PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 459-463 (1976)

Early Change of Specific Activity of Plasma and Red Blood Cell Cholesterol following Intravenous Administration of [4-^{14}C]Cholesterol in Man[1] (39568)

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Compartmental analysis of cholesterol requires the intravenously administered labeled tracer cholesterol to be mixed instantaneously in the rapidly exchangeable pool and subsequently to have the same metabolic fates as the unlabeled cholesterol (1). In most instances, the disappearance curve of the plasma total cholesterol specific activity is used for compartmental analysis (2-9). There are two major problems involved in such studies: (i) Cholesterol in the rapidly exchangeable pool exists in blood and various tissues in both free and esterified forms (10). The complete mixing of the labeled and unlabeled cholesterol in this compartment cannot be instanteneous. Complete equilibrium between plasma and red blood cell (RBC) -free cholesterol is rapid as shown in both *in vitro* and *in vivo* studies (11-18), whereas that between plasma-free and esterified cholesterol can be achieved only 48 hr or longer after giving [^{14}C]acetate or lipoprotein-bound [^{14}C]cholesterol to the dog (12, 17) or [^{14}C]acetate to man (13). Such equilibrium cannot be attained by *in vitro* incubation of plasma because of the lack of enzyme for hydrolysis of cholesterol esters (11, 14, 16).

(ii) Since cholesterol is virtually insoluble in water, the tracer cholesterol has been administered in the form of lipoprotein (17, 18) in acetone, ethanol, saline, or ethanol-saline solution (2-8, 19, 20), or as an emulsion stabilized wth detergent (18, 21). The serum cholesterol specific activity during the first few days after administration has been shown to be greatly affected by the form of tracer cholesterol administered. Cholesterol in the form of a suspension is rapidly removed from the bloodstream (18, 19, 21). There is direct evidence, at least in animals, that this is due to the uptake by phagocytic cells, especially the hepatic macrophages (19).

Ideally, the tracer cholesterol should be entirely in the form of lipoproteins. Methods for *in vitro* incorporation of cholesterol into serum lipoprotein have been described but require prolonged incubation (17, 18, 22) and have been adopted for human study by Sodhi and Kudchodkar (22, 23). However, the time for complete equilibrium between plasma-free and esterified cholesterol was not studied in their reports. Since the validity of the compartmental analysis depends upon the complete equilibrium of cholesterol in each pool, it is necessary to determine the time required for attaining such equilibrium, especially when the administered tracer cholesterol is not entirely in the form of lipoprotein. Such information is lacking in human studies and is the goal of our present research.

Materials and methods. Subjects. Two healthy adult white males, D. P. and K. B., measuring 169 and 175 cm and weighing 62.6 and 74.8 kg, respectively, were the volunteers for this study. Their plasma total cholesterol levels were, respectively, 208 and 199 mg/dl and free cholesterol levels were 52 and 56 mg/dl, as determined by the method of Sperry and Webb (24). Their serum lipoprotein electrophoretic patterns by agarose gel electrophoresis (25) were within normal limits.

Preparation and administration of the injectable [4-^{14}C]cholesterol. Approximately 60 μCi of [4-^{14}C]cholesterol in a stock solution containing 1 mCi in 6.8 mg of cholesterol (New England Nuclear, Boston,

[1] Supported by U.S. Public Health Service Grant No. HL-13612 from the National Heart and Lung Institute, Birmingham, Alabama, and Veterans Administration Medical Research Service, Albany, New York.

