Isotope-dilution techniques: a wave of the future in human nutrition

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In the valuable study reported in this issue, Haskell et al (1) remind us of the utility of using isotope-dilution techniques for evaluating the nutritional status of a group of subjects, in this case the vitamin A status of Bangladeshi surgical patients. In essence, she finds that the estimation of mean liver reserves of vitamin A by isotope dilution agreed well with the direct measurement of them by liver biopsy. Furthermore, a significant linear relation was found between values in individuals obtained by the two techniques. Her study confirms and extends the earlier studies of Furr et al (2) with a much smaller group of better-nourished American adults.

The concept involved is simple; namely, a dose of a labeled tracer, in this case \([10,19,19^{3}H]\)retinyl acetate, is given orally to a subject and then the investigator waits patiently for the equilibration, or a state approaching it, of the labeled tracer with endogenous body reserves of the vitamin. A sample of blood is taken, vitamin A is purified from the serum, and the ratio of the deuterated to the nondeuterated compound is measured by mass spectrometry. The amount of endogenous reserves is related to the extent of dilution of the labeled tracer. Pioneering studies of this technique were conducted in rats by Rietz et al (3) > 20 y ago.

Vitamin A lends itself well to this approach for several reasons: its absorption from the gastrointestinal tract is quite good; \(\approx 50\%\) at the doses used, it is primarily stored in the liver in well-nourished persons, it is rapidly recycled between retinol binding protein in the plasma and various tissues (4), and it is catabolized quite slowly (\(t_{1/2} = 120–150\) d) (5, 6). Thus, several reasonable assumptions can be made about its absorption efficiency, rate of equilibration, and rate of metabolism that allow a quantitative estimation of total body reserves (6). Both the results of Haskell et al (1) and Furr et al (2) show that the approach is clearly valid.

But why do we need isotope-dilution techniques? Why don’t we just depend on the tried and true measurements of serum retinol values? Or, indeed, on a variety of other technically simpler tests for determining vitamin A status (7)? Serum retinol values are unquestionably well tried but they are not very true. Indeed, because serum retinol concentrations are homeostatically controlled and are affected markedly by infections, fever, and other nutritional deficiencies, they are, in fact, a rather insensitive indicator of vitamin A status, particularly among individuals who are not frankly deficient but who are in the twilight zone of vitamin A inadequacy, sometimes termed preclinical or marginal vitamin A deficiency. Other methods, such as the response tests, dark-adaptation tests, and histologic indicators are useful, but only for limited ranges of vitamin A reserves (7). Thus, isotope dilution is the only method that provides a full range of useful measurements from very low endogenous reserves to excessive and even toxic amounts.

So why isn’t everyone using the method? First of all, deuterated substrates are needed, which are either costly if made commercially or require considerable skill in organic synthesis. Second, the isolation and analysis of retinol by mass spectrometry is not a trivial laboratory procedure. Third, the assumptions made thus far about its absorption and metabolism, although feasible, are not yet well defined by a body of experimental evidence. Fourth, the method has been validated by comparing the calculated value of endogenous reserves with an experimental value measured by biopsy. Biopsy values can vary considerably, however, inasmuch as vitamin A is not distributed uniformly in the liver (8). Thus, both the calculation and the measurement have limitations. The observation that they correlate quite well in individuals (1, 2), however, brightens the spirit, although the variance is considerable in these linear plots. Fifth, liver reserves are a smaller portion of total body reserves as the latter become smaller. If the assumption is made that 90% of the endogenous stores are in the liver when only 45% is actually present, the calculated value based on isotope dilution will be double that of the biopsy concentration. Such differences were in fact noted among Bangladeshi subjects with low liver reserves (1). Sixth, the percentage of a dose stored in the liver is higher when liver reserves are adequate than when they are inadequate. Seventh, lipid malabsorption will upset the calculation, ultimately giving overestimates of total body reserves because a smaller portion than expected of the labeled tracer will be absorbed. Eighth, heterogeneity in the population relative to rates of intestinal absorption, of storage, of equilibration, and of irreversible loss will introduce additional variance into the relation. Finally, outliers exist that confoundingly show little relation between calculated and biopsy values; namely, 4 of 31 in the Bangladesh study (1) and 1 of 11 in a US study (2). These subjects, for reasons that were not always clear, apparently did not absorb the labeled tracer efficiently.

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So let us turn the question around. In view of these shortcomings, why is anyone using the method at all? The crux of the matter is that good correlations between calculated and experimental values exist despite these uncertainties. As our assumptions become more precisely based, our experimental techniques simplified and improved, and confounding factors, such as lipid malabsorption, better evaluated, the procedure may well become a key facet of surveys as well as of laboratory-based clinical studies.

Thus far, we have only considered the use of labeled tracers in equilibration studies with particular reference to vitamin A. Such approaches are also applicable to other nutrients, such as vitamin E, folic acid, and vitamin C. Indeed, fruitful studies with labeled tracers have been conducted with all three of these vitamins.

In a broader context, labeled tracers are generally used in quantitative in vivo kinetic studies. Such studies can provide important information for defining the variance in biological rate processes for a given population as well as in determining the requirements and recommended intakes of a given nutrient for a selected population group. Indeed, data of this kind have been used in just this way for vitamin A (9, 10) and for vitamin C (9–11). Further uses of labeled tracers both in determining endogenous reserves of nutrients as well as in quantitating rate processes in human are clearly a wave of the future in human nutrition.

REFERENCES