bolism. Second, as in the evaluation of any diagnostic procedure, independent verification of the final disease state is essential. Dr. Wilson and his colleagues imply that their 22 patients had a subjective diagnosis of pulmonary embolism. Thus, I would conclude that while their supposition may be direct, their data do not yet support their conclusions.

BARBARA J. McNEIL
Brigham and Women's Hospital
Boston, Massachusetts

Re: Correlation of Contrast Angiography and Histological Pattern with Gallium Uptake in Primary Liver-Cell Carcinoma: Noncorrelation with Alpha-Feto Protein

In their article, Waxman et al. comment that "the hepatitis A antigen also did not correlate with the findings of the gallium scan" (*1*). As there is no known association between hepatitis A (as opposed to hepatitis B) and hepatocellular carcinoma, the relevance of this observation escapes me. Did this represent a repeated typographical error or a misunderstanding?

THOMAS G. COONEY
University of Oregon Health Sciences Center
Veterans Administration Medical Center
Portland, Oregon

REFERENCE

1. WAXMAN AD, RICHMOND R, JUTTNER H, et al: Correlation of contrast angiography and histological pattern with gallium uptake in primary liver-cell carcinoma: Noncorrelation with alpha-feto protein. *J Nucl Med* 21:324-327, 1980

Reply

In response to Dr. Cooney's letter, the statement should read, "the hepatitis B antigen showed no correlation with the findings of the gallium scan." The hepatitis B surface antigen has been shown to have a high association with hepatocellular carcinoma, especially in nonalcoholic cirrhosis (*1,2*). The hepatitis B surface antigen has also been called the hepatitis associated antigen or HAA. The abbreviation was initially used and subsequently changed in the final draft of the paper and incorrectly referred to as hepatitis antigen A, instead of hepatitis associated antigen for hepatitis B surface antigen. We are indebted to Dr. Cooney for his observation. We too know of no known association of hepatitis A and hepatocellular carcinoma.

ALAN D. WAXMAN
Cedars-Sinai Medical Center
Los Angeles, California

REFERENCES

1. PETERS RL: Pathology of hepatocellular carcinoma. In *Hepatocellular Carcinoma*. Okuda K, Peters R, Eds. St. Louis, John Wiley & Sons, 1976, pp 107-108
2. VOGEL CL, ANTHONY PP, MODY N, et al: Hepatitis-associated antigen in Ugandan patients with hepatocellular carcinoma. *Lancet* 2: 621-624, 1970

Re: A Radiometric Microbiologic Assay for the Biologically Active Forms of Niacin

Concerning the paper by Judith A. Kertcher et al: "A Radiometric Microbiologic Assay for the Biologically Active Forms of Niacin" published in this *Journal* (*1*), we have been seriously misquoted. They state: "Another microbiologic assay using the protozoan *Tetrahymena pyriformis* is said to be more sensitive and more specific. This organism, however, is slow and difficult to grow and was shown to respond to several naturally occurring, biologically inactive derivatives of niacin." Their reference for such statements was to our paper "Nicotinic acid assay in blood and urine" (*2*).

The point of our paper was that the niacin requirement of *T. pyriformis* closely—hence usefully—parallels that of man and higher animals (our paper, p. 575), and that niacin derivatives having *no animal activity* were *inactive* (our italics) for *T. pyriformis*, in contrast to their incorrect allusion (*vide supra*) to our work (*2*). Indeed, we stated that "Trigonelline (the betaine of nicotinic acid), which has *no animal activity, proved inert* for *T. pyriformis*" (our italics), and "*T. pyriformis* does not respond to nicotinuric acid, whereas *Lactobacillus plantarum* does, another point of specificity favoring *T. pyriformis*." We never stated, as these authors wrote, that *T. pyriformis* responded to "biologically inactive" niacin derivatives. We did note, as they confirm, that *L. plantarum* does respond to animal-inactive niacin derivatives, e.g., trigonelline and nicotinuric acid (an excretory product), thus making *L. plantarum* less specific than *T. pyriformis* for detecting and estimating biologically active niacin.