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Mass.) was purified by silica-gel thin-layer chromatography in petroleum ether:ethyl ether:glacial acetic acid, 80:20:1 (by volume). Cholesterol was eluted with diethyl ether and sterilized by filtration (Millex 0.22-μm filter, Millipore Corp., Bedford, Mass.) to two sterilized 10-ml test tubes in equal amounts and evaporated to dryness under a stream of nitrogen. Approximately 4 ml of fresh plasma obtained aseptically from each subject was added to the tube which was immediately incubated in a 37° water bath with constant shaking for 1 hr. At the end of 1-hr incubation, exactly 2.5 ml of the incubated plasma was injected intravenously back to the same subject. The remaining plasma was used for (a) routine bacterial culture, (b) immediate determination of the free and total cholesterol concentrations by the method of Sperry and Webb (24) and their radioactivities, (c) calculation of the total radioactivity administered to each subject, and (d) immediate agarose gel electrophoresis (25) for determination of the distribution of radioactivity among various classes of lipoproteins by cutting off the bands in the strip corresponding to chylomicron, β, pre-β, and α-lipoproteins and by counting their radioactivity in PPO–Bis–MSB toluene solution. The total radioactivities given to subjects D. P. and K. B. were 6.3×10^7 and 8.2×10^7 dpm, respectively. Six percent of [4-^{14}C]-cholesterol was esterified after 1-hr incubation in subject D. P. and 9% in subject K. B. The distribution of relative radioactivity in the point of application, β, pre-β, and α-lipoprotein was 65, 29, 5, and 1% in subject D. P. and 62, 33, 4, and 1% in subject K. B. Apparently two-thirds of [4-^{14}C]cholesterol was not incorporated into lipoproteins after 1-hr incubation and remained at the point of application during electrophoresis. The bacterial cultures were all negative.

Collection and analyses of sera. A 5-ml blood sample was collected from each subject in the following schedule: 12 min, 30 min, and 1, 2, 4, 8, 13.5, and 24 hr, and 3, 4, 5, and 7 days after the administration of [4-^{14}C]cholesterol. RBC, after separation from plasma by centrifugation, were washed three times with 20 vol of normal saline. The cholesterol content of RBC was determined by the method previously described (26) and the serum-free and total cholesterol levels were determined by the method of Sperry and Webb (24). Their radioactivities were counted in PPO–Bis–MSB toluene solution. Quenching, when present, was corrected by the channels ratio method (27). Serum-esterified cholesterol concentration and radioactivity were calculated from the data obtained for free and total cholesterol.

Results. Changes of plasma and RBC cholesterol contents. The means and standard deviations of the 13 determinations of plasma and RBC cholesterol levels over a period of 7 days in subjects D. P. and K. B. were, respectively, 208 ± 6 and 199 ± 18 mg/dl for plasma total cholesterol, 156 ± 6 and 143 ± 12 mg/dl for plasma-esterified cholesterol, 52 ± 8 and 56 ± 5 mg/dl for plasma-free cholesterol, and 99 ± 6 and 97 ±3 mg/dl for RBC cholesterol. Minor fluctuations were noted from time to time, as shown in Fig. 1.

Changes of cholesterol specific activity (Figs. 2–4). (i) *Plasma-free cholesterol specific activity.* The plasma-free cholesterol specific activity decreased sharply from the initial high value obtained 12 min after the injection in both subjects to a value only about 30% (28 and 31%) of the 12-min value in 2–4 hr. The specific activity then increased to a new high value 13.5 hr after injection. It then decreased again, steadily and gradually, to a value 10–16% of the initial high value on the seventh day.

(ii) *Plasma-esterified cholesterol specific*

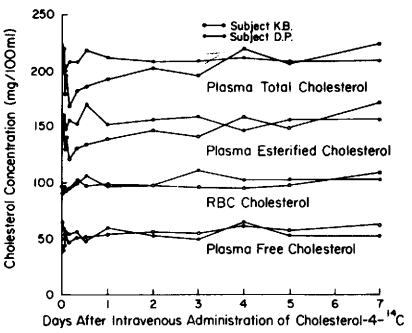

FIG. 1. Changes of plasma-free, esterified, total cholesterol levels, and red blood cell cholesterol contents in two subjects during the study.

