Arch. Inst. Razi, 1968, 20, 111 - 115

A SIMPLE METHOD FOR THE DETERMINATION OF SERUM CHOLESTEROL

By :

MANHOURI H. and KAMALI M.

Department of Biochemistry, Razi Institute

INTRODUCTION

For the determination of serum total cholesterol one may choose among the two main groups: direct and extraction. In the direct method, serum is allowed to react directly with the appropriate reagents. The most widely used techniques of this depend on the Pearson reaction (1), which employs toluen-p-sulphonic acid as stabilizer, and the method of Zlatkis (2) using ferric chloride and sulphuric acid. The main difficulties of these methods are the effect of interfering chromogens, such as bilirubin and haemoglobin. In the former group cholesterol is extracted from the serum into a suitable solvent. Typical of the extraction procedures are those of Bloor (3), Schoenheimer and Sperry (4), and the method of Kenny (5). The main disadvantages of the extraction methods are that they are time-consuming and require considerable manipulative skill.

This paper describes a method for the determination of total serum cholesterol which is sensitive in reaction, stable in colour development and is not much affected by such chromogens possibly present in the serum.

MATERIALS AND METHODS

Reagents

(1) Glacial acetic acid.

(2) Diluent- 2% distilled water in acetic acid.

(3) Colour development reagent- Dissolve 0.25 gm cupric acetate in 50 ml glacial acetic (w/v). Add 100 ml of 10% toluen-p-sulphonic acid in glacial acetic acid (w/v), then add 100 ml of concentrated perchloric acid, and finally 15 ml of concentrated sulphuric acid. This reagent is to be kept in a dark bottle and is stable for several weeks at room temperature.

(4) Standard cholesterol- In a 100 ml volumetric flask, dissolve 100 mg of recrystallized cholesterol and dilute to the mark with glacial acetic acid.

Method

Pipet 0.1 ml serum into a 12-ml conical centrifuge tube. Add 4.9 ml of glacial acetic acid. Stopper and shake vigorously at least 20 times. Allow to

stand 5 minutes. Centrifuge and decant the supernatant extract. Pipet 2.0 ml of the clear extract into a 19×150 mm of spectrophotometric tube marked "Test". Pipet 0, and 0.1 ml of standard cholesterol (100 μg) respectivly to the tubes marked "Blank" and "Standrad". Bring the volumes of the three tubes to 3.5 ml by adding diluent solution. Add into each tube 2.5 ml of the colour development reagent and, mix well. The tubes are placed in a water bath of 60° for exactly 10 minutes. A pinkish colour develops which is stable for at least two hours. The optical densities are read against the blank using a wavelenght of 500 mμ. The optical density obeys Beer's Law up to 500 mg of cholesterol per 100 ml serum.

EXPERIMENTAL

Wavelength

The optical densities at various wavelength of the colour produced in this method are investigated by comparing with serum cholesterol at the same concentration, the results are shown in Fig. 1. The maximum development of the colour occurs at 500 mμ.

Temperature

Fig 2 shows the various times required for maximum colour development at different temperatures. At 60° and 10 minutes heating, the maximum clolour development occurs.

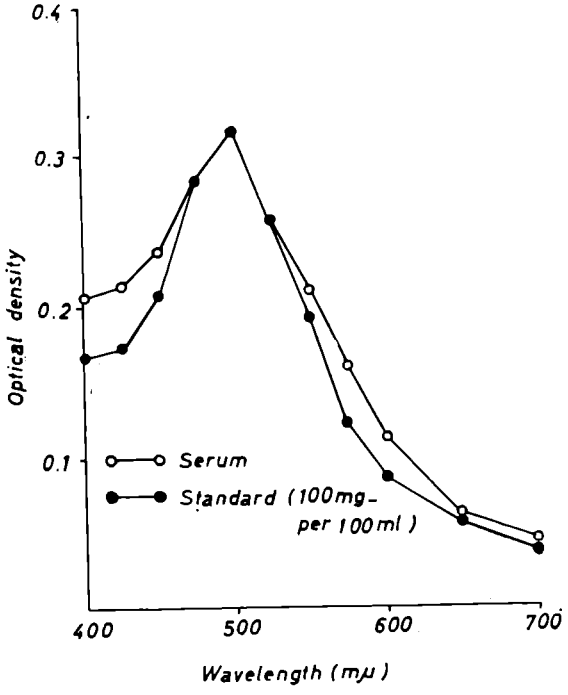

Fig 1. comparison of adsorption curves of serum with that of standard cholesterol

