Methods for Vitamin B₁₂ Assay: Some Comments

To the Editor:
We were interested in the Letter to the Editor, “Comparison of Two Radiosassay Methods for Vitamin B₁₂,” by Drs. Kubesik and Murray [Clin. Chem., 18, 740 (1972)]. We too have been interested in comparative methodology, and we are concerned with some of the comments in that report. We do believe the comparison between our method (1) and that of Wide and Killander (2) worthwhile and, in fact, think the two methods are quite similar. Several points, however, are worthy of note.

First, the authors state that our method required 10–12 h of technician time, which puzzles us, since we always run our assays in no more than 6 h, a time similar to that of the Sephadex method. Such a long interval suggests that Drs. Kubesik and Murray may not have followed the steps outlined (1), which leads to our questioning their comment regarding “precision” of the two assays.

Second, relative to the question of precision or duplicates it is noteworthy that replicate specimens by our method are taken from the original sample whereas in the Sephadex-kit procedure (Pharmacia Labs., Inc., Piscataway, N.J. 08854) replicates obtained from the extracted serum are used. Were our replicates to be done in this manner the test would only require 2 ml of serum (as compared to the 0.5 ml in the Sephadex method).

Third, our assay procedure suggests that an extra 2 ml of serum be used to determine recovery. This step adds re-assurance, since some sera such as those with high or abnormal proteins may pose problems. Incidentally, the recovery step and the incubation can be simultaneous (i.e., it can be done in the carriage of the gamma counter) and this may not have been clear to the authors.

Finally, the Sephadex method does require a complete standard curve with each run, whereas we have demonstrated the validity of a composite curve for laboratories that measure B₁₂ regularly. These comments do not merely express our vested interest in their method but are meant to emphasize some of the aspects that must be considered in the assay for serum vitamin B₁₂. As we mentioned, we do agree that the two methods are quite similar.

References
10. Scrimgeour, A. W., Oral prophylactic urea treatment in sickle cell disease and problems encountered. ibid.

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Furfural: Exogenous Precursor of Certain Urinary Furans and Possible Toxicologic Agent in Humans

To the Editor:
Advanced multiple analytical techniques make it possible to detect and identify many compounds in normal and pathologic urine samples. Recently, different groups of investigators have found traces of several furan derivatives in urines from both healthy and diseased subjects—namely, 2-furoylglycine (1–3), 2,5-furandicarboxylic acid (1–3), and 5-hydroxymethyl-2-furioic acid (3). Also, 5-hydroxymethyl-2-furoylglycine was identified in the urine of a leukemic patient who was excreting an abnormal amount of the free acid (3). It was suspected that at least the first two compounds originate from the diet (2), and several related metabolic pathways involving glucuronic and galacturonic acids for the formation of substituted furans in humans were proposed (3).

These researchers overlook the fact that preformed furfural—either ingested in foods or inhaled via cigarette smoke—must also be recognized as contributing to the overall “normal” synthesis and urinary excretion of certain furans. Small quantities of furfural occur in many foodstuffs, including—among many others—bread, coffee, processed fruits and fruit juices, and alcoholic beverages. In fact, whenever plant or animal tissue containing pentoses or hexoses is subjected to heat, the possibility arises that furfural, 5-hydroxymethyl furfural, and probably other furans as well will be produced. Perhaps most importantly, it is not widely recognized that cigarette smoke contains appreciable amounts of furfural (4) along with lesser quantities of 5-hydroxymethyl furfural (5). Much of our knowledge on the general physiologic action and metabolism of furfural comes from the petroleum industry, in which furfural is used in large quantities in solvent extraction. However, the need for more detailed biochemical and pharmacologic research on furfural has been stressed in numer-
ous articles concerning the congeners of whiskey (6, 7). It is not appropriate to review the literature here, except to state that when furfural is fed to rats, the major urinary end-product is 2-furoylglycine. The same compound occurs in the urine after feeding furoic acid, furfural diacetate, or furfuryl alcohol. It would appear, then, that furfural is changed to 2-furoylglycine by way of furoic acid and that furfuryl alcohol is dehydrogenated in the body to furfural and then further oxidized to furoic acid for conjugation with glycine. Feeding furoic acid also results in the urinary excretion of furfurylacrylic acid and furfurylroylglycine. These few examples should suffice to illustrate the known role that any exogenous furfural would play in the formation of urinary furans.

