patients with variegate porphyria truly excrete more coproporphyrin than protoporphyrin, but that this fact has gone unrecognized owing to suboptimal analytical techniques and inattention to the complex nature of fecal porphyrins?

I would appreciate the authors’ views on these questions.

References


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Two authors respond:

To the Editor:

With respect to the first question, the following possibilities might be considered. Moderately increased dicarboxylic porphyrins in feces should be attributed mainly to bacterial flora activity, but the occasional patient could have erythropoietic protoporphyrina. However, in the latter case erythrocyte protoporphyrina also is increased. Latent porphyria—whether acute intermittent porphyria, hereditary coproporphyrina, or variegate porphyria—often cannot be proven by measuring porphyrin in feces or urine. When fecal porphyrins are repeatedly found to be increased, one might consider decontaminating the patient’s gut, to see whether or not this increase is ascribable to bacterial activity. In cases where a porphyria is in an acute or subdormant phase, the detection and characterization of the disease is no problem at all. Indeed, porphyria produces its own characteristic abnor-

Effect of Heat Inactivation on Results of HIV Antibody Detection by Western Blot Assay

To the Editor:

Heating sera at 56 °C for 30 min to 1 h has been suggested (1) as a way to inactivate human immunodeficiency virus (HIV) and make specimen handling safer for laboratory personnel. Alexander (2) correctly points out that heated sera can give false-positive results with the immunoenzymatic HIV assay (3). We decided to investigate the effect of heating sera on results of the Western blot HIV assay, and found that there are changes in reactivity in this assay when human serum samples are heated to 56 °C for 30 min.

We selected four human serum samples for the test. These samples were thought to be negative for HIV antibody. They had been stored at −80 °C, and were not thawed until this use. Two samples were from men, two were from women. We heated 500 µL from each sample for 30 min at 56 °C in a heating block. All samples were then diluted 100-fold in Tween (0.3 mL/L) in phosphate-buffered saline, pH 7.4, for the assay.

The Western blot membrane was produced by the procedure of Tsang et al. (4). The HIV antigen load was determined by assaying serial dilutions. The largest load that gives no binding for an HIV-negative serum is used.

Western blot strips were incubated with the four heated and four nonheated sera for 1 h. Strips were washed four times for 5 min each with the Tween/phosphate-buffered saline, warmed to 37 °C, then incubated for 1 h with antibody–enzyme conjugate (rabbit anti-human IgG–horseradish peroxidase) diluted in phosphate-buffered saline. The strips were then washed four times with phosphate-buffered saline, 5 min each washing. The substrate (H2O2–3,3’-diaminobenzidine tetrahydrochloride dihydrate) was applied and the reaction was allowed to proceed for 15 min.

All four strips that were incubated with heated serum generated more brown dye than the strips incubated with nonheated serum. The background for the first four strips was darker, and the tray slots were stained brown, whereas slots for the other strips remained white. All four heated sera showed changes in reactivity, including increases in banding strength and changes in banding.

The sera (heated and unheated) were refrozen after the samples had been removed for assay. The next day, we thawed the samples again and assayed them by Western blot. The difference in activity was the same. Therefore, changes caused by heating would appear to be permanent.

Three of the four original serum samples (unheated) gave no banding. One sample had a sharp band at 35 kDa. The four heated portions of these samples gave multiple (nine) strong bands, which appeared to be the same for all. These bands do not resemble the banding pattern and the sera would not be considered positive for HIV. The molecular sizes (kDa) of the bands were as follows: 61, 59, 55, 54, 49, 46, 45, 43, and 38. For the three samples that converted from no banding to multiple banding, it is possible that the heating somehow caused an increase in sensitivity by the enzyme detection system, making clearly visible an antibody banding that had been below the limit of detection. However, in the fourth case, the unheated sample had a very sharp band (35 kDa) with no other banding visible; upon