed, the photopeaks detected in the samples may, of course, be converted into quantitative values in exactly the same way as in routine neutron activation analysis. The technique has been described in detail elsewhere [4].

3. Review and Discussion

The vital importance of adequate sampling was recognized by leading authorities a considerable time ago, yet it would appear that the level of practice among many investigators left much to be desired. Thus, the warning issued by Thiers in 1957 [5] that "unless the complete history of any sample is known with certainty, the analyst is well advised not to spend his time analyzing it" was largely ignored for several years.

Cotzias and his co-workers again attracted the attention to the problem [6,7]. In 1961, these investigators reported a mean plasma manganese concentration of 2.69 ng/mL which, five years later, they acknowledged to be unreliable because a careful search revealed that a systematic contamination with exogenous metal had persisted in their first study. At that moment, they came to the conclusion that the true value was 0.587 ± 0.183 ng/mL—a value nearly five times lower than the first. Shortly after, Davies and his colleagues [8] showed that routine plasma zinc determinations, whether fasting or at random, are of no value unless a few simple but stringent precautions are observed. They noted that a series of zinc estimations in plasma from blood samples of apparently healthy individuals, taken without special precautions, showed an aberrantly high mean (about 1.33 μg/mL) and an erroneously large spread (from about 0.86 μg/mL up to about 1.66 μg/mL) when compared to the true values obtained under adequately controlled conditions (mean of 0.95 μg/mL in men and 0.96 μg/mL in women; range from 0.76 μg/mL to 1.25 μg/mL for either sex).

In the author's laboratory, systematic studies were initiated at the end of the 1960s [4]. A general survey of the results, obtained in the course of the years, was published in Talanta in 1982 [9].

Using the direct approach, very important additions were observed in blood samples collected with irradiated, disposable, steel needles. In a series of experiments, the most striking contaminations were invariably found in the first 20-mL samples. Thus, the iron contamination in the first 20-mL sample amounted to about 15% of the expected, intrinsic level of the element in plasma or serum and to about 2% in subsequent 20-mL samples. The manganese contamination in the first 20-mL samples varied from about 13 to 77% and in the third or fourth 20-mL samples from about 2 to 10%. The transfers of cobalt and, more particularly, of chromium and nickel were even more important as they may equal and even largely exceed the intrinsic levels of the elements in human serum. For chromium, e.g., additions varying from 90 ng/mL in the first 20-mL sample to 10 ng/mL in the third 20-mL sample were observed whereas the true value in human serum has been estimated to be about 0.15 ng/mL. The additions of scandium, silver, tin, antimony, and gold are difficult to interpret because, in several instances, only upper limits could be established and because the uncertainty surrounding the plasma or serum levels of these elements continues. Of the additions examined, only those of copper and zinc turned out to be negligible [9].

To avoid these serious artifacts, it was decided to take blood samples with a polypropylene catheter (Intranule®, Vygon). Studies showed that the manganese additions were considerably reduced—the largest errors that were observed varied from 3 to 4%.

The transfers of manganese and copper to serum samples stored in polyethylene containers were also studied. Some of them were not cleaned, others were briefly rinsed with bidistilled water. The absolute amounts of the additions of both elements were found to be roughly of the same order of magnitude (mean values, non-

cleaned containers—manganese: 0.57 ng/mL, copper: 0.96 ng/mL; rinsed containers—manganese: 0.084 ng/mL, copper: 0.27 ng/mL). It is evident, however, that the significance is widely different: indeed, when compared to the normal mean plasma or serum levels of the elements (manganese: about 0.55 ng/mL, copper: about 1.0 μg/mL), the observed copper additions are negligible whereas, on the contrary, the observed manganese additions are very significant! These data also illustrate the vital importance of cleaning all containers with extreme care. If they are only rinsed with bi-distilled water, for manganese, errors of up to 15 or 20% may easily persist.

It may be argued that, in these experimental conditions, irradiation damage to the instruments may have increased the extraneous additions, particularly when instruments, like venipuncture needles, were irradiated for a long time at high neutron fluxes, e.g., for the study of chromium, iron, cobalt, and nickel additions (irradiation: five days, neutron flux: 10^{14} n·cm^{-2}·s^{-1}). Personal results obtained using the indirect approach, however, also prove that the artifacts may be extremely important.

For example, the concentration of manganese, copper, and zinc was assayed by neutron activation analysis in duplicate serum samples of 12 patients with minor ophthalmologic disorders but without apparent signs of other health problems. Initially, the mean ± standard deviation for manganese was found to be 6.7±6.6 ng/mL or 6.9±7.3 ng/mL. The variability between duplicate samples appeared to be very important ($\sqrt{\Sigma d^2/2N}$=5.7 ng/mL; d=difference between the two results in a duplicate determination, N=number of duplicate determinations performed). After the sampling procedure was substantially refined (use of a plastic catheter for venipuncture; thoroughly cleaned, high-purity synthetic quartz tubes and conventional polyethylene containers for collection, storage, lyophilization, and irradiation; transport of samples under carefully secluded conditions; working under clean room conditions), employing exactly the same radioanalytical technique, values turned out to be 0.63±0.10 ng/mL or 0.64±0.14 ng/mL in a comparable series of subjects. In this second series, the variability between duplicate samples appeared to be minimal ($\sqrt{\Sigma d^2/2N}$=0.074 ng/mL [9]. It is interesting to note that the results for copper and zinc in both series were nearly identical. This illustrates that a sampling procedure may be adequate for analyses at the μg/mL (serum copper and zinc) yet grossly deficient for determinations at the ng/mL level (serum manganese).

Table 1 shows another example. In the upper part, it catalogues the values obtained by neutron activation analysis for manganese, copper, and zinc in nine 1-mL serum samples transferred in the liquid state with a digital dispenser (manufactured by Hamilton; PTFE tubing system) into previously cleaned, conventional polyethylene containers for lyophilization and irradiation. Before use, the system was flushed with quartz-bidistilled water. In the lower part, it lists the results found in nine other samples that were first lyophilized and then transferred with a carefully cleaned, small quartz spoon (Spectrosil®, Thermal Syndicate) into thoroughly cleaned, identical conventional polyethylene containers for irradiation, as done in the laboratory for many years. It is evident that one misstep may heavily distort the figures for a low-level trace element like manganese (concentrations measured in samples transferred with dispenser—mean value: 4.83 ng/mL, range: 0.61 – 16.83 ng/mL; in samples transferred with quartz spoon—mean value: 0.72 ng/mL, range:

Table 1. Manganese, copper, and zinc levels measured in nine serum samples transferred with a digital dispenser (manufactured by Hamilton; PTFE tubing system) and in nine others transferred after lyophilization with a thoroughly cleaned, high-purity quartz spoon. All measurements were done by exactly the same radiochemical technique.

Samples transferred with Digital Dispenser	Mn (ng/mL)	Cu (μg/mL)	Zn (μg/mL)
1	0.71	1.03	0.84
2	1.38	1.14	0.94
3	0.61	0.98	0.88
4	2.39	1.01	0.99
5	8.56	1.07	0.99
6	5.66	1.07	0.90
7	16.83	1.01	0.91
8	3.92	1.10	0.88
9	3.39	0.97	0.84
Mean	4.83	1.04	0.91
Range	0.61–16.83	0.98–1.14	0.84–0.99

Samples transferred with Quartz Spoon	Mn (ng/mL)	Cu (μg/mL)	Zn (μg/mL)
1	0.74	1.11	1.05
2	0.56	1.01	0.98
3	0.89	1.07	0.98
4	0.64	1.01	0.90
5	0.66	1.03	0.96
6	0.74	1.07	1.02
7	0.70	1.03	0.94
8	0.82	1.04	0.97
9	0.69	1.08	0.84
Mean	0.72	1.05	0.96
Range	0.56–0.89	1.01–1.11	0.84–1.05

0.56–0.89 ng/mL) although the figures for copper and zinc are not markedly affected.

Scattered throughout the literature are reports of other investigators who cautioned against the errors from inadvertent sample contamination in biomedical trace element investigations.

Published data on contamination from venipuncture needles are scarce. Kumpulainen and his colleagues [10] compared chromium concentrations in serum from blood samples collected with conventional steel needles and with plastic catheters (Venflon®, Viggo): in the first, they found a mean value of 0.43 ng/mL, and in the second, of 0.12 ng/mL. Sunderman and his co-workers [11] measured nickel concentrations in serum samples (21 healthy adults) from blood collected from one arm with 22 gauge "Monoject" needles (about 71.1% of iron, 17.4% of chromium, 9.1% of nickel, and small amounts of other elements, e.g., 1.6% of manganese, 0.43% of molybdenum, 0.12% of colbalt, and 0.047% of tungsten) [12] and from the other arm with polyethylene intravenous cannulas. In the first case, the levels averaged 0.74±0.25 ng/mL, in the second 0.37±0.18 ng/mL. The mean difference between the paired nickel concentrations was found to be 0.38±0.23 ng/mL.

The risk of obtaining misleading serum zinc values because of extraneous additions from the rubber stoppers of evacuated blood collection tubes of various kinds (Labtube®, Abbot; Vacutainer®, Becton-Dickinson; Venoject®, Kimble-Terumo) has been identified by numerous investigators [13–18]. Thus, Williams [18] determined iron, copper, and zinc in serum or plasma collected from normal volunteers in plain, acid-washed test tubes on the one hand and in plain, lead-free, and heparinized Vacutainers® on the other. The mean values for both iron and copper were perfectly comparable, regardless of the tubes used. In contrast, mean zinc values were consistently higher when plain, lead-free and heparinized Vacutainers® were used (respectively 1.94±0.064 μg/mL, 2.50±0.127 μg/mL, and 1.84±0.072 μg/mL versus 1.05±0.023 μg/mL—value measured in serum from blood collected in plain, acid-washed, glass test tubes). These observations prompted Becton-Dickinson to develop a new type of stopper for trace element studies (tube with minimal trace element content, royal blue stopper). All studies showed that the contamination of the samples with zinc was strongly reduced [9,17,18]: thus, using this tube, Williams measured a serum zinc value of 1.16±0.023 μg/mL [18]. Our investigations,however, indicate that it offers only an incomplete solution. Indeed, considerable contamination with manganese was found to persist: additions varied from 0.044 to 0.292 ng/mL and from 0.064 to 0.918 ng/mL in samples that remained in contact with the stopper for respectively 30 and 120 minutes, whereas the true mean serum manganese concentration in healthy adults is now generally believed to be about 0.55 or 0.60 ng/mL [9,19]. Furthermore, its reliability in the assay of other low-level trace elements such as vanadium, chromium, cobalt, arsenic, and molybdenum remains to be established.

