New reagent for the direct determination of serum cholesterol

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The stable purple colour given by cholesterol (Zlatkis, Zak, and Boyle, 1953) and related to 5-ene, 3 β -ol steroids (Bischoff and Turner, 1958) with Fe^{3+} in an acetosulphuric medium (Zak reaction) constitutes the basis of an increasingly popular method for the colorimetric estimation of sterols. Nevertheless, many workers (Henly, 1957; Zak, 1957; O'Brien, Ibbott, and Pinfield, 1961; Connerty, Briggs, and Eaton, 1961; Zak and Epstein, 1961) have emphasized that the reproducibility of the Zak reaction is critically dependent on the quality of the reagents employed. A definite improvement in this connexion was the finding of Bowman and Wolf (1962) that glacial acetic acid is not essential for the Zak reaction and may be replaced by absolute ethanol or, conceivably, some other non-aqueous solvent.

In the present note, a further modification of the Zak-Bowman reagent is described in which ferric chloride is replaced by cupric chloride as electron donor.

The procedure for the determination of cholesterol is patterned after that of Bowman and Wolf (1962).

Serum or standard solution, 0.1 ml., is mixed in succession with 3.0 ml. of 0.2% $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$ in absolute ethanol and 2.0 ml. concentrated H_2SO_4 (Sp. gr. = 1.84). The purple colour which develops after the admixture of the two liquid layers is read, after cooling, at 540 m μ against a reagent blank. The Beer function is linear up to about 400 $\mu\text{g./0.1 ml.}$

The precision of the method as estimated by the coefficient of variation is 4.10 (serum) and 3.28% (standard solution), the instrument being a Beckman DU spectrophotometer. Recovery values were within similar limits.

The absorbancies at all arbitrarily selected concentrations of several substances of biological origin are given in Table I. Among non-steroids, tryptophan, indole, and bilirubin were found to be the strongest potentially interfering substances.

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TABLE I
REACTION OF SEVERAL SUBSTANCES
OF BIOLOGICAL ORIGIN WITH THE CUPRIC-
ETHANOL-SULPHURIC REAGENT

Substance	Concentration ($\mu\text{g./ml. 0.1 ml.}$)	Absorbancy (A1.0 cm.) (4540 m.)
Cholesterol	200	0.551
Estradiol	200	0.800
Testosterone	200	0.690
Pregnanediol	200	0.657
Hydrocortisone	200	0.309
Desoxycholic acid	200	0.080
Cholic acid	200	0.100
Taurocholic acid	200	0.162
Calciferol	200	0.050
Tryptophan	100	0.803
5-Methyl-tryptophan	100	1.123
Indole	100	1.100
Histidine	100	0.070
Bilirubin	50	0.490
Fructose	1,000	0.075
Glucose	1,000	0.215
Galactose	1,000	0.140
Arabinose	1,000	0.160

Comparison of the colours given by cupric chloride and ferric chloride respectively with cholesterol (spectral analysis) showed that they were essentially similar.

The colour with cupric chloride was not influenced by differences in the amount of heat developed on mixing concentrated sulphuric acid with ethanol ($116^\circ \pm 10^\circ\text{C.}$), making it possible to dispense with the temperature control required by the Zak procedure (Zak, 1957; O'Brien *et al.*, 1961; Connerty *et al.*, 1961). Further relative advantages of the present method include lower blank values, increased stability in solution of the colour reagent, and uniform batch to batch performance of cupric chloride as compared with ferric chloride.

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