other sources. The total hydroxyproline content of the nodules and the synovial tissue was accounted for completely by the collagen and elastin fractions. The suggestion is made on this basis that the fibrinoid material of the rheumatoid nodule is not of collagenous origin.

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The *In vitro* Interchange of Cholesterol Between Plasma and Red Cells.* (19064)

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In previous studies on the incorporation of C^{14}-acetate carbon into cholesterol in various tissues of intact animals(1), it was observed that newly synthesized cholesterol appeared in liver, plasma, and red blood cells with surprising rapidity and attained uniform distribution within a few hours. Further investigation in dogs led to the conclusion that a rapid interchange of cholesterol must be assumed to occur in the living animal to explain the rapidity of equilibration of isotopic cholesterol between liver, plasma, and red cells(2,3). It therefore seemed of interest to investigate cholesterol interchange *in vitro*. Hahn and Hevesy explored the *in vitro* interaction between P^{32}-labeled phospholipids of plasma and red cells and found a very limited interchange—5% in 4.5 hours in rabbit blood(4). Although only total phospholipid P was measured, these authors concluded that some phosphatides exchanged with great speed and others not at all.

It is now generally accepted that the phospholipids and cholesterol of plasma are present in lipoprotein form and this would appear to be true also for red cells. However, the unesterified cholesterol moiety of both plasma and red cell lipoproteins is chemically identical and is therefore particularly suitable for interchange studies.

Procedure and results. Whole blood containing C^{14}-labeled cholesterol of high specific activity was obtained from a dog previously maintained on a cholesterol-free diet by the oral administration of 100 to 400 μc of C^{14} as carboxyl-labeled acetate, followed by the exsanguination of the animal about 24 hours later. The blood was treated with an anticoagulant (A.C.D. solution or oxalate) and cells and plasma separated. The interfacial layer was rejected and the cells were washed 3 times with isotonic salt solution. Unlabeled dog blood was drawn and treated in the same way except that the cells were not washed. In most experiments labeled cells and labeled plasma from one dog were each incubated with their unlabeled counterparts from a normal dog. In one experiment, normal human red cells were incubated with radioactive dog plasma. Measured volumes of red cells and of plasma were mixed and incubated at 37°C with gentle agitation and a slow stream of 95% oxygen plus 5% carbon dioxide was passed through the flasks. Aliquots were removed at intervals up to 4 hours and centrifuged. The interfacial layer was rejected and total lipid extracts were prepared from aliquots of plasma and red cells by 3 extractions with hot alcohol-ether (3:1), to a total of 10 volumes of solvent. Aliquots of the clear lipid extract were analyzed for free and total cholesterol by the Sperry - Schoenheimer

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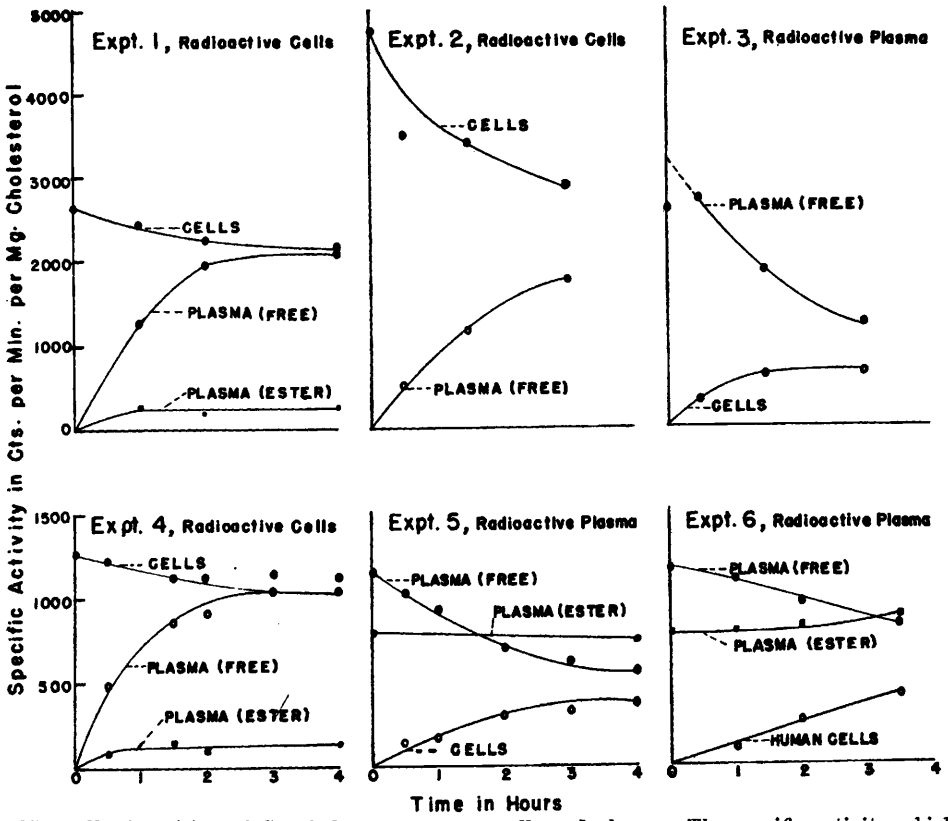

