of the total activity present in the skeleton of Rat I. There is a possibility that the bulk of the residual C^{14} activity may reside in the citric acid component of the skeleton since it is known that about 1% by weight of the dry fat-free bone is citric acid.[18]

The radioautograph of the incisor tooth of Rat I (Fig. 2) shows that the C^{14} was concentrated in a layer near to, or immediately adjacent to, the pulp cavity. Since this region is the location of the most recently formed dentin, the heavy concentration of C^{14} in this position is accounted for by deposition in the mineral phase of the dentin formed during the course of the experiment. The radioautograph of the kidney of Rat II shows that the C^{14} was concentrated in the cortical region.

The greater specific activity of C^{14} in the enamel of the incisor teeth than in the dentin (Rat II, Table IV) is probably due to the presence in enamel of a greater amount of inorganic carbon, which has a high uptake of C^{14}, than is present in dentin. No results as to the C^{14} content of the enamel of molar teeth were obtained because of poor yields of enamel from these teeth.

In the fat the greater part of the activity resides in the glycerol fraction but a definite incorporation of C^{14} in the fatty acids took place. The marked incorporation of C^{14} in the protein of the visceral organs is of interest. The relative amount of C^{14} incorporated by these proteins is in general agreement with their relative metabolic activity as determined by experiments with isotopic nitrogen.[19]

Other workers[2,5] were unable, in short term experiments, to demonstrate the incorporation of isotopic carbon (C^{11} or C^{13}), administered as inorganic carbon, in the muscle and liver glycogen of unfasted animals. Our results (Table IV) show, however, that such incorporation can be detected when the study is conducted under conditions which maintain the isotopic inorganic carbon content of the body fluids at a high level over a longer time.

Summary. Radioactive carbon was administered to unfasted mature rats either by intraperitoneal injection of $Na_2C^{14}O_3$ or by implantation in the peritoneal cavity of $CaC^{14}O_3$. Quantitative studies were made of the relative amounts of C^{14} excreted in the urine, the feces, expired air and incorporated by the tissues and derived components. A significant incorporation of C^{14} was found in the inorganic carbonate fraction of bone and in bone protein, the dentin, and enamel of the teeth, fatty acids, glycerol, hemin, red cell protein, plasma proteins, central nervous system protein, liver and muscle glycogen, muscle protein, and in the proteins of the testes and of the thoracic and abdominal viscera.

[18] Dickens, F., *Biochem. J.*, 1941, **35**, 1011.

[19] Schoenheimer, R., *The Dynamic State of Body Constituents*, Harvard University Press, Cambridge, Mass., 1942.

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Fractionation of Serum Cholesterol.*

J. C. FORBES, G. H. L. DILLARD, W. B. PORTER, AND O. PETTERSON.

From the Departments of Biochemistry and Medicine, Medical College of Virginia, Richmond, Va.

During the last few years considerable evidence has accumulated indicating that the cholesterol of normal plasma is, to a very large extent, bound with the plasma proteins. Since cholesterol bound with plasma proteins would be completely soluble in the plasma, it is possible that its physiological behavior might be quite different from that of cholesterol which exists in the plasma unbound with

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TABLE I.
Serum Cholesterol Fractionation.

Subject	Age and sex, yr	Total cholesterol, mg %	Free chol., mg %	Readily extractable cholesterol, mg %	Neutral fat + cholesterol, mg %	Diagnosis
D.M.	50 M	197	97	33	—	Normal subject
C.H.	29 M	307	92	26	511	" "
H.W.	24 M	276	78	28	440	" "
M.U.	22 F	267	70	26	315	" "
B.P.	25 F	229	43	19	363	" "
A.B.	39 M	297	69	23	482	" "
H.	28 M	170	32	12	256	" "
R.E.	24 M	246	81	20	529	" "
H.E.*	3 M	966	427	891	—	Nephrosis
		584	318	512	1664	"
		584	367	505	1635	"
A.J.*	38 F	585	198	484	—	"
		582	208	387		
W.E.	4 M	658	247	583	—	"
R.W.*	3 M	760	226	742	1768	"
		596	138	576	1376	
O.J.*	24 M	694	302	654	1897	"
		767	357	693	1682	
F.P.	4 M	1100	527	1070	—	"
B.H.	2 M	748	288	634	—	"
H.R.	42 M	1020	405	927	1397	Nephritis with nephrotic syndrome and diabetes
		817	268	728	1040	After a fat-free diet for 9 days