In contrast to what the authors state in their paper (*1*), *T. pyriformis* is easy to grow. The maintenance medium consists of glucose and proteose-peptone, and gives as heavy a growth in 3 days as does the basal medium (*2*). Also the *T. pyriformis* method does not require elaborate, costly arrays of equipment and supplies—as for example, a radioactivity counter, radiolabeled metabolites, etc.; one needs only flasks, tubes, pipettes, and a simple photometer. For over 20 years we have routinely used the *T. pyriformis* assay for niacin, and we process over 100 blood or tissue samples with ease in 2 hr, with a turnover time of 3 days. We think their present method (*1*) cannot render as good performance.

We reiterate that *T. pyriformis* mirrors the response of only biologically active niacin; *L. plantarum* does not. The authors should be aware of this point; their quotations badly misguide the reader. Indeed, the protozoan response to vitamins that are biologically active only for man makes them suitable and specific microanimals, parallel to the higher animals, for analyzing vitamins in biologic fluids and tissues. Their clinical correlations make them far superior to radionuclide methods (*3,4*), as for example in the comparison with radioisotope assays for vitamin B_{12} (*4*), a point they left unmentioned before decrying the use of protozoan assays (*1*).

It has long been recognized that assays for B vitamins, mainly based on protozoan assays, including that of *Tetrahymena* for niacin, yield results of especially close clinical validity, as recognized by our invitation to describe assays for vitamins in this (*3*) and the previous edition.

HERMAN BAKER
OSCAR FRANK
New Jersey Medical School
East Orange, New Jersey
S. H. HUTNER
Haskins Laboratories of Pace University
New York, New York

REFERENCES

1. KERTCHER JA, GUILARTE TR, CHEN MF, et al: A radiometric microbiologic assay for the biologically active forms of niacin. *J Nucl Med* 20:419-423, 1979
2. BAKER H, FRANK O, PASHER I, et al: Nicotinic acid assay in blood and urine. *Clin Chem* 6:572-577, 1960

3. BAKER H, FRANK O, HUTNER SH: Vitamin analyses in medicine. In *Modern Nutrition in Health and Disease*, 6th ed, Goodhart RS, Shils M, Eds. Philadelphia, Lea & Febiger, 1980, pp 611-640
4. COOPER BA, WHITEHEAD VM: Evidence that some patients with pernicious anemia are not recognized by radiodilution assay for cobalamin in serum. *New Engl J Med* 299:816-818, 1978

Reply

As pointed out in our earlier publications (*1-4*) we have shared for some time the concern of Drs. Baker, Frank, and Hutner regarding the nonspecificity of the competitive protein-binding assays (CPBAs) for vitamin B_{12} and folates. Indeed, we observed normal test results in patients who were clearly suffering from severe deficiencies of one or other of these essential nutrients. This fact, combined with the reality that the well-proven specific microbiologic growth assays are no longer available to the large majority of clinicians (because they are no longer acceptable to most clinical laboratories), led us to search for new, reliable, sensitive, and specific methods for assay of these and other essential nutrients.

We emphasize that our new radiometric microbiologic assays (RMAs) share nothing in common with these CPBAs; instead we have been able to utilize the selectivity and specificity of the microorganism, but have enhanced specificity, sensitivity, and precision of measurement by careful selection of the appropriate C-14-labeled substrate for each assay. As is detailed in our publications (*1,2,5*), we have reason to believe that coenzyme-dependent ("vitamin"-derived) pathways are being utilized in the presence of the biologically active forms of these essential nutrients, thus enhancing specificity. We believe that we are combining the unique properties of these microorganisms with the sensitivity and precision of measurement of a physical event (i.e., radioactive decay).

In addition, our required "growth" period is only *18 hr* and the measurements of many assay vials are fully automated. Turbid solutions, precipitates, or other debris do not interfere with our RMAs, which are thus readily applicable to previously much more tedious assays, such as that of foodstuffs (*6*).

It certainly was not our intent to offend the authors of the single most definitive publication on the use of *T. pyriformis* for the assay of nicotinic acid (*7*). On the other hand, we found the data as presented in that paper difficult to assess, since no "N", means, or standard deviations are given for any of their measurements. Tables 2 and 3 in that paper indicate a growth response to certain amounts of nicotinuric acid, such that if these were present in whole blood, this biologically inert substance could clearly mask a deficiency state of "niacin" in man.