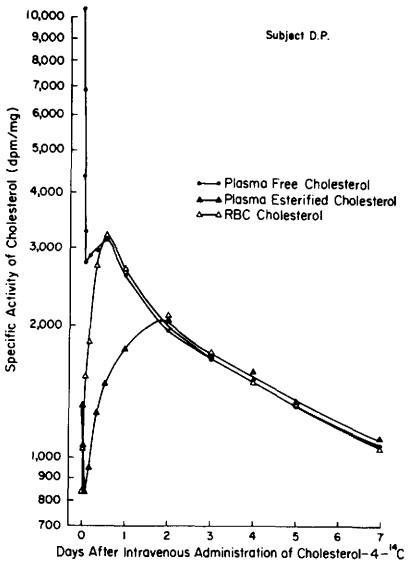

FIG. 2. Changes of the specific activity of plasma-free and esterified cholesterol, and red blood cell cholesterol after intravenous administration of [4-^{14}C]cholesterol in subject D. P.

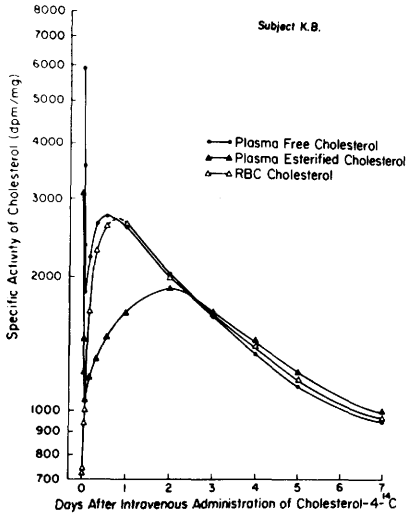

FIG. 3. Changes of the specific activity of plasma-free and esterified cholesterol, and red blood cell cholesterol after intravenous administration of [4-^{14}C]cholesterol in subject K. B.

activity. The plasma-esterified cholesterol specific activity obtained 12 min after injection was 52% of the plasma-free cholesterol specific activity in subject K. B. and only 13% in subject D. P. Nevertheless, it decreased sharply in both cases to a low value in 2–4 hr and then increased gradually to

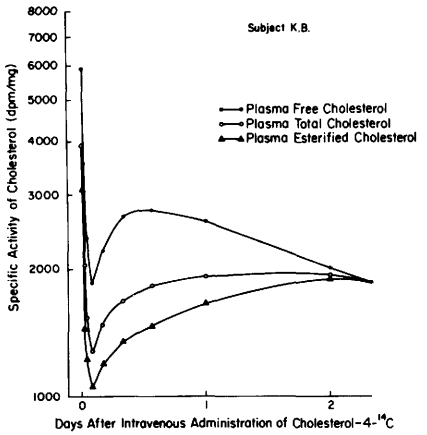

FIG. 4. Changes of the specific activity of plasma-free, esterified, and total cholesterol during the first 2 days after the intravenous administration of [4-^{14}C]cholesterol in subject K. B.

reach a value similar to that of free cholesterol 2 days later. It then decreased again parallel to the decreasing curve of the plasma-free cholesterol specific activity, but the former usually had a higher value than the latter at any given time.

(iii) *Plasma total cholesterol specific activity*. The change of the plasma total cholesterol specific activity fell between that of the free and esterified cholesterol specific activity; an initial rapid decline was followed by a gradual increase to a peak 2 days after injection and then a gradual decrease.

(iv) *RBC cholesterol specific activity*. The RBC cholesterol was not labeled at the time of injection but the specific activity decreased rapidly to reach a value equal to the plasma-free cholesterol specific activity 12–18 hr after the injection. The RBC cholesterol specific activity then decreased following a curve parallel to that of the plasma-free cholesterol specific activity.