Among the more interesting literature references on the toxicology of furfural is a report on the production of liver cirrhosis in rats by furfural feeding (8) and a recent study that suggests a cocarcinogenic effect of furfural and benzo(a)pyrene on the respiratory tract of hamsters (9). An examination of the existing literature on furfural leads to the impression that it could enter a wide variety of chemical reactions that may have physiologic significance. It is difficult to understand why this compound has not been investigated more thoroughly as to its potential involvement in lung cancer and (or) other diseases statistically associated with heavy cigarette smoking.

In view of the current resurgence on cancer research, and especially because of the continuing failures to identify the human carcinogenic agent(s) in cigarette smoke, it seems reasonable to propose long-term furfural inhalation (and feeding) experiments with different animal species. Such studies are laborious and expensive, and few institutions could undertake these endeavors. Thus, I am wondering if this could represent a type of project appropriate for Fort Detrick’s new assignments on basic cancer research, considering that this installation has an unparalleled assortment of facilities for doing “aerosol-animal” toxicology. Or perhaps the proposed research might be more suitable for the National Center for Toxicological Research at Pine Bluff, Ark.

References

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Validity of the Lechitin/ Sphingomyelin (L/S) Ratio for Amniotic Fluid Containing Blood

To the Editor:
Most amniotic fluid specimens submitted for measurement of the L/S ratio are free of blood. However, an occasional specimen is contaminated with blood. In an attempt to determine if these specimens were usable, we performed the following study, with use of Borer’s (personal communication to J. R. Elliott, Ph.D., Sept. 1971) modification of Gluck’s (1) method for L/S ratio.

Using only amniotic fluid that was free of blood on arrival in the laboratory, we measured the L/S ratio as usual. Venous blood was obtained from the same patient and was added in various amounts to part of the amniotic fluid. The remaining blood was centrifuged, and the plasma diluted 20-fold with physiological saline. Table 1 gives the L/S ratio for each such specimen from six patients.

Because the plasma L/S ratio apparently influences the L/S ratio in amniotic fluid, we measured the L/S ratio in plasma from 20 pregnant women (32–40 weeks gestation). With use of significant figures of 0.1/1, there was no standard deviation in duplicates. The range of L/S ratio in these plasma specimens was 1.6/1 to 2.4/1, with a mean of 2.0/1.

Assuming other factors in blood beyond the plasma L/S ratio could influence the amniotic fluid L/S ratio, we performed a two-patient study on specimens allowed to stand at room temperature. These specimens contained blood on arrival in the laboratory. The specimen was mixed, poured into three separate tubes, centrifuged, and run at different time intervals. The first specimen had an L/S ratio of 3.3/1 when run immediately. The ratio dropped to 3.2/1 in 10 min and to 2.7/1 in 30 min. The second specimen was 1.9/1 when run immediately, 1.8/1 in 10 min, 1.5/1 in 30 min. Hematocrits on both specimens were less than 1 vol/ dl.

From this study, we conclude that data on the L/A ratio of an amniotic fluid containing blood are only useful to the physician if the sample is centrifuged and assayed immediately, and if the concentration of any contaminating blood is less than 10% by volume.

Reference

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Editor’s Note: A second letter on this subject was more recently received. The authors (J. E. Aldrich and K. A. Nevell, Univ. of Minnesota) did the same experiment and came to the same conclusions: “... amniotic fluids contaminated with maternal blood should be rejected for L/S if the hemoglobin concentration of the

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* Added to amniotic fluid.