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A RAPID AND SENSITIVE ASSAY FOR DETERMINATION OF CHOLESTEROL IN MEMBRANE LIPID EXTRACTS

PETER OTT, YVONNE BINGGELI and URS BRODBECK

Medizinisch-chemisches Institut der Universität, P.O. Box, CH-3000 Berne 9 (Switzerland)

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A commercially available enzymatic assay (Boehringer Monotest) was modified to allow a rapid and sensitive determination of cholesterol in membrane lipid extracts. This was achieved by adding 0.5% Triton X-100 to the reagent solution. The detergent did not interfere with the assay. The relationship between the amount of cholesterol per assay and the absorbance at 500 nm was linear up to 100 μg. The recovery in the assay was better than 95%. The assay was applied to the determination of cholesterol in erythrocyte membrane lipid extracts.

It is well documented that cholesterol plays an important role in maintaining vital membrane properties. The interactions of cholesterol with other membrane components are known to influence the characteristics of the lipid phase [1–4] as well as the behavior of membrane-bound enzymes [5–7]. In this relation several properties of the erythrocyte membrane have been reported to be influenced by its cholesterol content, such as regulation of cell shape [8,9], blood group antigen expression [10] and membrane permeability [11–13]. Even the irregular red blood cell shapes, observed in certain liver diseases, have been ascribed to a change in membrane cholesterol content [14].

Thus, it is obvious that a rapid, sensitive and reliable method for cholesterol determination is of practical importance. However, the chemical methods described in the literature, such as the Liebermann-Burchard reaction, modified according to Watson [15], or the assay according to Webster [16], are cumbersome, time-consuming and insensitive.

Recently, rapid and sensitive enzymatic assays for cholesterol determination in blood serum have been developed and are now widely used in clinical chemistry [17]. However, these assays, based on cholesterol oxidase, cannot be applied to the determination in intact biological membranes, because the entire membrane cholesterol pool is not always susceptible to cholesterol oxidase [18,19].

The present communication shows that suitable modification of a commercially available enzymatic serum cholesterol assay (Boehringer Monotest, obtained from Boehringer, Mannheim, F.R.G.) permits reliable determinations of membrane cholesterol. Two aspects are of importance for the modified procedure: First, dried membrane lipid extracts, rather than intact membrane structures, are used as the material to be analyzed and, secondly, the detergent Triton X-100 must be added to the reagent solution to ensure solubilization of the lipid extracts during the assay procedure.

The lipids were extracted from erythrocytes or ghost membranes by standard procedures [20–22]. An aliquot (containing 10 to 50 μg cholesterol) of each extract or of a cholesterol stock solution in chloroform/methanol (1:1, v/v) was dried under a stream of nitrogen, and 2 ml of Triton-free assay solution were added to the dried extract. Incubation was carried out at 20°C for 30 min and the

absorbance was read at 500 nm against a blank. Calculation of the cholesterol content was performed as prescribed by the manufacturer. When more than 50 μg of cholesterol was present per assay, a turbid suspension was observed.

Therefore, a second set of determinations was carried out with 0.5% Triton X-100 (v/v; final concentration) in the reagent solution. Under these conditions no turbidities could be noticed. To monitor the influence of the added Triton X-100 on the assay procedure, a series of determinations were carried out with Moni-Trol I E and II E (obtained from Merz and Dade, Düdingen, Switzerland). These control sera, which contain the constituents of human serum (e.g. cholesterol) in known concentrations, are widely used in clinical chemistry and permit to check the accuracy of assay methods. Table I gives a summary of these determinations, which clearly show, that 0.5% Triton X-100 (v/v), included in the reagent solution, does not interfere with the enzymatic cholesterol determination.

The assay, if performed in presence of Triton X-100, shows a linear relationship of absorbance at 500 nm versus cholesterol concentration up to 100 μg/assay. In this range, 100% of the cholesterol was recovered in the assay and at 150 μg/assay still 97% were found (Fig. 1).

Fig. 2 summarizes the results of various cholesterol determination procedures performed on dried lipid extracts. It is obvious, that Triton X-100 must be included in the assay medium to prevent