* The different determinations were run several weeks apart.

protein. The immediate purpose of the present investigation was to develop a method which might give the concentration of the *unbound* fraction. We have found that when normal serum is dried *in vacuo* from a frozen state, a relatively *small but constant* fraction of the total cholesterol of the dried serum can be extracted with chloroform at a temperature of about 5°C. This fraction will be referred to as the "readily extractable cholesterol." We postulate that it represents cholesterol which is either not bound with protein or held in very loose union.

Experimental. One-half cubic centimeter of serum was transferred to a half-ounce thick-walled screw-capped bottle. The serum was rapidly frozen and dried for an hour and a half at a pressure of about 0.2 mm. Immediately after drying, the bottles were put in a desiccator over calcium chloride. Shortly before extraction, the bottles were tightly capped with a screw-cap lined with tin foil, and put in a cold room at a temperature of around 5°C. When the temperature of the bottles had dropped to that of the room, 10 cc of cold chloroform were added to each, the bottles recapped and shaken at intervals thereafter. A 3-hour extraction period was adequate for maximum extraction of normal serum but as a special precaution we always extracted different samples of the same serum for both 3 and 24 hours. At the conclusion of the extraction period, the extracts were filtered cold, taken to the laboratory and analyzed for cholesterol, using a previously described procedure.[1] Inasmuch as the readily extractable cholesterol fraction, even in normal serum, contains both free and ester cholesterol, the recorded values are slightly higher than the true cholesterol value since cholesterol esters of the long-chain fatty acids give more color with the Liebermann-Burchard reagents than does an equivalent amount of cholesterol. The free and total cholesterol content of the undried serum was determined, using the procedure of Gershberg and Forbes.[1] Since the readily extractable

[1] Gershberg, H., and Forbes, J. C., *J. Lab. Clin. Med.*, 1942, **27**, 1439.

TABLE II.
Serum Cholesterol Fractionation—Rabbits on a High Cholesterol Diet.

Rabbit No.	Total chol., mg %	Readily extractable chol., mg %	Neutral fat + chol., mg %	Remarks
1-12	41* (22-69)	9* (5-15)	—	Normal rabbits
13	1373	1115	—	31 days on fat + cholesterol diet
	2690	2661	4834	45 " " " " " "
	1883	1712	3407	56 " " " " " "
	2373	2127	3157	77 " " " " " "
14	3167	2698	—	31 " " " " " "
	1391	1081	2277	45 " " " " " "
	2916	2863	3942	56 " " " " " "
15	2076	1873	—	31 " " " " " "
	2316	2325	4000	56 " " " " " "
	1850	1769	3448	71 " " " " " "
16	1142	1046	1540	38 " " " " " "
	3174	3158	6064	71 " " " " " "
17	2878	2880	5583	38 " " " " " "
	1679	1664	2556	71 " " " " " "

* Average of 12 rabbits, range in parentheses.

cholesterol fraction was not hydrolyzed prior to color development, the values for serum total cholesterol recorded in the tables also are given as the value obtained prior to hydrolysis of the cholesterol esters. The fraction designated as "neutral fat plus cholesterol" consists of neutral fat, free and ester cholesterol. A chloroform extract of undried serum obtained by a method previously described[2] was used for the analysis. The serum is adsorbed on an artificial zeolite (Doucil) which allows complete extraction of neutral fat and cholesterol by chloroform, but prevents extraction of the phospholipids. After filtration, an aliquot of the chloroform extract was evaporated to dryness and its neutral fat and cholesterol content determined by Bloor's oxidation method.[3]

