Interference of Thiouracil in the Ferric Chloride-Sulfuric Acid Cholesterol Reaction

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Thiouracil, previously recommended as a preservative to be added to serum for butanol-extractable iodine determinations, inhibits the ferric chloride-sulfuric acid color reaction for cholesterol. The color inhibition may be eliminated by shaking the serum for about 5 min. with solid silver iodate prior to cholesterol analysis.

It is usual practice to determine both butanol-extractable iodine (BEI) and cholesterol on a given sample of serum. Recently, Man proposed adding thiouracil in approximately 0.001M concentration to stored sera as a preservative for BEI determinations (1). An impression was gained in the author's laboratory that sera so treated with thiouracil often yielded cholesterol values that appeared to be somewhat lower than anticipated. The cholesterol procedure employed at the time of this observation was that of Zak et al. (2).

Investigation revealed that the Zak ferric chloride-sulfuric acid reaction for cholesterol gives erroneously low results in the presence of thiouracil. Figure 1 shows the absorption curve of the color reaction (Curve 1), and also that the addition of increasing amounts of thiouracil (TU) (curves 2–6) suppresses the 550 mμ absorption maximum of the color complex. Addition of 0.13 mg. of thiouracil per milliliter of serum (approximately 0.001M) causes an average decrease in the apparent cholesterol value of 30–40 mg. per 100 ml. of serum.

In Man's paper (1) no attempt was made to ascertain the exact concentration of thiouracil, but "the practice was to add a minute particle, as small an amount of thiouracil as could be dropped from a pointed..."
stirring rod, to 5–10 ml. of serum. If these instructions are followed exactly, only a minimum error will result. However, frequently the tendency would be to add the same amount of solid to 2–3 ml. of serum, thereby making the error more appreciable.

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\begin{align*}
(1) &= 0.15 \text{ mg. CHOLESTEROL} \\
(2) &= (1) + 5 \mu\text{g. TU} \\
(3) &= (1) + 10 \mu\text{g. TU} \\
(4) &= (1) + 15 \mu\text{g. TU} \\
(5) &= (1) + 25 \mu\text{g. TU} \\
(6) &= (1) + 50 \mu\text{g. TU}
\end{align*}
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**Fig. 1.** Interference of thiouracil (TU) in the Zak cholesterol method.

Obviously, it would be preferable to remove an aliquot of serum for cholesterol analysis before adding thiouracil to the sample. However, if thiouracil is added beforehand, the color inhibition may be overcome simply by shaking the serum for about 5 min. with approximately 25–50 mg. of solid silver iodate per milliliter of serum. After the sample is centrifuged briefly, the clear supernatant serum may be analyzed according to the Zak procedure.

The first 5 samples in Table 1 illustrate the effect of the addition of thiouracil to serum by comparison of the decreased values obtained upon adding thiouracil (TU) to untreated sera. As shown in the last column, this interference is removed by treating the samples with solid silver iodate. It is fortuitous that this is the identical treatment previously developed to eliminate the enhancement of the Zak cholesterol color reaction caused by the presence in sera of exogenous bromide or
iodide (3). Serums 6, 7, and 8 in Table 1 demonstrate that the errors caused by the simultaneous presence of both bromide and thiouracil may be avoided by treating the serum with solid silver iodate.

Subsequently, the laboratory adopted the ferric chloride-cholesterol procedure devised by Babson et al. (4). Thiouracil was found to interfere with this procedure to about the same extent as with the Zak method. The Babson method employs 1:1 ethyl acetate-absolute ethanol as an extraction reagent and bilirubin is removed from the extract by adsorption on nonreactive aluminum hydroxide. The technic does not require evaporation of the extract to dryness. It permits a direct colorimetric analysis, is fast, and has proved to be more suitable for routine use than the original Zak procedure.

Addendum

While this manuscript was in press, a short report by Pileggi and Farber presented data which did not support the thesis that thiouracil addition to serum is required if the BEI procedure is delayed after venipuncture. (Clin. Chim. Acta 9, 93, 1964).

References