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Simplified Blood Cholesterol Method

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SIMPLIFIED BLOOD CHOLESTEROL METHOD

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In the treatment of diabetics it is desirable to use the blood cholesterol value as an indication of effectiveness of the control measures. The diabetic individual is usually accustomed to the finger prick for obtaining blood for sugar determinations; so a method using capillary blood has been devised for cholesterol determination.

Materials

Filter Roll: From a four inch diameter circular filter paper cut two equal sectors, each less than a quadrant, so that the resulting paper cut resembles a cross section of a toadstool. Fold the semicircular part of this in half and make a roll enclosing the edges. Fasten near the end with a paper staple perpendicular to the axis of the roll. This should make a roll that just fits into the extraction tube.

Extraction tube: A Folin-Wu sugar tube, Pyrex No. 7820, with additional markings at 5 ml. and 10 ml., and fitted with an interchangeable ground glass stopper No. 16. (Furnished by Central Scientific Co., Chicago, Ill.)

Condenser: A straight water jacket condenser or the Allihn type (Fig. 1) is suitable. Fit with a No. 5 or No. 6 cork stopper. Condensers may be obtained with ground ends to fit the ground top of the extraction tube.

Acetic anhydride: Reagent quality.

Chloroform: Reagent.

Sulfuric acid: C. P.

Cholesterol Standard: Chloroform solution. One cc. equals 1 mg.

Method

From a prick in the finger tip draw blood up to the mark in a pipette calebrated to deliver 0.2 cc. Stick the tip of the pipette well into the filter roll and blow out the blood. Dry at room temperature about ten minutes. Put five cc. of chloroform into an extraction tube and insert the filter roll so that the paper stem can be just turned down over the lip and fastened to the outside by adhesive tape. Connect the water condenser so that the refluxed chloroform will drop into the roll. Set the tube in a boiling wa-

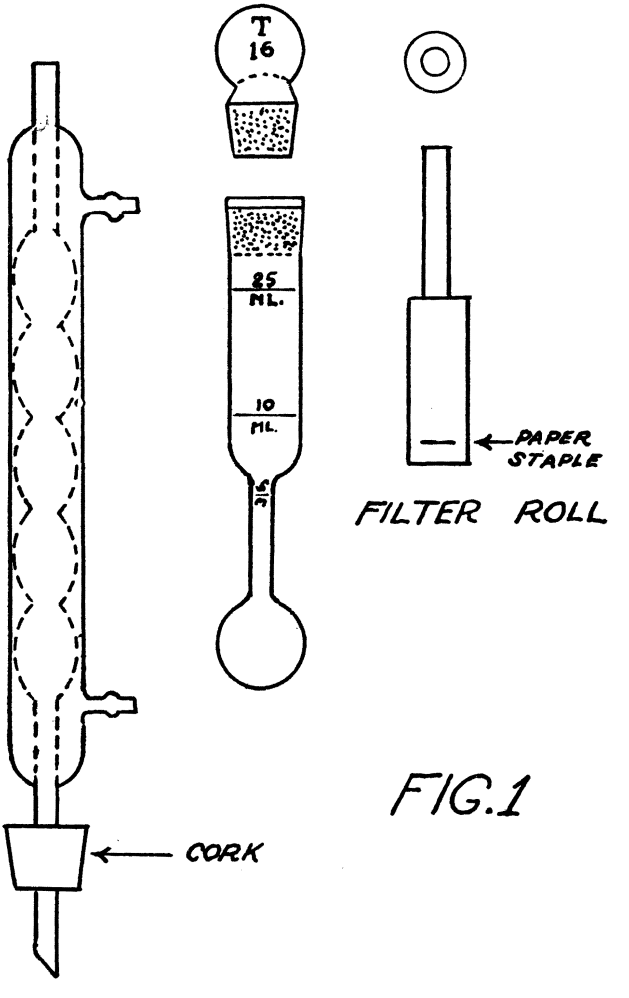

FIG. 1

CHOLESTEROL APPARATUS

ter bath and allow the refluxing to go on for thirty to forty minutes. If the chloroform fails to boil, a glass bead dropped in through the condenser top will start it. After the extraction is complete make the volume up to five cc. Two standards are made by diluting 0.2 cc. and 0.5 cc. of the standard to 5 cc. with chloroform in two of the special extraction tubes. To the unknown and standards add two cc. acetic anhydride and 0.1 cc. sulfuric acid. Insert the glass stoppers and invert the tubes. The heat generated usually necessitates the opening of the tubes once before a second inversion. Allow the color to develop for 15 minutes in the dark at room temperature. Compare with the closest standard in a colorimeter.

Calculation:

$$\frac{\text{Standard reading}}{\text{Unknown reading}} \times \text{cc. of standard used} \times \frac{100}{0.2} = \text{mg. \% cholesterol}$$

Discussion

By the use of several clinical methods, including the one given here, the total cholesterol content of capillary blood has been found equal to that of venous blood. Consequently, the use of capillary blood will give valid results. The finger prick is preferred to venipuncture whenever possible on patients whose veins are already subject to multiple entry. Since cholesterol determinations are frequently done for diabetic and genito-urinary patients, who already have had considerable intravenous work, this method will permit an adequate number of determinations without further vein injury.

Results by this technique compare well with those on alcohol-ether extracted blood. As with any continuous extraction process, the precaution of allowing sufficient time for complete extraction applies. As a check we place the extracted filter role in a test tube with five cc. of chloroform and add 2 cc. of acetic anhydride and 0.1 cc. sulfuric acid. If more than a trace of green color is apparent in fifteen minutes the extraction has been incomplete.

This method makes use of equipment which is employed in other analytical procedures. When the extraction tubes are used for blood sugars the convenience of the glass stopper makes them far superior to the standard type of Folin-Wu tube.

Summary

A simple extraction method using capillary blood has been devised for cholesterol determination.

A conventional Liebermann-Burchard reaction is carried out in the glass-stoppered reaction tube.

The method is particularly useful when venipuncture is to be avoided.

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