Typical experimental results obtained on normal subjects and certain hospital patients are shown in Table I. Twenty-three normal individuals have been studied altogether. In these the readily extractable cholesterol fraction varied from 12-37 mg per 100 cc of serum, while 21 of the subjects gave values below 30 mg. The blood samples were usually taken 2 to 3 hours after breakfast. It is possible that the values might have been a little lower in the normal subjects if fasting blood samples were used. Eight nephrotic patients have been studied and the readily extractable cholesterol fraction was very high in all, being in some cases over 90% of the total serum cholesterol. Two cases of definite hypothyroidism gave elevated values for the readily extractable fraction, 49 and 58 mg, respectively. Two other subjects who showed no evidence of hypothyroidism except a somewhat elevated total cholesterol and a slightly subnormal B.M.R. gave values slightly above that of our highest normal value, the average of several determinations in each of these individuals being 40 and 43 mg. Fifteen diabetic patients have been studied. The readily extractable cholesterol fraction was quite high in one, slightly elevated in 4 and normal in 10. The results so far obtained do not allow any definite conclusion as to whether diabetic patients on the whole tend to have an elevation of this fraction or not. It should be noted that the diabetes was reasonably well controlled in all cases at the time the subjects were studied.

[2] Forbes, J. C., and Irving, H., *J. Lab. Clin. Med.*, 1931, **16**, 520.

[3] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

This problem is being studied in greater detail, with a particular attempt being made to correlate the laboratory data with the clinical findings.

Typical results obtained on a total of 20 hypercholesterolemic rabbits are shown in Table II. The experimental animals were fed Rockland rabbit pellets to which 5 g of cholesterol and 50 g of a hydrogenated vegetable oil (Mazola oil) dissolved in chloroform were added per kg, the whole thoroughly mixed and the chloroform allowed to evaporate. Blood was obtained by cardiac puncture at intervals and analyzed. It will be seen that the readily extractable fraction was very high in all cases, apparently comprising all of the cholesterol of the serum in some cases. All of the experimental animals at the time of autopsy showed some atherosclerotic lesions. Animals fed a similar diet but without added fat showed the same general picture except for a considerably greater fluctuation in the different cholesterol fractions amongst the individual animals.

Discussion. Although the significance of the variations in the concentration of the readily extractable cholesterol of the serum remains to be determined, it is of considerable interest that the two conditions in which it was found to be exceptionally high, namely clinical nephrosis and experimental hypercholesterolemia of rabbits, both tend toward the rather rapid development of atherosclerotic lesions. More extensive studies, however, are necessary before any conclusions regarding its significance can be drawn.

Summary. Chloroform extraction of normal serum, dried *in vacuo* from a frozen state, removed a relatively small but constant fraction of the total cholesterol. A very high percentage of the total cholesterol of the serum of nephrotic patients was extracted under the same conditions. The same was true of rabbits rendered hypercholesterolemic by the administration of cholesterol with their food. The readily extractable fraction was slightly elevated in all the hypothyroid cases studied and in some diabetic patients.

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Disappearance of Cholesterol Following its Intravenous Injection in Physiologically Emulsified Form.*

L. HORLICK,† M. FELDMAN, JR., AND L. N. KATZ.

From the Cardiovascular Department,‡ Medical Research Institute, Michael Reese Hospital, Chicago.

Despite the implication of cholesterol in many vital processes, such as in the formation of hormones and in the genesis of atherosclerosis, very little is known concerning its metabolism. As technics in metabolic studies oral tolerance tests have consistently failed because of the slow and irregular absorption of cholesterol suspensions from the gastrointestinal tract, and the resultant insignificant rise in blood levels.[1-4] Artificial emulsions prepared with high pressure emulsifiers and various emulsifying agents, such as the lecithins and phospholipids have been unsatisfactory, either because the particle size obtained was too large for safe intravenous administration,

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† Dazian Fellow.

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1 Brun, G., Changes in the Lipid Contents of Serum in Patients with Manic-Depressive Psychosis, H. K. Lewis, London, 1940.

2 Wendt, H., *Biochem. Z.*, 1932, **250**, 212.

3 Gardner, J. A., and Gainsborough, H., *Biochem. J.*, 1928, **22**, 1048.

4 Turner, K. B., and Steiner, A., *J. Clin. Invest.*, 1939, **18**, 45.