in mammals. Indeed, Kewitz(12) found traces of a choline ester of urocanic acid, probably Mur, in extracts of cervical ganglia of cattle and horse.

Summary. 1. Murexine (Mur) and dihydromurexine (DhMur) are both hydrolyzed by human plasma cholinesterase. The hydrolysis rate of DhMur is as fast as that of acetylcholine (ACh) and is 18 times faster than that of Mur. 2. Neither Mur nor DhMur are hydrolyzed by human red cell cholinesterase. 3. Both Mur and DhMur inhibit the hydrolysis of ACh by red cell cholinesterase. Mur also inhibits plasma cholinesterase.

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Determination of Serum and Tissue Cholesterol. (22992)

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It was found by Swinnen(1) and by us that the direct ferric chloride method of MacIntyre(2) yielded high values for total cholesterol of the serum. Furthermore, this procedure was obviously not directly applicable to tissues. By combining this method with the extraction procedure of Abell(3), with minor modifications, a satisfactory method was obtained that eliminated the false high values but retained the desirable color stability and sensitivity.

Methods. 1. Alcoholic KOH; 6 ml of a 30% w/w KOH solution are added to 94 ml of redistilled absolute ethyl alcohol and well mixed. A white precipitate may form. This reagent should be prepared just before use. 2. Petroleum ether; 60-70°C boiling range and redistilled. 3. Glacial acetic acid; reagent grade glacial acetic acid is refluxed (2) over chromium trioxide for 3 to 4 hours and then redistilled. Start application of heat cautiously and with constant stirring until mixture is close to boiling point, then remove stirrer and install the reflux condensor. This precaution should be followed to prevent a possible violent exothermic reaction. 4. Color reagent; to about 150 ml of reagent grade sulfuric acid add 10 ml of 10% $FeCl_3 \cdot 6H_2O$ in redistilled glacial acetic acid and mix well, then dilute to 1 liter with concentrated sulfuric acid. 5. Standard cholesterol solution; 20 mg of cholesterol/100 ml of glacial acetic acid. *Tissue preparation*: To known weight of tissue, 100 to 200 mg, add 0.5 ml of 10% tetraethylammonium hydroxide and heat in boiling water bath, with occasional mixing, until solution is attained (about 45 min). Allow solution to cool to room temperature, then dilute to exactly 1 ml with distilled water. The use of tetraethylammonium hydroxide makes possible a further analysis of the sample for alkali earth metals. An aliquot of this is used for determination of cholesterol.

Procedure. Place 0.1 ml of serum (0.2 ml in the case of tissue) or less, as required by

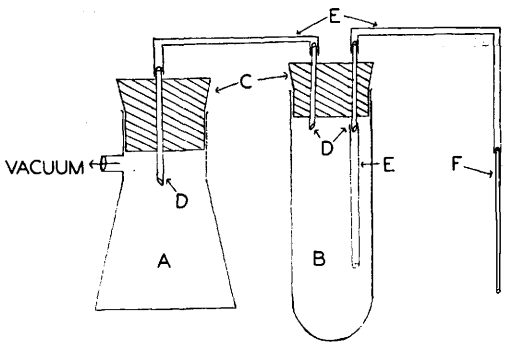

FIG. 1. Aspiration apparatus. A, suction flask; B, colorimeter tube; C, rubber stoppers; D, No. 22 gauge hypodermic needles with slip-on hub removed; E, polyethylene tubing PE50; F, glass capillary which is placed into the petroleum layer to be transferred.

the amount of cholesterol present, into a glass tube (culture tube 14 x 100 mm outside dimensions) and add 1 ml of alcoholic KOH. Mix well and incubate tubes in water bath at 37-40°C for 1 hour. Remove tubes from water bath and, when cool, introduce 2 ml of petroleum ether into each tube via hypodermic syringe with sufficient force to provide mixing. One ml of distilled water is then added and the cholesterol extracted by up and down movement of footed glass rod for 2 minutes. This is most easily accomplished by extracting 10 to 12 tubes simultaneously by a manifold which consists of wooden block holding 10 to 12 footed glass rods spaced so as to fit into tubes in the test tube rack. The emulsion which forms is broken by low speed centrifugation and the top petroleum ether layer containing extracted cholesterol is quantitatively transferred into clean colorimeter tube. This is most readily done by aspiration, (Fig. 1). Petroleum ether is evaporated at room temperature to dryness by gentle stream of air directed into tube from a manifold, (Fig. 2). The stream of