FIG. 1. Equipartition of C^{14}-cholesterol between cells and plasma. The specific activity which is proportional to the concentration of labeled cholesterol in the total cholesterol is plotted against time. In Exp. 6, human cells were incubated with dog plasma, and in all other experiments dog plasma and cells were used.

method as recently described(5). Larger digitonide samples were prepared for C^{14} assay by essentially the same procedures except that only a slight excess of digitonin was used. The assay technic is described elsewhere(6). All specific activities are expressed in terms of counts per minute for an infinitely thick layer of cholesterol (arbitrarily defined as counts per minute per mg cholesterol).

Fig. 1 shows the results of all 6 experiments, the cholesterol specific activities being plotted against time. It is evident that interchange of labeled cholesterol was very rapid in both directions. The total decrease in specific activity of the labeled component varied in each experiment depending on the relative masses of free cholesterol in the 2 phases.

Specific activities of esterified cholesterol in plasma were calculated from the specific activities and analyses for free and total cholesterol. Fig. 1 shows that esterified cholesterol of plasma neither acquired significant quantities of isotopic cholesterol (Exp. 1 and 4) nor did the labeled cholesterol ester fraction show any significant decrease in specific activity (Exp. 5 and 6). From this it may be concluded that esterified cholesterol was not capable of exchanging and also that the cholesterol esterases were not active under the conditions used. In the intact animal, however, cholesterol esters have been found to come to equilibrium with the free plasma cholesterol.

Analyses on all aliquots showed no significant change during incubation. Table I gives

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TABLE I. Cholesterol Analyses and Total Isotopic Cholesterol Content.

Exp. No.	Labeled component	C^{14}-blood from dog No.	Plasma component Vol, ml	Plasma component Cholesterol level Free, mg %	Plasma component Cholesterol level Ester, mg %	Red cell component Vol, ml	Red cell component Cholesterol level, mg %	Total C^{14} in cholesterol† Initial $\times 10^{-3}$	Total C^{14} in cholesterol† Final $\times 10^{-3}$
1	Cells	1	25	31	80	25	152	99.8	97.6
2	”	2	22	27	79	14	155	99.4	84.9
3	Plasma	2	34	30	64	14	147	32.7	29.2
4	Cells	3	50	37	91	50	131	82.4	86.6
5	Plasma	3	50	62	148	34	152	35.7	36.5
6*	”	3	48	62	148	20	92	33	35.6

* Radioactive dog plasma was incubated with human red cells.

† Total C^{14} in cholesterol was obtained by multiplying the cholesterol specific activity (c/m/mg) by total mg for each component and summating.

Bloods from dogs No. 1 and 2 were collected into A.C.D. solution so that the plasmas were diluted considerably. Potassium oxalate crystals (.2 g/100 ml) were used as anticoagulant in the blood from dog No. 3.

average values for each experiment.

Both labeled red cells and plasma contained C^{14}-labeled constituents other than cholesterol. Such labeled compounds will not interfere with the cholesterol studies unless they are converted into cholesterol during the incubation. Estimates of the total amount of isotopic cholesterol present in each incubation mixture did not show any significant change during the experiment as shown in Table I. It is of interest to note that cholesterol had a considerably higher specific activity than any other constituent examined.

As the curves in Fig. 1 appear similar to those for a first order process the data have been plotted in exponential form. In Fig. 2 $\log \frac{a}{a-x}$ is plotted against time, a being the final equilibrium specific activity for each experiment (obtained by extrapolation) and x being the change in specific activity from the initial value to the value at time t. In this way both ascending and descending specific activity curves can be plotted on the same basis. A close approach to linearity was shown by each of the individual experiments. Average values for all 5 dog experiments are shown in Fig. 2. The time required to reach 50% equilibration was 1.1 hours for red cells and 0.95 hour for plasma or an average of very close to 1 hour.