In general, the sample container is one of the potentially largest sources of sample contamination. Much of the analytical accuracy will depend upon the choice of the material and the method of cleaning. For this important issue, the reader is referred to the work of Moody and Lindstrom [20] who examined the levels of impurities in various plastics (conventional polyethylene, linear polyethylene, polypropylene, several types of Teflon®, and a number of other materials) and made recommendations for cleaning methods.

The paper of Reimold and Besch [17] also contains information on other potential sources of contamination with zinc (plastic tubes, Parafilm®, wooden applicator sticks, chemical reagents, laboratory tissues like Kimwipes® and Kleenex® for wiping pipettes and glassware, filter paper, etc.). Detailed information on impurities in chemical reagents has been published by Murphy [21] and Zief and Micholotti [22]. Techniques for preparing exceptionally high-purity reagents, which are especially valuable for the stabilization of trace metals in solution and for the dilution of samples, have been described by Kuehner et al. [23], Mitchell [24], and Moody and Beary [25].

Heydorn and associates [26,27] drew attention to the potential errors caused by airborne particulate matter,usually referred to as dust, in serum samples intended for manganese determinations. An analysis of 11 duplicate serum samples, taken with great care to avoid contact with materials likely to contaminate the samples with traces of the element, revealed the presence of unknown sources of variation. The precision of the analytical technique being well established, it was concluded that the duplicates were not identical but caused an additional, estimated standard error of about 0.35 ng/mL. Another set of samples was obtained by the same technique but under more secluded conditions, keeping them covered essentially all the time. The resulting measurements clearly showed that a highly significant reduction of variation between duplicate results was obtained (standard error of 0.04 ng/mL) as a consequence of shielding the samples from airborne contamination.

In addition, the potential for trace element contamination from air particulates has been examined by many other investigators [21,28–32]. Leading experts in trace and ultratrace analysis consider a clean laboratory to be an essential requirement to reduce this source of contamination. It can be assumed that plasma or serum

samples for the determination of elements in the μg/mL range, e.g., iron, copper, zinc, and a few others, can be adequately handled in ordinary analytical and clinical laboratories—at least when some basic technical rules and principles are strictly observed. On the other hand, when concentrations go down to the ng or sub-ng/mL range, e.g., aluminum, vanadium, chromium, manganese, cobalt, nickel, arsenic, molybdenum, and others, the whole prospect changes and all routine cleanliness or precaution practices become insufficient. In those cases, the key to successful measurements is found to be in the control of the analytical blank and a clean laboratory environment is one of the major tools available to the researcher. In 1982, the design of the clean laboratories for trace element analysis at the National Bureau of Standards has been authoritatively described by Moody [33].

The foregoing survey will have made it clear that unsuspected extraneous additions during the collection and preparation of plasma or serum samples may have devastating effects on the results of trace element assays. All potential sources need the greatest attention from the investigator. Rigid control of one source is not sufficient. Thus, controlling the environmental blank caused by air particulates, however important it may be, will be of little value if other sources of contamination such as collection devices, containers, and reagents are out of control. Scrupulous efforts must be undertaken to eliminate deficiencies at all stages.

4. Conclusions

Sample collection and preparation have been relatively neglected areas in trace element research. Advances in contamination control made it clear that they may be the origin of more serious errors than any other step in the analytical process. This is particularly true in the case of blood plasma or serum because the intrinsic levels of many elements are extremely low [3,9–11,34–38]. Control of contamination holds the key to further progress in accuracy and will permit the exploitation of the full sensitivity and specificity range of the existing analytical procedures in practical studies. Hopefully, this paper will contribute to the ultimate disappearance from the literature of papers reporting reference values or variations in physiological and pathological conditions based on estimations of trace element levels in obviously contaminated samples!

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Storage and Pre-Neutron Activation Analysis Treatment for Trace Element Analysis in Urine

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The problems regarding storage and pre-neutron activation analysis treatment for the elements aluminum, calcium, vanadium, selenium, copper, iodine, zinc, manganese, and magnesium in a urine matrix are reviewed. The type of collection and storage procedure and pre-neutron activation analysis treatment of urine depend on the specific trace element; that is, its inherent physical and chemical properties. Specifically polyethylene in teflon containers are the most suitable for general determinations. Whether any preservative is added would depend upon the stability of the trace element and its tendency for surface adsorption. Preferably preservatives should contain no radioactivatable elements for maximum efficacy. Freeze drying or packing urine shipments under dry ice needs to be explored on an individual basis. Each pre- or post-neutron activation analysis treatment is specific and optimized for the trace element analyzed.

Key words: aluminium; calcium; iodine; magnesium; manganese; neutron activation analysis; pre-NAA treatment, urine; selenium; storage; vanadium; zinc.

Introduction

Cornelis et al. [1] describe one of the most comprehensive studies to date regarding neutron activation analysis for bulk and trace elements in urine, problems in sampling, collection, storage, sample preparation and contamination hazards during neutron irradiation. Our intent in this review is not to repeat Cornelis's study but to add to it from our experience in work involving the urine matrix [2–7].

The quantitative analysis of urine for trace elements is important for metabolic and nutritional research. Because of its ready availability and easy access, under proper conditions, it can be a vehicle for mass screening of individuals for normal and disease states. Urine is an aqueous admixture composed of dissolved and suspended waste products as well as inhaled and absorbed substances such as pollutants and/or their metabolites. The two major radioactivatable elements whose presence can interfere in the radioassay of urine are sodium and chlorine. Perhaps that is why there is a paucity of Instrumental Neutron Activation Analysis (INAA) techniques for trace element determination in the urine matrix. For the sake of brevity this discussion will be limited to Ag, Cd, Hg and the radionuclides depicted in

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[1]Figures in brackets indicate literature references.

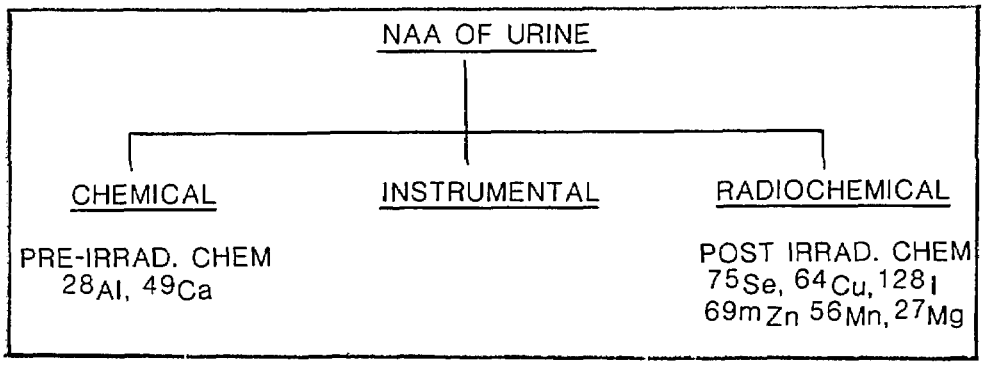

Figure 1–Trace elements determined in urine.

figure 1. One of the drawbacks of neutron activation is the limited number of reactor facilities where research and routine analysis can be performed; consequently, in many cases samples must be transported to the facility. It is of paramount importance that special precautions be taken in the collection and storage of urine samples prior to reactor irradiation. In other words, the analyst must know the history of the sample before it reaches his or her hands.

Storage of Urine Specimens for Specific Elemental Analysis

Not all storage vessels or containers are suitable for all trace elements to be investigated. It is important that the containers chosen show no absorption or adsorption of the desired trace element and that there is no loss of the trace elements during storage. West et al. [8] showed that the adsorption of silver in potable water samples to the walls of borosilicate, flint, and polyethylene was pH dependent and could be prevented for 30 days by collecting samples in sufficient sodium thiosulfate to produce a 10–15% solution.

King et al. [9] have shown that in order to prevent losses of Cd by adsorption to the walls of glass containers, water samples should be acidified with HNO_3. Since polymer surfaces do not interact with Cd aqueous solutions, sampling of Cd would be better performed employing plastic containers.

Struempler [10], found that polyethylene containers did not absorb cadmium and zinc; and acidification with dilute HNO_3 to a pH value of 2 prevented Ag, Pb, Cd, and Zn adsorption on borosilicate and silver adsorption on polyethylene surfaces. He also found that silver solutions must be kept in the dark, even under acidified conditions, to maintain stability and to minimize adsorption loss and that new polypropylene containers could not be cleaned satisfactorily for Cd and Zn. Feldmen [13] has shown that distilled water solutions containing >0.1 ng Hg/mL can be stored in glass without deterioration for as long as five months if the solution contains 5% by volume HNO_3 and 0.01% dichromate. Storage of such standards is safe in polyethylene containers for at least 10 days if the solution contains 5% HNO_3 and 0.05% dichromate.

Thiers [11] found that borosilicate glass can seriously contaminate solutions and should not be used for storage unless the analyst is confident that the contamination of the element being determined is negligible. Murphy [12] states that "Teflon FEP bottles and beakers have been used at NBS for the past several years with favorable results after thorough cleaning with nitric and hydrochloric acids to remove contaminants introduced during fabrication." High purity acids were stored for at least two weeks with no significant levels of contamination observed. It is important that any substance added to the urine for purposes of preservation does not form complexes with the desired trace element, or introduce that trace element. Some authors have suggested that the samples be lyophilized since dry, solid samples lessen the possibility of leaking or adsorption during storage. However with urine we have found that due to its high NaCl concentration there is both a volume limitation and the possibility for a potential loss due to bumping of the sample during the vacuum process. As a minimum the urine must be diluted with deionized water to allow solid freezing.

Determining what kind of stabilizing additive should be mixed with the solution can be very complex, as can be seen in the following figures showing the loss of the element with storage time for several types of containers.

As seen in figures 2 and 3 [13], the aqueous results obtained in both glass and polyethylene confirm the generally held view that aqueous solutions of mercury salts rapidly lose strength on storage because they exist as a colloidally suspended hydrolysis product.