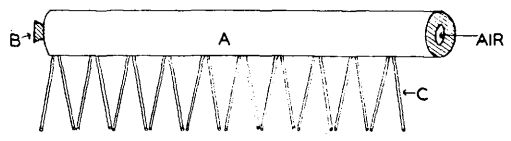

FIG. 2. Air manifold. A, rubber suction tubing; B, cork; C, No. 18 gauge hypodermic needles with slip-on hub removed. A short piece of polyethylene tubing PE160 may be attached if desired.

air is turned off as soon as dryness is attained. To the dry residue add 6 ml of glacial acetic acid and then forcefully introduce 4 ml of color reagent directly into the glacial acetic acid by a 10 ml hypodermic syringe. This allows for instant mixing and avoids uneven heating of solution. Immediately after the color reagent has been added a footed glass rod is employed to insure thorough mixing. The tubes are allowed to cool to room temperature and the color density is then read at 570 mμ. The color is stable for at least 2 hours. A standard curve is prepared by adding from 0.1 to 1.0 ml of standard cholesterol solution to the colorimeter tubes and enough of glacial acetic acid to make total volume 6 ml. Color is developed as stated above.

Results. The present method was compared with the direct ferric chloride method of MacIntyre(2) on 32 normal and hypercholesterolemic rabbit sera ranging from 49 to 495 mg% of cholesterol. The values obtained averaged 21% lower (15-25% extremes) than those obtained with the direct ferric chloride procedure. The present procedure was also compared with that of Abell (3) on 8 rabbit serum samples. The results are shown in Table I with good agreement.

TABLE I. Comparison of Rabbit Serum Cholesterol Concentrations, in 8 Samples.

Serum cholesterol, mg %	
Present method	Method of Abell(3)
68	65
36	38
40	41
124	125
101	105
45	43
45	42
44	46

Color of solution obtained from digestion of most tissues is reddish-brown, which on vigorous agitation turns to a greenish color. This color change had no effect on subsequent cholesterol determination. Furthermore, the tissue digest can be stored in freezer for at least 2 weeks without any effect on cholesterol value. A 500 mg liver sample from a rat on a hypercholesterolemic diet was digested as

TABLE II. Recovery of Cholesterol Added to Tissues.

Tissue	Rat No.	Cholesterol added (μg)	Cholesterol recovered (μg)
Liver	1	250	242
	2	250	238
	1	1500	1494
	2	1500	1418
Serum	1	250	229
	2	250	255
	1	1500	1419
	2	1500	1530

described. After very gentle mixing of the digest, an aliquot was removed for analysis; another was taken from the reheated digest after vigorous stirring which produced the greenish color; and a third aliquot was taken for analysis after 14 days storage in the freezer. The cholesterol concentrations were 263, 264 and 262 mg/100 g of liver, respectively. Recovery of added cholesterol from serum and liver was also studied. Results presented in Table II indicate that good recoveries are obtained.

Summary. A simple and sensitive method for determination of serum and tissue total cholesterol is described.

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Glycolytic Intermediates of Human Platelets: Their Separation and Identification.* (22993)

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Studies of human platelets have indicated the presence of an active carbohydrate metabolism(1,2) with a respiratory quotient of 0.96. Direct investigation of this metabolic system has demonstrated the presence of carbohydrate intermediates, following which partial separation and identification of the esters was obtained. Addition of radioactive phosphorous to the platelets prior to separation led to knowledge of the esters of highest specific activity, and thus to their place in the cycle. Important differences were found between fresh normal platelets, normal stored platelets, and platelets obtained from patients with hematologic disease.

Materials and methods. (a) Preparation of Platelets. Blood was obtained using silicone coated surfaces with one-tenth volume of 1% EDTA as the anticoagulant. Platelet rich plasma was carefully separated by differential centrifugation at 4°C. The volume of platelet rich plasma was standardized at 100 ml, the platelet count adjusted to 275,000/mm^3 and the pH maintained at 7.4 with .001M phosphate buffer. One millicurie of radioactive phosphorous was added and the platelet rich plasma incubated for 2 hours at 23°C. Platelets were separated by centrifugation at 1500 PM for 15′ and then washed twice with 100 ml of normal saline at 4°C. (b) Extraction of glycolytic intermediates from the washed platelet mass was obtained through modification of a method previously used in the study of erythrocytes (Fig. 1 and 2)(3,4). The platelets were ground after adding 25 ml of 4°C, 7.5% trichloracetic acid, the extract separated by centrifugation at 4°C, and the residue extracted with 25 ml of 4°C, 5% trichloracetic acid. The com-

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