The single experiment with dog plasma and human red cells gave similar results (Fig. 1, Exp. 6), showing that the phenomenon is not species specific. The rate appeared somewhat slower but additional experiments will be necessary to determine if this difference is significant.

Discussion. During the last 20 years the concept that cholesterol and phospholipids are bound to specific proteins as lipoproteins has been developed and has recently been given strong support by the isolation and characterization of purified β_1 lipoprotein by Oncley, Gurd, and Melin(7). The nature of the link-

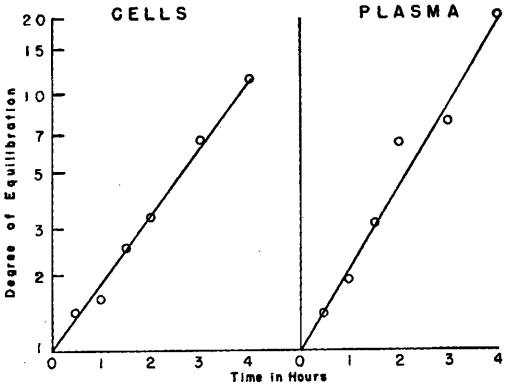

FIG. 2. The degree of equilibration of C^{14}-cholesterol between red cells and plasma expressed as $\frac{a}{a-x}$, plotted on a logarithmic scale against time. Each point represents the average of all data for that time interval for the movement of labeled cholesterol both into and out of dog red cells (on the left) and plasma (on the right). Data from the human red cell experiment are not included. Equilibration is half completed when $\frac{a}{a-x} = 2$, which is at approximately one hour in each case.

age between lipids and proteins is not understood but it has been generally considered to by a stoichiometric linkage with some stability rather than an adsorption complex. The experiments reported in the present paper show that unesterified cholesterol molecules initially present in plasma lipoproteins interchange with those in red cell lipoproteins during incubation. It appears highly unlikely that the entire lipoprotein molecules interchange since plasma lipoproteins contain both free and esterified cholesterol whereas red cells contain only the free form. Furthermore, red cell lipids occur in the water insoluble stroma in contrast to the highly water soluble plasma lipoproteins. All the free cholesterol molecules of plasma and of red cells are apparently capable of interchange. Although the radioactive cholesterol molecules measured in these experiments were recently synthesized, there is no reason to believe that a plasma lipoprotein molecule containing newly synthesized cholesterol will behave any differently from one containing "older" cholesterol. In the case of red cells, the fact that complete equipartition with free plasma cholesterol is attained both *in vivo* and *in vitro* is strong evidence for the assumption that all the free red cell cholesterol is exchangeable. The time required to reach 50% equipartition can be seen from Fig. 1 or 2 to be about one hour, corresponding to a turnover time of 1.44 hours. Thus, any given sample of plasma will have a minimum of 1.16% of the free cholesterol present leaving each minute and being replaced by an equal amount of red cell cholesterol. For example, in a liter of average blood the total cholesterol exchanged between plasma and red cells would be about 460 mg per hour.

The mechanism of this exchange is not simple diffusion since equalization in the free cholesterol concentrations of the 2 phases does not occur. The simplest assumption is that equilibria exist between cholesterol in each of the various lipoproteins concerned and cholesterol in unbound form. This last might be present in minute concentration, possibly in true aqueous solution, and still be capable of acting as intermediate in a rapid equilibration of lipoprotein cholesterol. Experiments to test this hypothesis are now in progress.

Summary. Plasma and red cells containing C^{14}-labeled cholesterol were obtained by feeding dogs with C^{14}-acetate. The labeled blood components were separated and incubated at 37°C for several hours with their unlabeled counterparts under an atmosphere of oxygen-carbon dioxide. The exchange of cholesterol between the two components was followed in each mixture by measurement of the cholesterol specific activities of each component at various time intervals during the incubation. It was observed that equipartition of red cell and plasma unesterified cholesterol was closely approached in 4 hours and 50% equilibration took place in one hour. Esterified cholesterol of plasma did not take part in the exchange phenomenon under these conditions. The possible significance of these results in relation to the nature of lipoproteins is discussed.

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Complement-Fixing Murine Typhus Antibodies in Vitamin Deficiency States.* II. Pyridoxine, and Nicotinic Acid Deficiencies. (19065)

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The authors have presented data(1) concerning the effects of adequate vit. B complex, 1/10th optimal level vit. B complex, total

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