stable zero reading is obtained. Fill the syringe-pet with glutaraldehyde and leave 5 to 10 min. Flush out with rinse solution. Wipe the septum cord with glutaraldehyde.

Since this procedure has been in use, no culture has yielded growth. Monthly CVs for a freeze-dried control serum (mean 4.5 mmol/L), analyzed about 300 times per month, have ranged from 2.9 to 4.9%. Average useful membrane life is three months. Thus we have seen no detrimental effects arising from this treatment.

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Chloral Hydrate Interferes with Radioassay of Vitamin B₁₂

To the Editor:

Elderly patients are often screened for possible vitamin B₁₂ deficiency related to poor diet or suspected pernicious anemia. This state may be suggested clinically, or, more commonly, by the findings with a blood film.

Two methods are commonly used to estimate the serum concentration of vitamin B₁₂. The older method involves microbial assays with Euglena gracilis or Lactobacillus leichmannii (1, 2). More recently, competitive-binding radioassays have been developed. Comparative studies between the two show good correlation (3, 4).

This hospital has a large population of geriatric patients, whom we routinely screen for vitamin B₁₂ deficiency with a radioassay soon after admission. Many of these patients, although not receiving replacement therapy, have shown vitamin B₁₂ values exceeding 1000 ng/L (reference range 100–880 ng/L). The blood films of some of these patients also showed evidence of megaloblastosis, with symptoms of vitamin B₁₂ deficiency.

However, when we estimated the vitamin B₁₂ concentration on the same serum samples with the microbial method, the values obtained were either low or in the normal range, which agreed well with the clinical picture. We then analyzed the records of these patients for a single common factor to explain the discrepancy, and found that all were being prescribed chloral hydrate as a sedative.

Additionally, two patients who presented after attempted suicide by chloral hydrate overdose had high serum concentrations of vitamin B₁₂ by the radioassay, 1600 and 710 ng/L, respectively. Subsequent microbial assay of serum from the second patient gave much lower results (400 ng/L).

We then designed and implemented a controlled trial to verify the hypothesis that the taking of chloral hydrate prevents the accurate assessment of serum vitamin B₁₂ by the radioassay method.

Eleven healthy adults on no regular medication except oral contraceptives volunteered to take part in the trial; their ages varied from 20 to 45 years of age. Each participant took a 500-mg capsule of chloral hydrate on three consecutive nights. Venous blood was sampled during the day before the first dose, and a second sample was taken on the morning after the last dose. Both specimens from each participant were assayed by microbial and radioassays. The microbial assay involved Euglena gracilis and followed the method originally described by Hunter et al. (1). The radioassay method involved the following steps:

(a) heating of serum samples in the presence of cyanide,
(b) competition of ⁵⁷Co-labeled cyanocobalamin and the serum B₁₂ derivative for purified intrinsic factor,
(c) separation of free vitamin B₁₂ by adsorption onto dextran-coated charcoal followed by centrifugation, and
(d) counting of the radioactivity of the soluble complex of purified intrinsic factor and vitamin B₁₂.

Analysis of results before and after ingestion of chloral hydrate (Table 1) indicated no significant difference (by paired observations t-test) between pre- and post-chloral-hydrate concentration of vitamin B₁₂ by the microbial assay (t = 0.1; t₀.₀₅ = 1.8), but there was a difference when the radioassay was used (t = 5.3; t₀.₀₅ = 3.2).

These results substantiate the hypothesis that medication with chloral hydrate prevents the accurate assessment of serum concentrations of B₁₂ by the radioassay method. The dosage given to the trial participants was only one fourth of the usual dosage prescribed, but the interference in the radioassay is evident.

The mode of interaction between chloral hydrate (or its metabolites) and the radioassay is not known but could be an interference in the binding of intrinsic factor with vitamin B₁₂.

Chloral hydrate is very rapidly converted to its active intermediates trichloroethanol and trichloroacetic acid in the liver. Because significant amounts of chloral hydrate have not been detected in blood after oral administration, the central depressant effects are generally believed to be caused by the trichloroethanol (5).

Trichloroacetic acid continues to accumulate for one to two days after the administration of chloral hydrate, which suggests an oxido-reductase system quite different from the one involved in the initial reduction of chloral hydrate (6).

Intrinsic factor is destroyed by several agents, including trichloroacetic acid (7, 8). If the concentration of trichloroacetic acid in the serum is sufficiently high, therefore, the extraneous intrinsic factor added during the radioassay may be destroyed, and fewer binding sites would be available for the vitamin B₁₂ molecules (both patient’s and labeled). The percentage bound fraction would thus be abnormally low and lead to erroneously high test results.

Continual taking of the drug might have a cumulative effect, we expect, at least seven days being required to clear the chloral hydrate intermediates from the circulation. The plasma half-life of trichloroacetic acid, which is highly bound to plasma albumin and not metabolized, is 67 h. Hence daily chloral hydrate treatment leads to trichloroacetic accumulation in the plasma.

Concentrations greater than 150 mg/L may occur after 10 days of receiving 1 g of chloral hydrate daily (6).

We are currently investigating modifications of the radioassay technique to avoid this effect.

Table 1. Vitamin B₁₂ Concentrations in Serum, ng/L, Estimated by Microbial Assay and Radioassay before and after Chloral Hydrate Ingestion

<table>
<thead>
<tr>
<th>Microbial assay</th>
<th>Radioassay</th>
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<tr>
<td>Before</td>
<td>After</td>
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<tr>
<td>270</td>
<td>300</td>
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<tr>
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References
Phospholipid-bound HDL, VLDL, and LDL were electrophoretically separated on plates of cellulose acetate by using a procedure similar to that proposed by Helena Laboratories for HDL cholesterol determination (3). The cellulose acetate plate was placed in Tris buffer (50 mmol/L, pH 7.8) for about 15 min before the electrophoretic separation interval was over, and afterwards impregnated with 1.5 mL of Takayama reagent in a concentration threelfold that prescribed for the original reagent for determining phospholipids. The relative density of the fractions was determined in an Auto Scanner Flur-Vis densitometer (Helena Laboratories) supplied with a 505-nm filter.

For determination of HDL phospholipids after precipitation of VLDL and LDL, we used the procedure of Yamaguchi (4) and determined phospholipids in the supernate in the ABA-100.

In a population of 33 samples with a range of concentration from 250 to 2500 mg/L, linear regression by Deming's method (5)—which recently has been validated (6)—shows a good correlation between the data given for HDL phospholipids by both the electrophoretic and precipitation methods (r = 0.965). Nevertheless, the mean obtained by the electrophoretic method (1203.8 mg/L) significantly (p < 0.001) exceeded that obtained by the precipitation method (1023.9 mg/L). At low HDL phospholipid concentrations the electrophoretic method gave analogous or even lower values than did the precipitation method, but at higher HDL phospholipid concentrations electrophoretic values were consistently higher. Durand et al. (7) and Schifman et al. (8) found results analogous to ours for HDL cholesterol on comparing the electrophoretic technique of Helena Laboratories with precipitation methods.

References
3. Helena Laboratories, Beaumont, TX 77704; HDL cholesterol method by electrophoresis (U.S. patent 4,105,521).

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