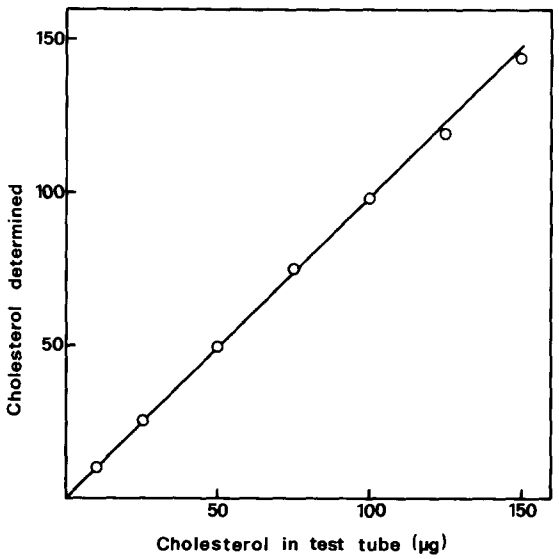

Fig. 1. Recovery of cholesterol with the Boehringer Monotest assay in presence of 0.5% Triton X-100. Assays were carried out as described in the text. The amount of cholesterol added from a stock solution in chloroform/methanol (1 : 1, v/v) to each test tube was plotted versus the determined amount.

formation of a turbid solution. Furthermore the high sensitivity of the enzymatic method, which was up to 10-fold increased if compared to the Liebermann-Burchard reaction [15], is clearly visible. The detection limit is approx. 5 μg/assay, which represents a great advantage in studies with biological material available only in limited amounts.

The cholesterol content of red blood cell membranes, determined by different methods is shown in Table II. Although no major differences could be found, it still has to be mentioned that higher values were obtained with the non-enzymatic methods, which is due to nonspecific reactions [17]. The amounts of cholesterol found per red blood cell are well within the range of earlier published values [23].

The major advantages of the procedure for determination of the total cholesterol content of membrane lipid extracts described in the present study are the high sensitivity, easy handling and the rapidity by which the assays can be performed. Furthermore, since dried lipid extracts are used, the assay procedure is insensitive to influences of different solvents used for lipid extraction.

TABLE I

INFLUENCE OF TRITON X-100 ON ENZYMATIC CHOLESTEROL DETERMINATION

Results are given as mean values ± S.D. of ten experiments. Nominal values for Moni-Trol I E and II E were obtained from Merz and Dade.

	Cholesterol (mg/100 ml)	
	Moni-Trol I E	Moni-Trol II E
Without Triton X-100	118.1 ± 1.4	94.3 ± 1.5
With 0.5% Triton X-100	118.1 ± 2.3	97.0 ± 1.02
Nominal cholesterol content [a]	120	95

[a] According to the manufacturer's declaration.

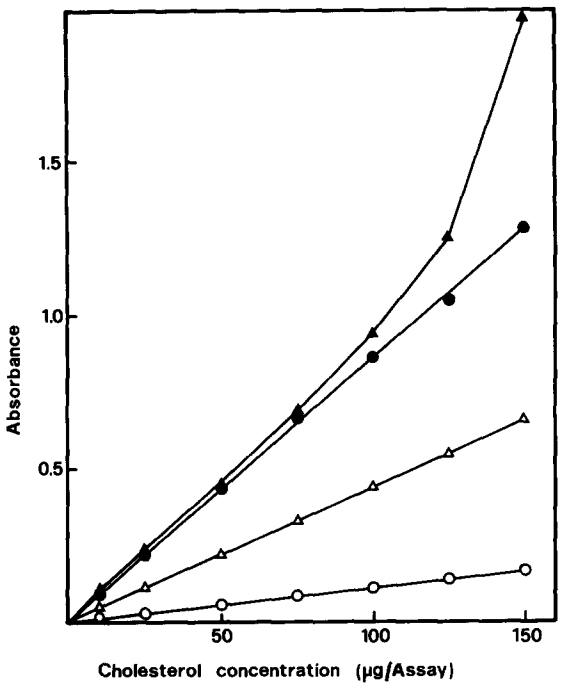

Fig. 2. Comparison of enzymatic cholesterol determinations with non-enzymatic assays. Aliquots from a cholesterol stock solution in chloroform/methanol (1:1, v/v) were taken to dryness in test tubes under a stream of nitrogen. After the assay reaction the absorbance was read at 500 nm for the Boehringer Monotest in presence (●———●) and absence (▲———▲) of Triton X-100, at 578 nm for the Liebermann-Burchard assay (○———○) and at 560 nm for the Webster assay (△———△).

TABLE II

CHOLESTEROL DETERMINATION IN RED BLOOD CELL MEMBRANES

A fresh blood sample was washed three times with a 10 mM Tris-HCl buffer, containing 144 mM NaCl, pH 7.4, lipids were extracted according to Rose and Oklander [21] and cholesterol determined by the indicated methods. Cell counts were obtained with a Coulter Counter Model S.

Assay method	Cholesterol content (mg/cell) ($\times 10^{10}$)
Boehringer Monotest (+0.5% Triton X-100)	1.24
Liebermann-Burchard (Roche-Kit)	1.27
Webster	1.47

It is obvious, that the method can be used for the assessment of cholesterol in lipid extracts obtained from a variety of membrane systems and, therefore, is of general interest.

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