Depicted in figures 4 and 5 [13], nitric acid at the 1% level appears to be almost as ineffective as water alone in glass and polyethylene vessels. It is more effective at the 5% level but still quite unsatisfactory.

As presented in figures 6 and 7 [13], the mixture of H_2SO_4 and potassium permanganate produces colloidal Mn oxides. These over a period of time remove the Hg

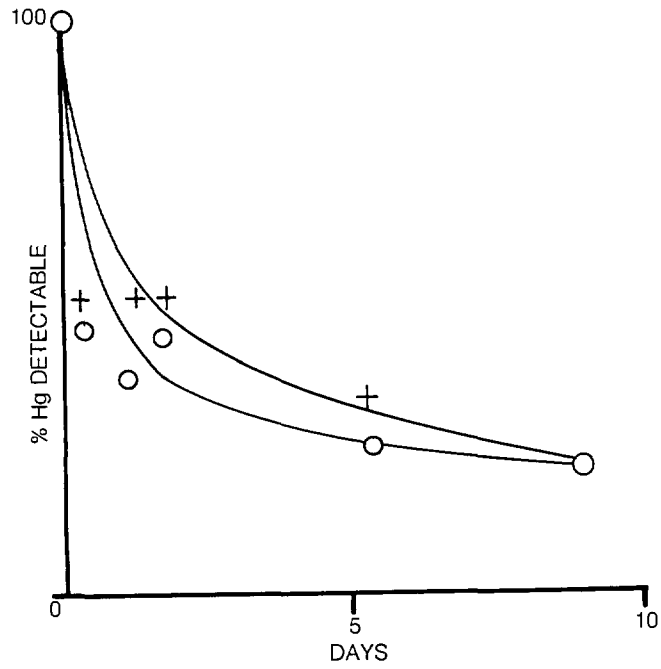

Figure 2–Stability of Hg solutions in glass (H_2O); 0, 10 ng lmL; +, lng/mL.

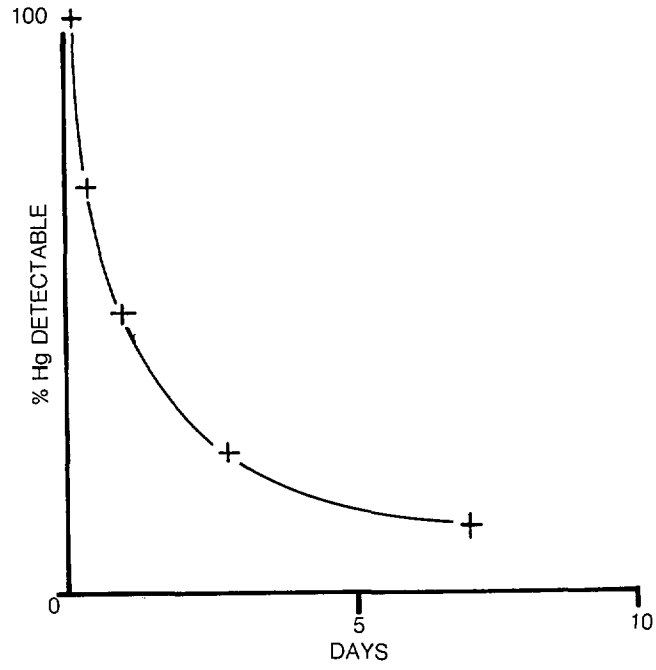

Figure 3–Stability of Hg solutions in polyethylene (H_2O) +, 0.2 ng/mL.

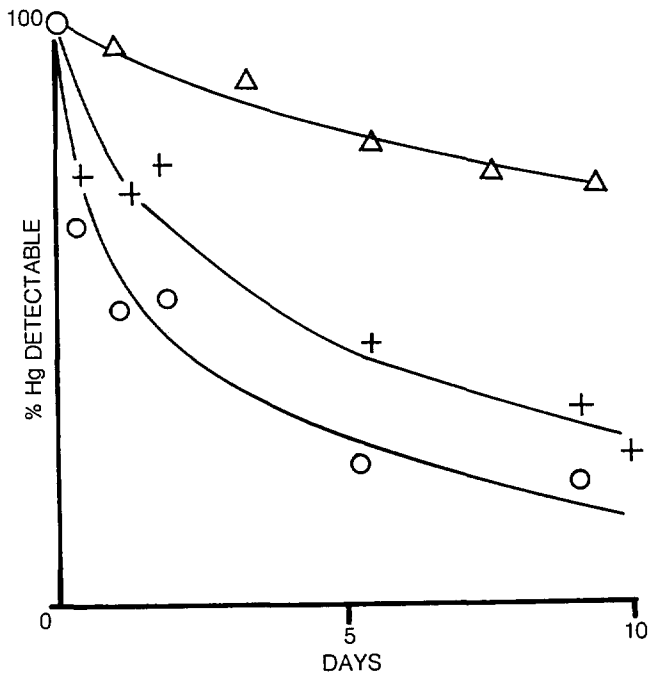

Figure 4–Stability of Hg solutions in glass (HNO_3); Δ, 10 ng/mL, 5% (v/v): +, 1 ng/mL, 1% (v/v); 0, 10 ng/mL, 1% (v/v).

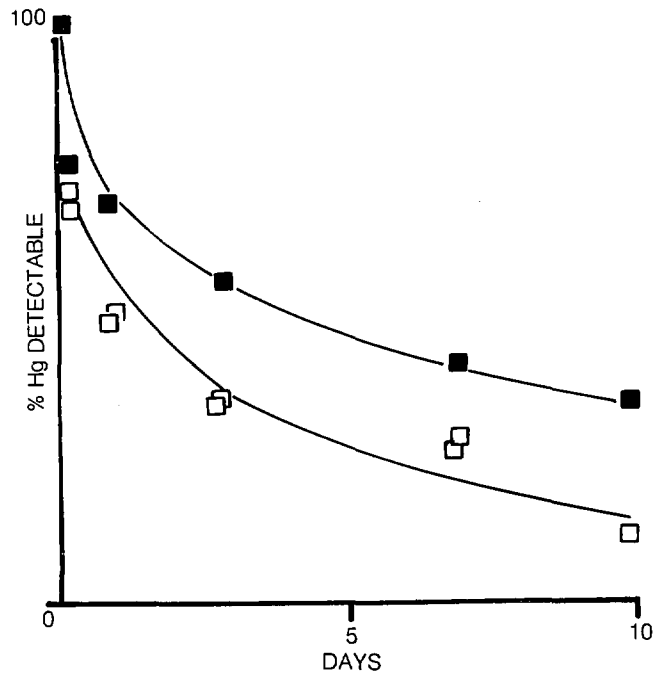

Figure 5–Stability of Hg solutions in polyethylene (HNO_3); ■ 0.2 ng/mL, 5% (v/v); □, 0.2 ng/mL, 1% (v/v).

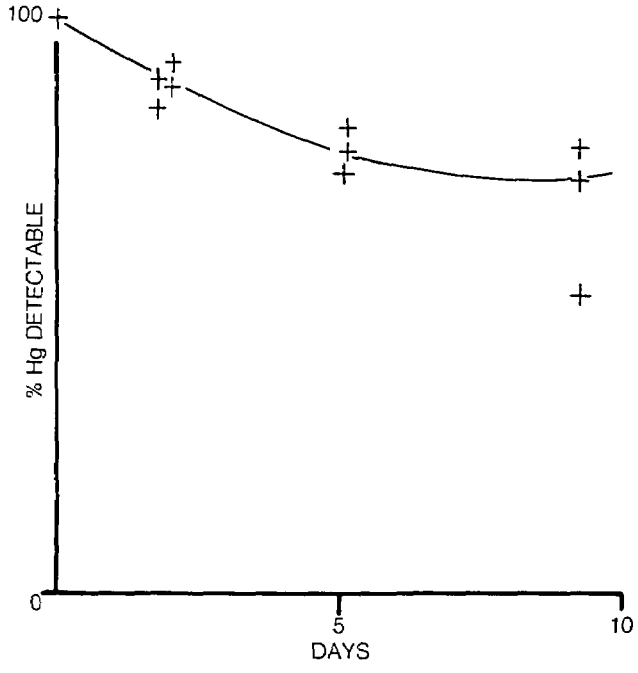

Figure 6–Stability of Hg solutions in glass (0.5% (v/v) H_2SO_4 +0.01% $KMnO_4$); +, 10 ng/mL.

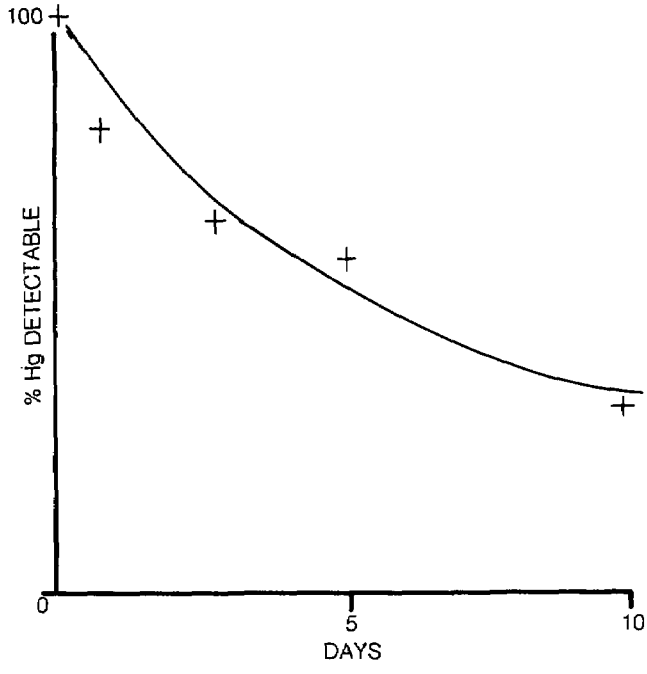

Figure 7–Stability of Hg solutions in polyethylene (0.5% (v/v) H_2SO_4+0.01% $KMnO_4$); +0.2 ng/mL.

from the solution and plate it on the side walls of the container.

In glass the combination of 1% HNO_3 and 0.01% dichromate does not prevent a rapid initial drop of Hg concentration with increasing storage time, possibly due to adherance of hydrolyzed Hg salts to the walls although little or no loss occurs after the first day. This effect is presented in figure 8 [13]. However, as can be seen in the figure, the combination of 5% HNO_3 and 0.01% dichromate is quite successful because of its ability to prevent the hydrolysis of dissolved mercury and to prevent its reduction to oxidation states lower than +2. For polyethylene containers it is necessary to increase the dichromate concentration to 0.05% as can be seen in figure 9.