Discussion. The changes of the specific activity of plasma and RBC cholesterol after an intravenous administration of [4-^{14}C]cholesterol followed a very similar pattern in these two subjects. The initial rapid decrease of the plasma-free, esterified, and total cholesterol specific activity over a period of 2–4 hr indicated the disappearance of some of the administered [4-^{14}C]cholesterol from the plasma. Apparently some of the disappeared [4-

^{14}C]cholesterol was incorporated into RBC, but the uptake by RBC could only account for a small portion of the total loss. Therefore, these must be a net loss of [4-^{14}C]cholesterol from the bloodstream within the first few hours after the injection. Two-thirds of the administered [4-^{14}C]cholesterol was not incorporated into lipoproteins and existed in a particulate form which would be subject to phagocytosis by the reticuloendothelial system, particularly hepatic macrophages as shown in rats by Nilsson and Zilversmith (19). After such initial fall of specific activity, [4-^{14}C]cholesterol started to reappear in the blood because plasma cholesterol specific activity increased to a peak 13.5 hr after injection. Obviously the uptake and release of the particulate cholesterol by reticuloendothelial system are rather rapid processes.

The exchange of free cholesterol between plasma and RBC was also very rapid. A complete equilibrium between the plasma-free cholesterol and RBC cholesterol was attained within 12–18 hr. It has been shown that the equilibrium between RBC and plasma-free cholesterol both *in vitro* and *in vivo* is closely approached in 4–8 hr (11-18). A detailed study of the *in vitro* quantitative change of free cholesterol between plasma and RBC by d'Hollander and Chevallier (16) revealed an exchange rate of 0.065 mg/hr/mg of blood and a turnover time of 9.2 hr for RBC cholesterol. The exchange required no energy derived from glucose metabolism (15). Because of such rapid and complete exchange, RBC and plasma-free cholesterol can be considered as in the same pool.

The exchange between plasma-free and esterified cholesterol was a slower process as compared with that between RBC and plasma-free cholesterol. We found in this experiment that 2 days were required for their complete equilibration in man. A similar result was obtained in dogs given lipoprotein-bound free [^{14}C]cholesterol (17). This process requires the enzymes involving esterification of free cholesterol such as lecithin: cholesterol acyltransferase (28) and hydrolysis of esterified cholesterol such as cholesterol esterase (29). The exchange rate would depend upon the activities of these enzymes. This is apparently the rate-limiting step for complete equilibration between the labeled and unlabeled cholesterol in the rapidly exchangeable pool.

The cholesterol-esterification activity seemed to be more active in subject K. B. than in D. P., because after 1-hr incubation with their plasma, 9% of [4-^{14}C]cholesterol was esterified in K. B. whereas only 6% esterified in D. P. The initial serum-esterified cholesterol specific activity was also higher in K. B. than in D. P. However, the subsequent changes of serum-free and esterified cholesterol specific activity were quite similar in both subjects.

Although the technique for administration of tracer cholesterol for compartmental analysis described in this paper was not a perfect one because of the incomplete incorporation of [4-^{14}C]cholesterol into lipoprotein, an initial phagocytosis of the particulate [4-^{14}C]cholesterol, and 2-day requirement for complete equilibration between the plasma-free and esterified cholesterol, it was simple, rapid, and safe. Since the specific activity of plasma total cholesterol 24 hr after injection was underestimated, the compartmental analysis should be based on the plasma total cholesterol specific activity obtained 2 days after the injection.

Summary. The tracer [4-^{14}C]cholesterol, incubated at 37° for 1 hr with the subject's own plasma, was administered intravenously to two subjects. The initial rapid decrease of plasma cholesterol specific activity indicated a net disappearance of [4-^{14}C]cholesterol from the bloodstream, presumably due to phagocytosis of the particulate cholesterol by the reticuloendothelial system. The initially disappeared [4-^{14}C]cholesterol quickly reappeared in the blood. Complete equilibrium between RBC and plasma-free cholesterol was attained within 12–18 hr, whereas the equilibrium between plasma-free and esterified cholesterol required 2 days. Therefore, the compartmental analysis should be based on the plasma total cholesterol specific activity obtained 2 days after the injection.

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Received May 24, 1976. P.S.E.B.M. 1976, Vol. 153.