We do agree that trigonelline does not promote the growth of *T. pyriformis;* however, other compounds such as nicotinmethylamide and nicotinethylamide (although biologically active in dogs (*8*)), do not have the full potency of nicotinic acid and its amide in promoting the growth of *T. pyriformis*. We do know that *L. plantarum* responds to nicotinuric acid (measured turbidimetrically) as pointed out in our paper (*5*); however, we have also indicated that this nonspecificity was largely circumvented when the compound was measured using our RMA.

Our earlier detailed review of available literature on pellagra indicated that all studies, regardless of the methods then available, showed a great overlap of values between patients with pellagra and normal subjects. It has recently been noted that we continue to see this disease in our own medical institutions (*9*) and that the often long-delayed proper diagnosis of the described patients almost certainly represented only the "tip of the iceberg." It was these facts that led us, after development of RMAs for vitamin B_{12} and folate, to apply this entirely new combination of standard technologies and readily available equipment to the assay of "niacin," and then to test it carefully for specificity. For the initiate to this field, we should point out that any equipment for detecting β radiation (from a simple ionization chamber to an automated liquid-scintillation counter) can be adapted for use with our RMAs (*10-12*). Certainly, the automated equipment used by us has only recently been spreading to clinical laboratories. On the other hand, we share the concept of "regionalization" for infrequently needed tests and/or those requiring special expertise and equipment.

We wish to thank the authors of the above letter for sharing their concerns with the readers of the *Journal of Nuclear Medicine*. We hope they will agree with us that ultimately it will be these readers who will make the choice of the method most suited to their needs.

PATRICIA A. McINTYRE
TOMAS R. GUILARTE
JUDITH A. KERTCHER
MARIANNE F. CHEN
AGATHA A. RIDER
Johns Hopkins Medical Institutions
Baltimore, Maryland

REFERENCES

1. CHEN MF, McINTYRE PA, WAGNER HN JR: A radiometric microbiologic method for vitamin B_{12} assay. *J Nucl Med* 18:388-393, 1977
2. CHEN MF, McINTYRE PA, KERTCHER JA: Measurement of folates in human plasma and erythrocytes by a radiometric microbiologic method. *J Nucl Med* 19:906-912, 1978
3. McINTYRE PA: Use of radioisotope techniques in the clinical evaluation of patients with megaloblastic anemia. *Semin Nucl Med* 5:79-94, 1975
4. McINTYRE PA: Newer developments in nuclear medicine applicable to hematology. In *Progress in Hematology*. Vol. IX. Brown EB, Ed. New York, Grune & Stratton, 1977. pp 361-409
5. KERTCHER JA, GUILARTE TR, CHEN MF, et al: A radiometric microbiologic assay for the biologically active forms of niacin. *J Nucl Med* 20:419-423, 1979
6. CHEN M, McINTYRE PA: Measurement of the trace amounts of vitamin B_{12} present in various foods by a new radiometric microbiologic technique. National Bureau of Standards Special Publication 519, Trace Organic Analysis: A New Frontier in Analytical Chemistry, Proceedings of the 9th Material Research Symposium, Gaithersburg, MD, April, 1979, pp 257-265
7. BAKER H, FRANK O, PASHER I, et al: Nicotinic acid assay in blood and urine. *Clin Chem* 6:572-577, 1960
8. Niacin. In *The Vitamins*. Sebrell WH and Harris RS, Eds. New York, Academic Press, 1954, p 453
9. SPIVAK JL, JACKSON DL: Pellagra: An analysis of 18 patients and a review of the literature. *Johns Hopkins Med J* 140:295-309, 1977
10. DeLAND FH, WAGNER HN JR: Early detection of bacterial growth with cabon-14-labeled glucose. *Radiology* 92:154-155, 1969
11. DeLAND FH, WAGNER HN JR: Automated radiometric detection of bacterial growth in blood cultures. *J Lab Clin Med* 75:529-534, 1970
12. BUDDEMEYER EH: Liquid scintillation vial for cumulative and continuous radiometric measurement of *in vitro* metabolism. *Appl Microbiol* 28:177-180, 1974