The initial concentration of Cd present in water also affects the degree of loss of Cd during storage. Figure 10 shows the percentage loss of Cd as a function of time for distilled water samples at pH 10 with different Cd concentrations. The curve for 25 ppb Cd reaches a maximum value of about 35% Cd loss after 20 hours of storage in soft glass [9]. For most environmental monitoring programs the significant level of Cd is in the 1–100 ppb range.

Loss of Cd is definitely pH dependent as seen in figure 11. At pH 6.9 there is no Cd loss. Cd loss does not occur in plastic containers [9].

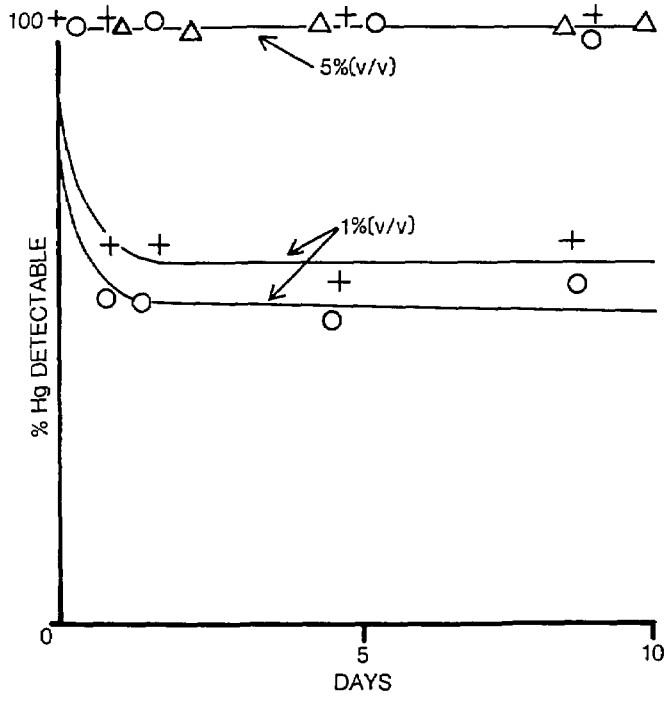

Figure 8–Stability of Hg solutions in glass (HNO_3+0.01% $K_2Cr_2O_7$); Δ, 0.1 ng/mL; +, 1 ng/mL; 0, 10 ng/mL.

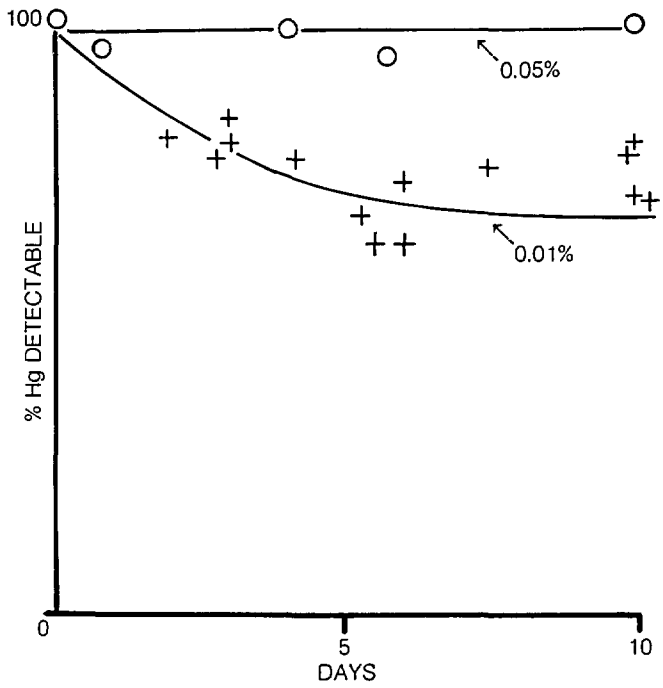

Figure 9–Stability of Hg solutions in polyethylene (5% (v/v) $HNO_3 + K_2Cr_2O_7$); +, 0, 0.2 ng/mL.

Figures 12, 13, and 14 show the loss of silver in aqueous solutions stored in different types of containers [8]. The maximum and minimum values represent the variation in multiple runs. There appears to be a narrow range of adsorption values in the case of the flint glass but adsorption begins after a shorter contact time.

Thus, we can see that the container loss must be investigated for each element analyzed and for each concentration range. Because of the nature of urine, several practical considerations must also be addressed if urine samples are to be shipped to a nuclear reactor for neutron irradiation and subsequent radioassay. Rapid freezing and dry ice shipment may offer the least potential damage to the matrix; however, for each individual elemental assay it must be determined if freezing has any deleterious effects on the analysis.

Pre-Neutron Activtion Analysis Treatment

Each trace element has its own inherent physical, chemical, and radioactivatable properties which must be considered in the pre-irradiation chemistry prior to neutron irradiation and its detection and analysis. Because of the large amount of the radioactivatable elements sodium and chlorine in urine, it is not practical or wise to employ instrumental NAA regardless of the sensitivity and selectivity of the radioassay equipment. This can graphically be seen in figure 15 which shows the spectra of raw saliva and an extracted CCl_4 phase. It can be observed that the iodine peak appears in the top spectra but the compton continuum contribution to the iodine photopeak is 77% of the total counts in the photopeak [14]. For an individual trace element several

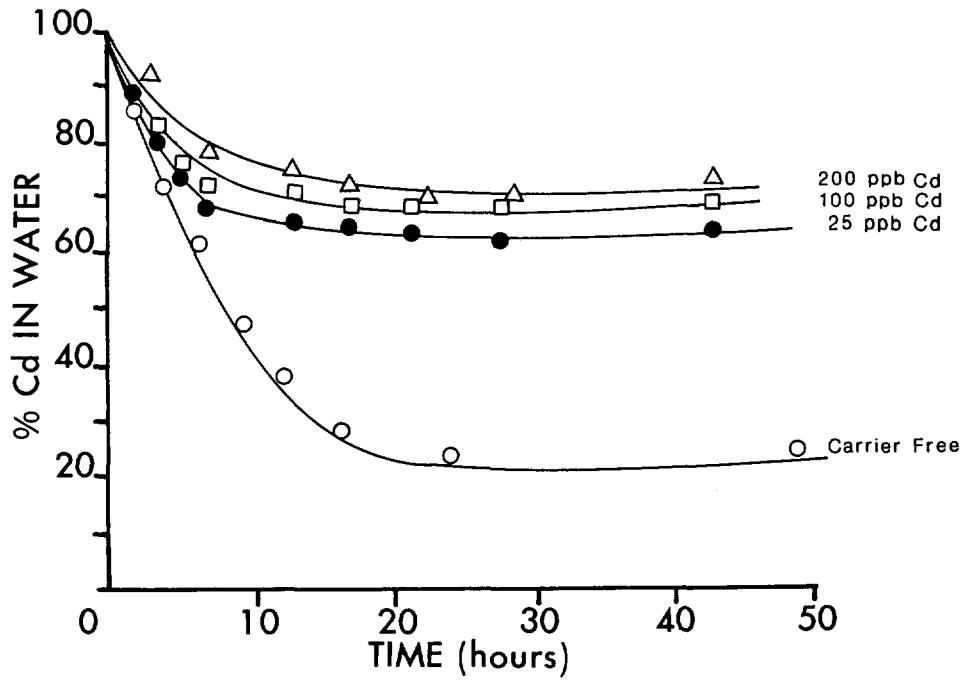

Figure 10–Percent of ^{109}Cd in water during storage with respect to the initial concentration of cadmium in solution. Soft glass.

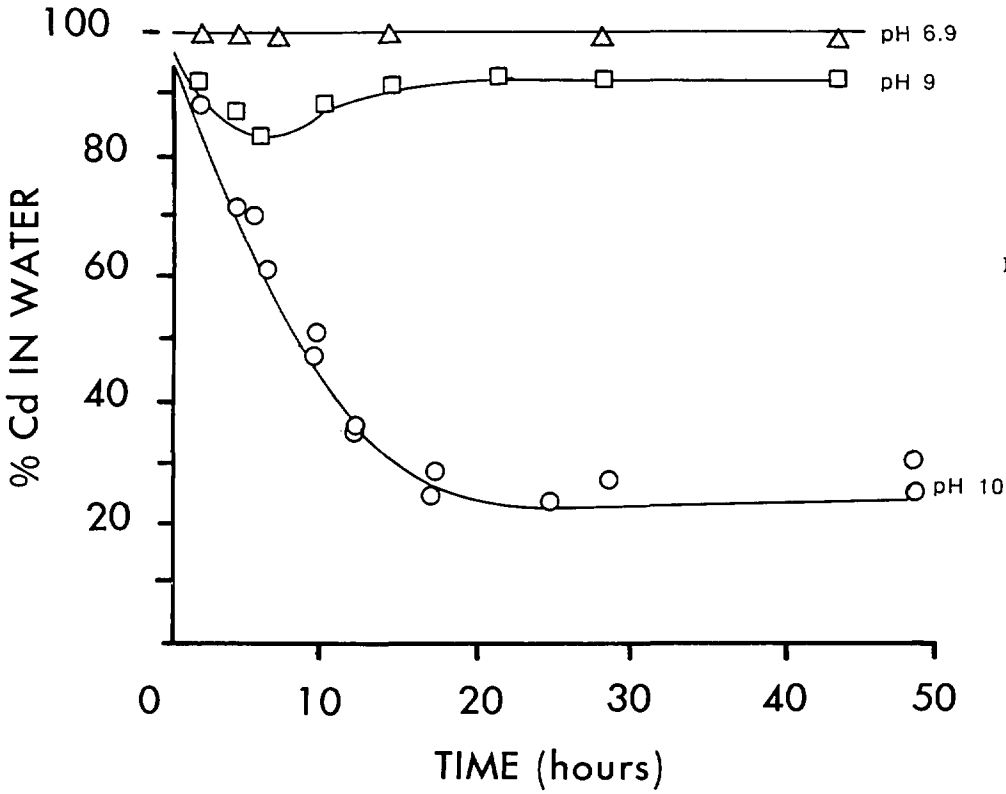

Figure 11–Percent of ^{109}Cd in water during storage asa a function of pH of solution. Soft glass.