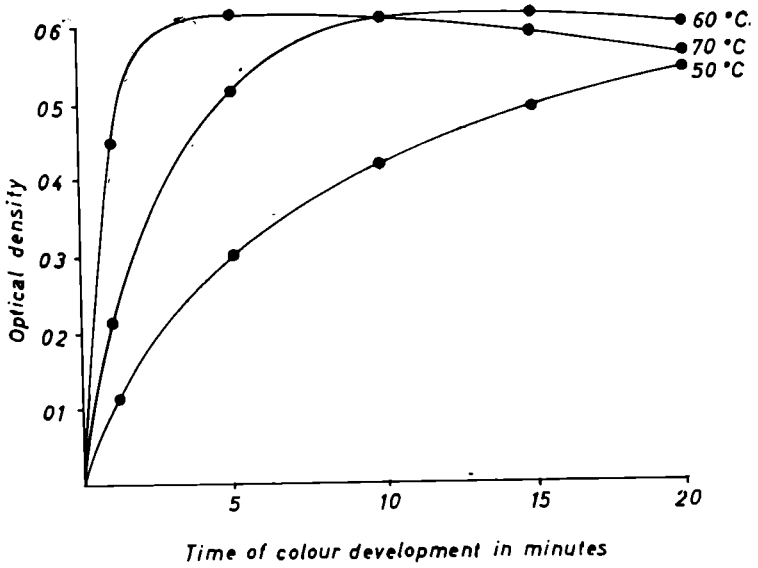

Fig 2. Relationship of colour development to time and temperature of incubation when using 200 μg cholesterol

Comparison

Table 1 shows the results of comparison of presented method with both direct modified Pearson (6) and Kenny's (5) extraction methods.

Table 1. Comparison of cholesterol determination by three methods (mg/100 ml)

Test	1	2	3	4	5	6	7	8	9	10	11	12	Mean
Kenny (5)	219	212	200	218	166	200	212	226	261	180	197	218	209
Modified Pearson (6)	217	211	196	217	164	198	210	220	263	174	200	224	207
presented method	217	213	199	214	162	200	216	221	264	175	202	222	208

Influence of bilirubin and haemoglobin

Bilirubin (N.B.C) was dissolved in 0.1 N sodium hydroxide and added into normal serum to give a series of concentration ranging from 0 to 20 mg

bilirubin per 100 ml serum. A solution of haemoglobin was prepared and added to serum covering a rang of 0 to 500 mg haemoglobin per 100 ml serum. The cholesterol content was determined in duplicate and the average value recorded and the results are shown in table 2.

Table 2. Effect of bilirubin and haemoglobin on cholesterol determination.

Effect of bilirubin			Effect of haemoglobin		
Bilirubin mg/100 ml serum	Cholesterol mg/100 ml serum	Difference per 5 mg bilirubin	Haemoglobin mg/100 ml serum	Cholesterol mg/100 ml serum	Difference per 100 mg haemoglobin
0	170	—	0	210	—
5	173	3	100	213	3
10	175	2	200	218	5
15	177	2	300	220	2
20	179	2	400	226	6
			500	229	3

DISCUSSION AND RESULTS

The need for a simple and, accurate method for determination of total serum cholesterol prompted the development of the present precedure. The method is rapid, sensitive, and requires only a very small amount of serum.

In the course of this experiment, it was noted that premixing of reagents has no effect on colour development even if it is kept for weeks, at room temperature. In addition to simplicity, this reduces the sources of error, since it involves only one pipette for transfering the components of colour development reagent.

As demonstrated in table 2, high bilirubin levels up to 20 mg/100 ml of serum do not significantly interfere with the test, nor does added haemoglobin as high as 500 mg/100 ml serum.

The presented method is almost 3 times as sensitive, compared with those of modified Pearson (6) and Libermann reaction (7).

SUMMARY

A simple method for the determination of serum cholesterol is described. The method is rapid, sensitive and compares well with both direct and extraction methods and requires only 0.1 ml of serum.

RESUME

Une méthode simple est décrite pour la détermination du cholesterol de sérum.

La méthode est simple, sensible et se compare bien avec les deux méthodes directe et extraction et il lui fait seulement 0,1 ml de sérum.

REFERENCES

1 S. Pearson, S. Stern and T.H. McGavack, J. Clin. Endocrinol, Metab; **12**, 1245 (1952).
2 A. Zlatkis, B. Zak and A.J. Boyle, J. Lab. Clin. Med; **41**, 486 (1953).
3 W.R. Bloor, J. Biol. Chem; **17**, 377 (1914).
4 R. Schoenheimer and W.M. Sperry, J. Biol. Chem; **106**, 745 (1934).
5 A.P. Kenny, Biochem. J;**52**, 611 (1952).
6 A. Jamieson, Clin. Chim. Acta, **10**, 530 (1964).
7 C. Libermann, Ber. Deut. Chem. Ges; **18**, 1804 (1885).