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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

[1] Hopps, H. C., and B. L. O'Dell, Research Needed to Improve Data on Mineral Content of Human Tissues, Federation Proceedings **40** (8): 2112–2114 (1981).

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[3] Hopps, H. C., editor; General Procedures and Protocol for Mineral Analysis of Human Tissues and for Establishing a Coordinated Data Bank (Manuscript has been submitted for publication—1984).