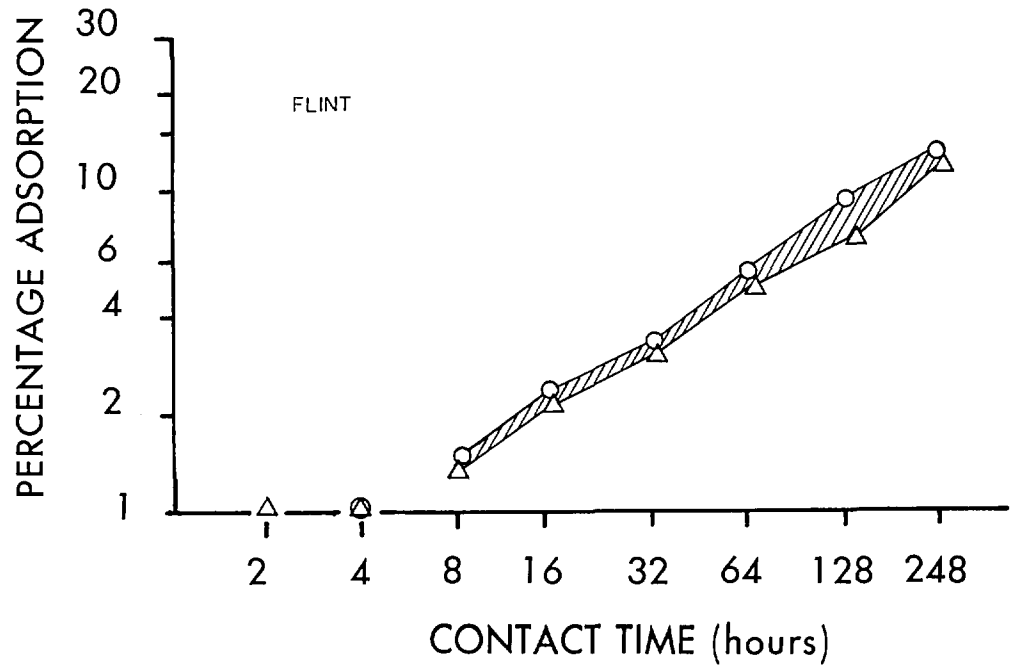

Figure 12–Adsorption range profiles for flint beakers at the 1.0 mg per liter silver concentration. Maximum (o) and minimum (Δ) plotted values are from the distilled water series.

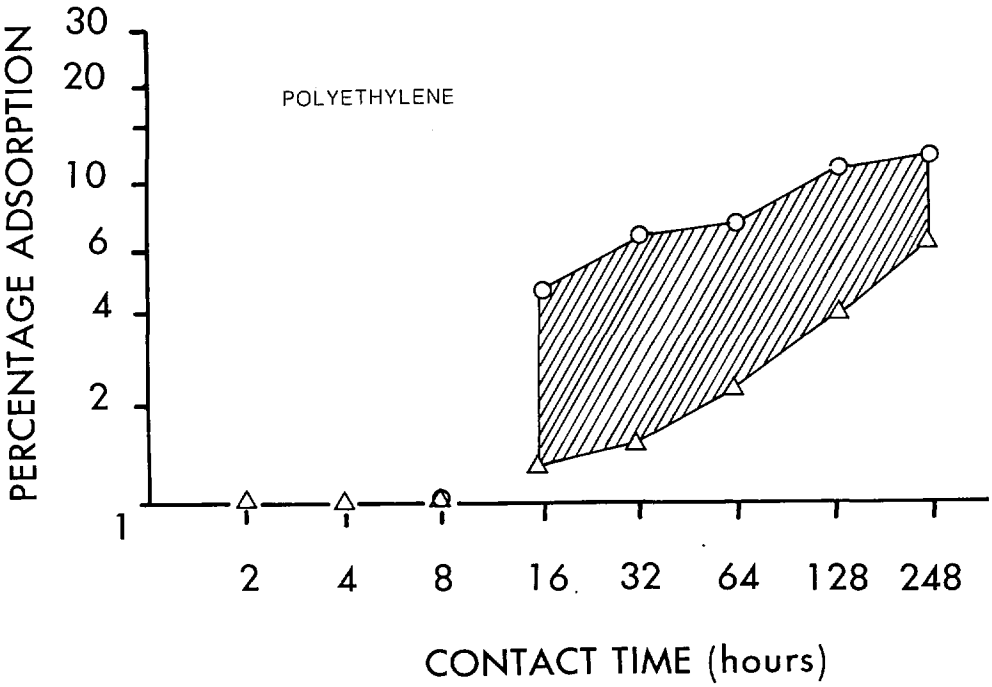

Figure 13–Adsorption range profiles for polyethylene beakers at the 1.0 mg per liter silver concentration. Maximum (o) and minimum (Δ) plotted values are from the distilled water series.

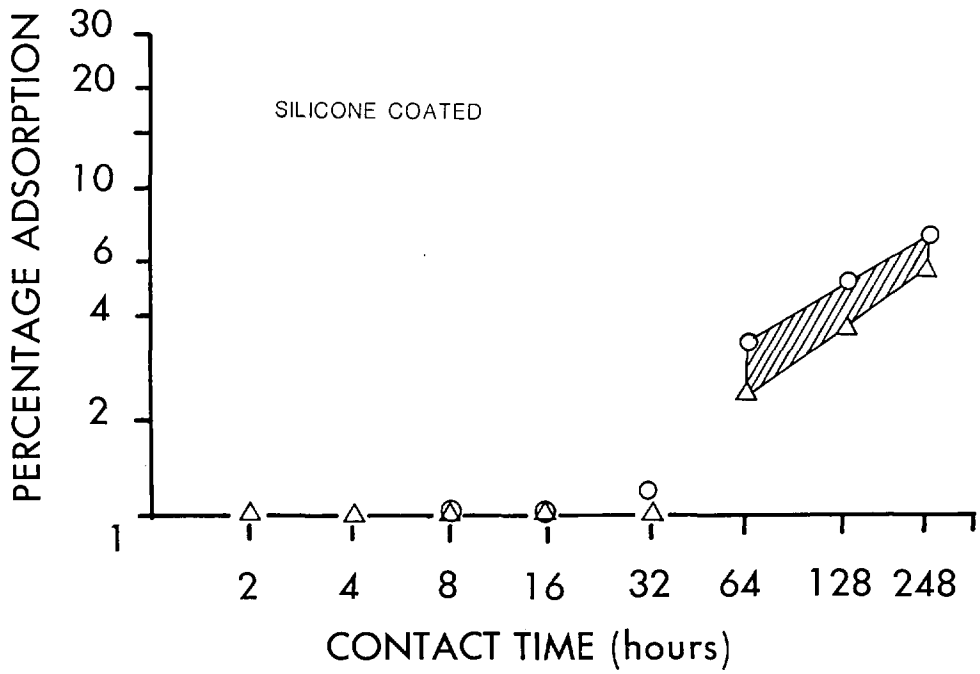

Figure 14–Adsorption range profiles for silicone coated beakers at the 1.0 ng per liter silver concentration. Maximum (o) and minimum (Δ) plotted values are from the distilled water series.

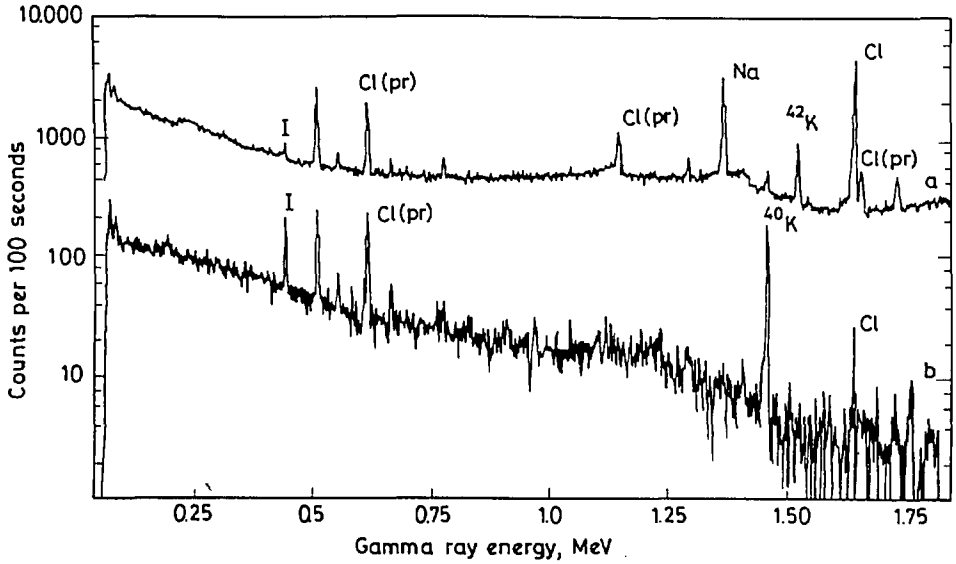

Figure 15–Typical γ-ray spectra of neutron irradiated saliva using a Ge(Li) detector—(a) raw saliva (b) extracted CCl_4 phase.

varied and different techniques can be employed for its isolation. If wet chemistry must be performed on the sample prior to neutron irradiation, it is important that the reagents employed be free of interfering activity. For example, for our aluminum determination various reagents have trace quantities of aluminum as can be seen in table 1.

Nitric acid is generally the reagent of choice in wet digestion procedures and table 2 shows that different grades of nitric acid contain varying amounts of many trace elements [12]. The reagent of choice in our laboratory for wet digestion is Baker Ultrex nitric acid.

Urine is a nonhomogenous admixture whose concentration can vary over wide limits. In some determinations it may be necessary to take into consideration the specific gravity of the urine. For example, it may be quite critical in solvent extraction procedures such as those in our determination of total iodine in urine by the Szilard-Chalmers technique, as can be seen in table 3.

Table 1. Aluminum contents of reagents.

Reagent	Mfg.	Concentration	Al	Assay
HNO_3	Baker-Ultrex	Conc.	0.02	μg/ml
HNO_3	Du Pont	Conc.	0.04	μg/ml
HNO_3	Fisher	Conc.	0.22	μg/ml
HNO_3	Mallinckrodt	Conc.	0.36	μg/ml
HF	Mallinckrodt Ar	48% WT	0.25	μg/ml
HF	Matheson Reagent	48% WT	0.69	μg/ml
H_2O	Fontenelle Springs Distilled		0.01	μg/ml
H_2O	McGaw-Distilled For Irrigation		0.03	μg/ml
RESIN	Bio-Rad Ag 50W-X8		0.25	μg/g Resin
RESIN	Dowex 50W-X8		4.7	μg/g Resin

Table 2. Impurity concentration in nitric acid.

Element	Sub-boiling Distilled (ng/g)	ACS Reagent Grade acid (ng/g)	Commercial High purity (ng/g)
Pb	0.02	0.2	0.3
Tl	—	0.2	—
Ba	.01	8	—
Te	.01	0.1	—
Sn	.01	0.1	1
In	.01	—	—
Cd	.01	0.1	0.2
Ag	0.1	0.03	0.1
Sr	.01	2	—
Se	.09	0.2	—
Zn	.04	4	8
Cu	.04	20	4
Ni	.05	20	3
Fe	.3	24	55
Cr	.05	6	130
Ca	.2	30	30
K	.2	10	11
Mg	.1	13	—
Na	1	80	—
Total Impurity	2.3 ppb	220 ppb	240 ppb

Table 3. Variation of extraction yield with specific gravity and osmolarity for urine collected at different times from one individual.

Extraction Yield (%)	Specific Gravity
63.2	1.031
63.9	1.026
67.3	1.017
70.5	1.011
81.2	1.007
84.9	1.006

Since urine contains sodium and chlorine, it is necessary to perform a separation of the desired trace element from dissolved NaCl in order to obtain a viable analysis. Table 4 is a summary of the pre- or post-neutron activation chemistry required for the trace elements listed in figure 1. While this list is not all inclusive for all trace elements that can be detected in urine, it represents some of those which have the greatest importance in the study of disease and health states. It should be noted that the procedures summarized for Ca, V, Cu, Mn, and Mg are specific for tissue and will probably need to be modified accordingly for a urine matrix.

Conclusions

It would seem that the type of collection and storage procedure and pre- or post-NAA treatment of urine depends on the specific trace element; that is, its inherent physical and chemical properties. The following generalizations can be made.

1) Polyethylene or teflon containers may be the most suitable.
2) Whether any preservative should be added would depend upon the stability of the trace element and its tendency for surface adsorption. Preferably preservatives should contain no radioactivatable elements for maximum efficacy.
3) Freeze drying or packing urine shipments under dry ice needs to be explored on an individual basis considering all factors involved.
4) Each pre- or post-NAA treatment is specific and optimized for the trace element analyzed. As a general suggestion, it is important to minimize the number of operational steps and choose reagents that do not contribute radioactivity in the activation step.

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Table 4. Summary of pre- or post-NAA treatment for trace element analysis.

Element	Ref	Digest	Acid Sample Ratio	Temp (°C)	Resin	Elution Solution	Elution Volume	Fraction Analyzed	Pre or Post Chem.
Al	[2]	HNO_3	1:1	65	50WX8	1 M HNO_3	19 mL	Resin	Pre
V	[15]	HNO_3	1:1	65	50WX8	1 M HNO_3 0.5 M HNO_3 4 M HN_4OH	10 mL 10 mL 6 mL	Eluent	Pre
Se	[16]	H_2SO_4	1:5	50	Oxidation to red amorphous selenium			Precipitate	Post
I	[5]	No			HNO_3–H_2O_2 oxidation			CCl_4 Extraction	Post
Ca	[17]	No			Precipitate with Sat. $(NH_4)_2$ C_2O_4			Precipitate	Pre
Cu	[18]	HNO_3	1:1	65	Solvent Extraction (S.E.) HCl, dithizone, CCl_4			Organic	Post
Zn	[18]	HNO_3	1:1	65	S.E., Acetate, Sodium Thiosulfate, Dithizone, CCl_4			Organic	Post
Mn	[18]	HNO_3	1:1	65	S.E., Acetate, Chloroform, dithiocarbamate			Organic	Post
Mg	[18]	HNO_3	1:1	65	S.E., Acetate, n-butamine, TTA in chloroform			Organic	Post

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Journal of Research of the National Bureau of Standards

Technical News Briefs

NBS Technical Developments

NEW 10-FOLD QUASICRYSTAL STRUCTURE FOUND

A new type of quasicrystal structure has been found at the National Bureau of Standards (NBS) by NBS scientists and guest workers from Johns Hopkins University. The 10-fold (decagonal) quasicrystal is a totally different kind of aperiodic crystal from the first one of its kind discovered at NBS three years ago by a guest worker [1] from the Israel Institute of Technology.

The present discovery [2] proves that other types of nonperiodic crystal structures exist and can be made in materials. The 10-fold crystal structure was found in an alloy of aluminum and manganese produced by the same melt spinning technique to rapidly solidify metals that Shechtman used when he found icosahedral symmetry (six intersecting 5-fold axes) in a related alloy.

The crystal structures in these alloys defy a 100-year old theory in crystallography that is based on the assumption of periodicity, which requires all crystal structures to have only 2-, 3-, 4-, and 6-fold symmetry axes in various combinations to fit in one or another of the 32 classical diffraction pattern symmetries. Icosahedral and decagonal symmetry do not fit in these rotational patterns and therefore cannot be periodic. They belong to a new classification of crystals that have quasiperiodicity and are called "quasicrystals." Bendersky's 10-fold crystal is periodic along one axis and quasiperiodic along the other two; Shechtman's alloy is quasiperiodic in all directions.

Until Shechtman's discovery, there had been no exceptions to the theory and all crystals conformed to the 32 symmetries. Without this restriction of periodicity in crystal structures the number of possible symmetrics is infinite.

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BOND ENERGY VALUES IN SIMPLE HYDROCARBONS INCREASED

The values for the energies of the primary, secondary, and tertiary C-H bonds, which for nearly 40 years have been accepted to be 410 ± 4, 397 ± 4, and -385 ± 8 kJ/mol, respectively, have been revised upwards by recent work at NBS. The new values for the bond energies are 422 ± 2, 414 ± 2, and 402 ± 2 kJ/mol.

Bond energies are basic quantities for any general theory of chemical reactivity, and are needed for the modelling of any complex chemical processes such as high temperature pyrolysis or oxidation. The revised values for the C-H bond energies are a major step towards obtaining a complete understanding of the hydrocarbon cracking problem in terms of elementary chemical reactions.

The revisions result from a critical evaluation of data on elementary chemical kinetic processes. The work, supported by the Basic Energy Sciences Division of the Department of Energy, is aimed at the development of a data base of elementary kinetic processes for the computer simulation of combustion-related phenomena.

Because the rate constants of elementary chemical reactions are crucially dependent on reaction energetics, chemical kineticists are able to

infer bond energies from rate constants. Although the previously accepted values for C-H bond dissocia-tion energies were derived from experimental methodologies now known to be deficient, work in the 1960's led to results which appeared to support those values. However, in recent years, there has been a gradual accumulation of rate constant data which differed by 1 to 3 orders of magnitude from predictions based on the accepted values of the bond dissociation energies. Since these results are usually assessed individually, the discrepancies have usually been dismissed as the effects of unknown experimental artifacts.

The NBS effort [1] involved a critical examination of these previously rejected results on the rate constants of alkane and alkyl radical decomposition processes. Although these data were in gross disagreement with the values which would be expected on the basis of the accepted thermodynamics of the processes and the well-established kinetics of the reverse combination reactions, it was found that none of the measurements could be rejected. The next step was to establish thermodynamic self-consistency as a requirement for the data base, in order that the second law of thermodynamics should not be violated. Using recent theoretical and experimental entropy data, the NBS effort then demonstrated that the extensive body of rate data did lead to internally-consistent higher values for the bond dissociation energies.

The proposed new values for the bond energies make possible the resolution of many controversies in the chemical literature. For example, use of the previously accepted bond energy values led to a prediction that there should be an energy barrier for certain di-radical recombination processes (for example, ring closure of a tetramethylene species to give cyclobutane), but theoreticians had never been able to reproduce such a barrier. The revised bond energy values remove the predicted barrier. Using the new values, the rate data for alkane-akyl radical systems now show the systematic trends which would be predicted by any reasonable theoretical model. Therefore, the results of this critical data evaluation can be considered to be a validation of the kinetic methodologies for the determination of molecular stabilities.

The NBS work demonstrates the contribution that data evaluation within a proper theoretical framework can make toward a fundamental understanding of physico-chemical problems. In particular, the work demonstrated the importance of distinguishing between errors of measurement and errors of interpretation.

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NBS RESEARCHER DEVELOPS PARAMETRIC ELECTROMETER TO IMPROVE CHEMICAL ANALYSIS

Scientists who use mass spectrometers to perform medical, environmental, or industrial analyses at low concentrations—parts per million or below—should find a new device helpful in improving their measurement systems. Called a parametric electrometer, the invention was designed to measure extremely low levels of electrical current through the computer-controlled data collection systems of mass spectrometers. The device is an improvement over existing models because of its ability to measure signals at levels close to the theoretical limit of noise. It features variable response times at the flick of a switch, allowing an operator to control response time and noise level, two important factors in performing a mass spectrometric analysis at low levels. Though the electrometer was designed for use with NBS-developed thermal ionization mass spectrometers, it may be used with other similar types of instruments and measurement systems.

For further information contact Ronald W. Shideler, National Bureau of Standards, Gaithersburg, MD 20899.

FIRST NEUTRON OBSERVATION OF MAGNETISM IN A MULTILAYER MATERIAL

NBS scientists in collaboration with colleagues from the University of Illinois, have performed the first neutron-scattering study [1] of the magnetic order in a epitaxial multilayer of the rare earth metals dysprosium (Dy) and yttrium (Y). Multilayers are a very new class of materials which are prepared by molecular beam epitaxy techniques and consist of single-crystal layers of magnetic Dy and non-magnetic Y, each only 40 angstroms thick and stacked 64 bilayers high. These novel materials, combined with the unique probe of magnetic neutron scattering, have enabled the determination of the remarkable result that the helical magnetic order in the Dy layers is propagated through the intervening Y layer and into the next Dy layer without loss of phase coherence.

The neutron experiments allowed details of the helical magnetic order and its range, which was greater than 5 bilayers, to be examined directly in a manner not possible with conventional techniques.

These results are a dramatic confirmation, not previously available for ordinary alloy systems, of the existence of a peak in the generalized susceptibility of metallic yttrium at a wavelength close to that of the helical ordering found in other rare earth metals. These results have significant implications on the tailoring of such layered structures for specific magnetic applications.

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COMPOSITIONAL MAPPING: QUANTITATIVE ELECTRON MICROPROBE IMAGING DEVELOPED

Techniques to produce the first fully quantitative *compositional images with micrometer spatial* detail by means of the electron probe microanalyzer have been developed [1,2]. The imaging techniques involve the acquisition of digital images based upon direct measurement of x-ray counts at each pixel in an image. The full quantitative analysis procedure which is normally employed for analysis at individual points is then applied at each pixel in the scan, including correction for detector dead-time, background, spectrometer, defocussing, standardization, and matrix correction. The resulting images consist of quantitative compositional values which have equivalent accuracy and precision to conventional single-point analyses. Techniques have also been developed for quantitative display of these compositional images. The most successful technique has been the use of color encoding based on the so-called "thermal color" scale, in which the sequence of colors assigned to the intensity scale is that which is observed when an object is heated: various shades of red, orange, yellow, and white. Such encoding provides an intuitive scale in which the position in the color sequence is proportional to the sensation which the human eye receives. With this scale, concentrations ranging from 0.1 to 10 weight percent have been successfully displayed in a single image. The first application of the digital compositional mapping technique has been in the study of diffusion-induced grain boundary migration in polycrystalline binary alloys. In this application, zinc distributions with concentrations as low as 0.1 weight percent were mapped with micrometer spatial resolution at grain boundaries in polycrystalline copper.

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NON-TOXIC "NATURAL" BONE CEMENT DEVELOPED BY DENTAL SCIENTISTS

A new calcium phosphate cement for the repair of teeth and bone that is *wholly compatible with living tissues in the body* has been developed by dental scientists at NBS. Researchers from the Paffenbarger Research Center (PRC) of the American Dental Health Foundation at the bureau have developed the cement as a part of the PRC research program to study calcium compounds for use in dental treatments. The new material, which can be described as a "natural cement" because of its biocompatibility, is based on a mixture of two calcium phosphates, tetracalcium phosphate and brushite [1]. When the two compounds are combined they form hydroxyapatite, the primary mineral in teeth and bone. Laboratory studies by the American Dental Association (ADA) in Chicago confirm the cement's biocompatibility with soft and hard body tissues, and the ADA reports there is no toxic reaction. Laboratory research also shows that because of the new cement's setting properties, the material has significant potential use in a wide variety of dental treatments and in other health care applications. In dentistry, the cement may be used as a base for dental fillings, as a filler for root canals, as a desensitizing agent for the roots of exposed teeth, and as a filler in bone sockets after tooth extraction. Preliminary studies also indicate that the new material *may have use as a bone cement to set prosthetic knee and hip implant devices*. The scientists report it will be at least two years before clinical studies on some of the cement's dental applications are completed and the material is available to dentists. It probably will be five years before the cement is approved for use in medicine.

References

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International Comparisons of National Standards

U.S. AGREES WITH ITALY AND AUSTRALIA TO RECOGNIZE THE EQUIVALENCE OF EACH OTHER'S NATIONAL STANDARDS FOR THE SIX BASE UNITS OF THE SI SYSTEM.

In signing ceremonies in October 1985, the NBS Director, Dr. Ambler, ratified an agreement with the Istituto Elettrotecnico Nazionale "Galileo Ferraris" of Italy to recognize the calibrations in length and temperature done by either institution as valid in the other country. The agreement is to facilitate the sale of products between the two countries.

In a similar ceremony in Gaithersburg, MD, NBS signed an agreement with the National Measurement Laboratory of the Australian Commonwealth Scientific and Industrial Research Organization (CSIRO) to recognize the equivalence of the national standards for the six base units of the SI system.

Both Italy and Australia are signatories to the Convention of the Meter and determine, like the U.S., their basic units of measurements in accordance with the definitions for SI units adopted by the General Conference of Weights and Measures, an intergovernmental body that is the recognized world authority on physical quantities and the way they are measured.

COLLABORATION IN ELECTROLYTIC CONDUCTANCE STANDARDS WITH HUNGARY

Hungarian electrolytic conductance standards were recently compared with those maintained at NBS by the Inorganic Analytical Research Division. Mrs. Anna Tomek from the National Office of Measures (OMH) in Budapest, Hungary, spent three weeks at the NBS Conductance Facility to confirm the compatibility of the two nations' measurement systems in this important field. Further intercomparisons will be conducted when NBS issues a Standard Reference Material for electrolytic conductivity. The collaboration between NBS and OMH continues as research is conducted into improving the accuracy of electrolytic conductivity measurements and standards and this work complements the effort in standardization of pH measurements that was begun last year between NBS and OMH.

For further information contact William F. Koch, National Bureau of Standards, Gaithersburg, MD 20899.

INTERNATIONAL COMPARISON OF CLOCKS

This past year NBS scientists in Boulder, CO succeeded in comparing two clocks thousands of miles apart with an accuracy that amounts to one second in the course of a million years. David W. Allan achieved the most accurate clock comparison across the Pacific with colleagues at the Radio Research Laboratory in Tokyo. They checked the U.S. primary standard in Boulder, CO against the Japanese primary standard. The intermediary was the U.S. Department of Defense's Global Positioning System (GPS) of navigation satellites with their on-board atomic clocks.

This intercomparison brings Japan into the community of nations that contibute to the definition of the international atomic second, thus making International Atomic Time still more accurate. Besides the U.S., other nations that contribute to the definition of the second are Canada and the Federal Republic of Germany.

As a result of this intercomparison, the National Aeronautics and Space Administration, and the scientific community will be able to obtain a better time reference for deep space probes and the investigation of pulsars, the still somewhat mysterious emitters of radio waves in the universe.

Most industrial nations use laboratory clocks based on the vibrations of cesium atoms as their national standards of time. Prior to the availability of access to the GPS, international comparisons had to be done with portable clocks carried between national laboratories. Now the comparison can be done quickly by reviewing an electromagnetic signal from a satellite that is in common view of both time laboratories.

For further information contact David W. Allan in Boulder, CO 80303.

New Services From NBS

BUREAU STARTS TELECOMMUNICATIONS TESTING SERVICES

NBS is establishing a voluntary laboratory accreditation program (LAP) for laboratories that perform electromagnetic compatibility and telecommunications equipment testing. The program was

New Standard Reference Materials*

MATERIALS AIMED AT BOOSTING ACCURACY OF LEAD-IN-BLOOD TESTS

To improve the reliability of blood tests for determining long-term exposure to lead, NBS has developed a Standard Reference Material (SRM) for calibrating the laboratory instruments that measure minute amounts of lead in blood. Wide-ranging results—some in error by as much as 200 percent—have been obtained in past studies when blood samples with known lead concentrations were sent to various clinical and analytical laboratories for analysis. Though analyses have improved in recent years due to refinements in technology and increased quality control, there is still a need for a lead-in-blood standard as a reference for checking instrument accuracy and analytical methods. The new SRM is issued to fill this need. It is available in units that each contain four bottles of varying lead concentations in porcine (pig) blood: 5.7, 30.5, 49.4, and 73.2 micrograms per deciliter.

For technical information contact D. Reeder.

STANDARD REFERENCE MATERIAL FOR BETTER BREATH AND BLOOD ALCOHOL MEASUREMENTS

Ensuring accuracy in the instruments law enforcement agencies use to measure alcohol content in breath and blood is NBS' goal in producing a SRM that is now available. Called Ethanol-Water Solutions, the SRM was developed after law enforcement agencies requested an NBS alcohol/water reference material be produced that could be cited in court as a reliable standard for gauging breath or blood alcohol concentration. (Courts in some states and localities require validation of measurement techniques in drunk-driving cases.) The SRM can be used to calibrate and standardize alcohol-measuring equipment as well as to evaluate the daily laboratory reference solutions used in alcohol determinations [1,2,3]. SRM 1828 consists of five vials of ethyl alcohol (ethanol)/water solutions certified for these percentages by weight: 95.629 0.2992 (two vials are included at this concentration, and 0.1487 (two vials).

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SRMs can be ordered from the Office of Standard Reference Material, NBS, Gaithersburg, MD 20899, telephone 301-921-2045.

MADE-IN-SPACE POLYSTYRENE SPHERES ARE NEW NBS STANDARD REFERENCE MATERIAL

A new size primary particle standard is available from NBS. The Standard Reference Material, designated SRM 1960, is a suspension of 10 micrometer polystyrene spheres in water. Previously only .3 and 1 micrometer standards were available. The spheres are used for calibrating particle size measuring instruments and optical and electron microscopes.

The 10 micrometer size is especially useful because blood cells are approximately this same size and the accurate measurement of blood cell size is the basis for many medical diagnostic instruments. The accurate measurement of particles of this size is also necessary to implement a new air pollution standard which defines 10 micrometer particles as a cut off. The U.S. Pharmacopia is also developing a standard for pharmaceutical materials based on 10 micrometer size particles.

The spheres from SRM 1960 were grown aboard the space shuttle Challenger using a process developed for NASA by Lehigh University. It is very difficult to grow acceptable spherical particles larger than a few micrometers on earth. The process for growing spheres making use of the very low gravity during space flights results in more spherical and uniformly sized particles and much greater yield than particles of this size grown on earth. Because the process of growing the particles is time consuming, the time in space of five shuttle flights was required to obtain 10 micrometer spheres. The spheres are the first product manufactured in space.

The particles were provided by NASA to NBS for measurement and distribution. The NBS interest and expertise in micro-dimensional metrology was used to measure the diameter of the particles by a new technique developed specifically for the task. The new measurement technique is called "center distance finding." It is an optical technique related to array sizing [1]. The diameter measurements were also checked using electron microscope technqiues developed by NBS for one micrometer spheres [2].

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HOLMIUM OXIDE SOLUTION WAVELENGTH STANDARD FROM 240 TO 650 NM

The NBS Office of Standard Reference Materials has issued SRM 2034, Holmium Oxide and Perchloric Acid Solutions for use as a wavelength standard. This SRM is intended to be used as a reference standard for verifying the accuracy of the wavelength scale of spectrophotometers in the ultraviolet and visible spectral region (240 to 650 nm).

SRM 2034 consists of a solution of 4 percent holmium oxide in 10 percent perchloric acid in a fused silica cuvette of a nominal light path of 10 mm that fits the sample compartment of conventional spectrophotometers. The cuvette has a filler neck that was flame sealed after the cuvette was filled with the holmium oxide solution. Measurements of spectral transmittance of the solutions [1] were made by means of a high-precision spectrophotometer over the wavelength range 200 nm to 680 nm. The wavelength scale accuracy of this instrument was verified by extensive measurements of mercury and deuterium emission lines.

The measurements of spectral transmittance of the holmium oxide solutions were made as a function of temperature, purity, concentration, and spectral bandwidth. Analysis of the uncertainties associated with these parameers and the uncertainties associated with the calibration of the instrument wavelength scale and the data analysis have resulted in an estimated uncertainty of ± 0.1 nm for the determination of the wavelengths of minimum transmittance of the holmium oxide solution.

The certificate issued with SRM 2034 provides instructions for its use. The full details of the preparation, certification, and use of this SRM are described in NBS Special Publication 260-102. *Holmium Oxide Solution Wavelength Standard from 240 to 650 nm*.

References

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[2] NBS Special Publication 260-102, Holmium Oxide Solution Wavelength Standard from 240 to 650 nm.

New Standard Reference Data

STEAM GROUP ISSUES NEW STANDARDS FOR PLANT DESIGN/OPERATION

The International Association for the Properties of Steam (IAPS) has issued a new set of equations and formulations that will help scientists and engineers in designing power plants, specifying equipment, and describing the performance of boilers, turbines, and other equipment used to generate steam power worldwide. The new information was approved by IAPS representatives from eight of the world's leading industrial nations; the United States, Canada, Czechoslovakia, Federal Republic of Germany, France, Japan, the United Kingdom, and the U.S.S.R. The new equations and formulations are for use in predicting the thermal conductivity and viscosity of ordinary water in liquid and vapor, as well as in determining the surface tensions of heavy water. The group also released new "skeleton" tables for determining the specific volume and enthalpy of ordinary water and steam that include evaluated data at specific grid points over a wide range of temperatures (freezing point of water to 1000 degrees Celsius, and pressures from zero to 1GPa (=10 kilobar).

To obtain copies of the new 1985 IAPS standards, contact Howard J. White, Jr., National Bureau of Standards, Gaithersburg, MD 20899.

COMPILATION OF MAGNETIC DIPOLE LINES

NBS has just completed the first compilation of all the atomic and ionic magnetic dipole (M1) lines that have been observed, along with all possible predicted values, arising from transactions within the $2p^n$ and $3p^n$ ground configurations [1] for $2 \leqslant 42$. Such spectral lines, usually called "forbidden lines," have long been used for the analysis of astrophysical plasmas (measurement of temperature, abundances of elements, Doppler shifts, etc.) and have recently become important for similar diagnostics of fusion energy devices. Identifications of these lines have appeared in the astrophysical literature for the past 40 years and in many other journals since their discovery in tokamaks for the last 15 years. Magnetic dipole radiation is typically 1 million times weaker than ordinary light (electric dipole) and is usually seen only in density (10^{13}/cm^3) extended light sources such as the solar

corona, nebulae, and laboratory magnetically confined plasmas such as tokamaks. The latter is the principal machine designed to generate fusion energy. This radiation is of special importance because it is usually the only long wavelength radiation (>2000 Å) produced by highly ionized atoms. It may therefore, be conveniently observed through air.

This is the first compilation of its kind to include all atoms from Be to Mo and will serve both the astronomical and laboratory plasma communities. The compilation contains 384 known lines and 1660 calculated wavelengths in the range of 100 A (10 nm) to 26 nm. The calculated values are obtained from accurately known energy levels for Be to Ni and from semi-empirical calculations made for Cu to Mo that have led to the discovery of many more of these lines. Transition probabilities obtained mainly from relativistic calculations are included, as well as ionization energies for the radiating ions.

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Journal of Research of the National Bureau of Standards

Conference Reports

THE INVESTIGATION OF FUNDAMENTAL INTERACTIONS WITH COLD NEUTRONS

by G. L. Greene

Quantum Metrology Group
Center for Basic Standards
National Bureau of Standards

The past decade has seen the development of a remarkably fruitful line of experimental inquiry in which beams of low energy neutrons are used for the investigation of fundamental interactions. This work has included studies of parity and time reversal symmetry violation, baryon nonconservation, weak interactions, fundamental constants, charge conservation, and neutron interferometry as well as a variety of other studies. This work has had important implications in particle physics, nuclear physics, astrophysics and cosmology. In the past, the geographical focus of this work has been the High Flux Reactor at the Institut Laue-Langevin (ILL) in Grenoble, France with substantial efforts at other reactors in Germany and the Soviet Union. While researchers from the United States have provided a degree of leadership in this field, a stronger U.S. contribution has been frustrated by the absence of suitable low energy neutron facilities in the U.S.

The proposed National Bureau of Standards Cold Neutron Facility will provide the United States with a world class facility for such investigations. The NBS facility will be unique in the U.S. and it is not likely that a competitive U.S. source will be available within the next decade. The United States and the National Bureau of Standards are therefore presented with the opportunity to provide leadership in an exciting and important area of scientific endeavor.

In order to provide guidance for such a program, the Department of Energy and the National Bureau of Standards sponsored a workshop to review "The Investigation of Fundamental Interactions with Cold Neutrons." This workshop, held at Gaithersburg, 14–15 November 1985, brought together more than 50 leading practitioners in this field from U.S. laboratories and universities as well as from Europe and Canada. A total of 25 talks were given.[1]

In the first talk, N. F. Ramsey (Harvard) reviewed the current status of the experimental knowledge of the properties of the neutron. Following this introduction, J. M. Rowe (NBS) discussed the design plans for the NBS National Cold Neutron Facility and W. Mampe (ILL) described existing facilities at the High Flux Reactor at ILL.

The following session concerned the investigation of the details of neutron β-decay. J. Byrne (Sussex) discussed the theoretical implications of neutron β-decay, both for the theory of weak interactions as well as for astrophysics. It was clear from Byrnes' presentation that an accurate (< 1%) measurement of the mean neutron lifetime, τ_n, would provide an important input into current theories of nucleosynthesis, stellar dynamics and cosmology. Byrne also noted that the uncertainty in τ_n provides a major uncertainty contribution to the theories which predict the solar neutron flux. Following this talk which emphasized the theoretical importance of τ_n, J. Robson (McGill) reviewed the current experimental status and described the na-

[1] G. L. Greene, editor, *The Investigation of Fundamental Interactions with Cold Neutrons*, NBS Special Publications 711 (1986).

ture of the problems which must be faced in any accurate experimental determination of τ_n. Among these problems is the relatively prosaic but, nonetheless technically daunting problem of the absolute determination of a neutron flux. This was reviewed further by D. M. Gilliam (NBS).

There followed a series of talks concerning new experimental efforts to measure τ_n. J. Wilkerson (Los Alamos) described an elaborate electron-proton coincidence counter which, by its large volume, promises very high count rates. Most exciting was his description of a new cryogenic, calorimetric neutron detector which promises to provide a substantial improvement in the accuracy of neutron flux determinations. J. Byrne (Sussex) then described an innovative new technique for measuring τ_n by counting decay protons which have been stored in a Penning trap and subsequently accelerated. This proposal was striking for its careful consideration of systematic effects. D. Dubbers (ILL) and W. Mampe (ILL) then discussed several ongoing experiments at ILL aimed at the determination of τ_n. Perhaps most promising is the effort by W. Paul and associates to trap extremely low energy polarized neutrons in a hexapole magnetic field and observe their population decrease.

The last two talks on the first day of the workshop concerned the details of neutron β-decay and tests of the standard V-A model of the weak interaction. In a very stimulating contribution S. J. Freedman (Argonne) discussed the importance of the measurements of the various polarization and momentum correlation coefficients in neutron β-decay. He also reported very beautiful results from the Heidelberg/ILL/Argonne collaboration measuring the electron asymmetry in polarized neutron decay. Tom Bowles (Los Alamos) then described a new experiment aimed at improving, by at least one order of magnitude, the knowledge of T-violating triple correlation in neutron decay.

The second day's morning session was focussed on studies of parity and time reversal symmetry violation in interactions involving neutrons. E. Adelberger (Univ. of Washington) reviewed the importance of low energy nuclear physics experiments which probe weak interactions by the observation of parity violation. He pointed out that while such experiments are very difficult, they affort a unique window on certain details of the weak interaction P. K. Kabir (Virginia) then reviewed the nature of time reversal symmetry violation and suggested possible experimental tests.

In the following two talks, R. Wilson (Harvard) and B. Heckel (U. of Washington) reviewed existing experimental studies of parity violation and suggested future experimental directions. It seemed clear that the most desirable, and unfortunately the most difficult measurements, involve the interaction of neutrons with very simple nuclei. The simplest of course is the unbound proton in liquid hydrogen targets.

The focus of the workshop then changed from thea investigation of the neutron as a particle to the study of the neutron as a wave. S. Werner (Missouri) reviewed recent experiments involving single crystal neutron interferometry. He was followed by H. Rauch (Vienna) who proposed a variety of ingenious perfect crystal optical devices to manipulate monochromatic neutron beams. A. Zeilinger (Vienna) discussed the prospects for extending neutron optical devices to long wavelengths (>20Å).

In discussing how neutron interferometers might be made more sensitive, R. D. Deslattes (NBS) proposed the construction of multi-lithic interferometers having dimensions approaching 1 meter. Such devices would be extremely difficult to construct and operate. However their realization would represent a tour-deforce in precision engineering. Some of the environmental difficulties in neutron interferometry were discussed by J. Arthur (ORNL).

The remainder of the workshop returned to questions of interest in elementary particle physics. In particular, attention was paid to two experiments which probe the frontiers of current particle theory. M. Baldo-Ceolin (Padua) described the efforts to detect baryon nonconservation in the hypothesized $n-\bar{n}$ reaction. J. M. Pendlebury (Sussex) discussed the search for a nonzero neutron electron dipole moment. It was very gratifying to hear of the enormous improvement in neutron fluxes available at ILL for this important experiment. The workshop closed with reports by R. Golub (Max Planck Institut) and T. Dombeck (Los Alamos) on advanced methods for the production of extremely low energy neutrons.

The proceedings of this workshop have been published.