The Lipids of Whole Blood

2. THE EXCHANGE OF LIPIDS BETWEEN THE CELLULAR CONSTITUENTS AND THE LIPOPROTEINS OF HUMAN BLOOD*

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In Part 1 (James, Lovelock & Webb, 1959) and in the earlier work of Altman, Watman & Solomon (1952), it was reported that the cellular constituents of the blood are able to incorporate activity from sodium [*Me*-^{14}C]acetate into their lipid components. It was also shown that the radioactive lipids synthesized by the cells are transferred during the course of incubation *in vitro* to the plasma lipoproteins.

From this earlier work little or no information is available on the contribution of the cells to the lipid metabolism of the blood, or indeed to that of the whole animal. This paper reports experiments designed to determine quantitatively the turnover of the major lipid components, namely the phospholipids, the neutral fat and the unsaponifiable lipids, between the cells and the lipoproteins of the plasma.

METHODS

Incubation of blood. During the incubation sterile procedures were used throughout. Freshly drawn whole blood (100 ml.) was added to a mixture (15 ml.) of citric acid–sodium citrate–dextrose, penicillin, streptomycin sulphate (100 units each/ml. of whole blood) and ethylenediaminetetra-acetic acid (0·05 ml. of 0·1% solution). Sodium [*Me*-^{14}C]acetate (1 μc/ml.; specific activity 13·6 μc/mg.) was added. The mixture was incubated at 34·5° with gentle agitation in a roller-tube apparatus.

Separation of cells and fractionation of lipoproteins. At different time intervals, 10 ml. samples were withdrawn from the incubation mixture and centrifuged for 5 min. The cells were washed once with 0·9% NaCl (3 ml.) and then added in a thin stream to 100 ml. of ether–ethanol (1:2, v/v). To the plasma and 0·9% NaCl washings dextran sulphate (0·5 ml. of 1% solution) was added and mixed. After 30 min. the precipitated β-lipoprotein–dextran sulphate complex was removed by centrifuging (Oncley, Walton & Cornwell, 1957) and transferred to ether–ethanol. The supernatant liquid which contained the α-lipoprotein was added slowly to ether–ethanol.

Fractionation of the lipids. The protein precipitated by ether–ethanol was removed by filtration and the filtrate concentrated to dryness *in vacuo* at low temperature. Where necessary the last traces of water were removed by the repeated addition of ethanol, followed by distillation. The residue was obtained as a thin film inside the flask. This was immediately extracted twice with 1 ml. of acetone (extract A) and the residual acetone removed by warming *in vacuo*. Phosphorus analyses indicated that extract A contained negligible quantities of phospholipid.

Hydrolysis of phospholipids. The residue from acetone extraction, dissolved in a mixture of aqueous 50% (v/v) ethanol (5 ml.) and aqueous 40% (w/v) KOH (1 ml.), was heated at 60° for 4 hr. After the mixture had cooled to room temperature, water (3 ml.), acetic acid (4 drops) and 10 N-H_2SO_4 (2 ml.) were added and mixed. When cool the mixture was extracted with light petroleum (40–60°; 4 ml., 1 ml. and 1 ml.).

Hydrolysis of neutral fat. The acetone extract (A) was concentrated to dryness under reduced pressure and hydrolysed for 4 hr. at 60° in a mixture of ethanol (5 ml.) and aqueous 40% (w/v) KOH (1 ml.). Water (3 ml.) and acetic acid (4 drops) were added and the hydrolysate was extracted three times with light petroleum (4 ml., 1 ml. and 1 ml.) to remove unsaponifiable fat. Sulphuric acid (10 N; 2 ml.) was added and the mixture again extracted with light petroleum as before to obtain the saponified fatty acids.

After the hydrolyses of lipids the unmetabolized sodium [*Me*-^{14}C]acetate remained substantially in the aqueous layer during the extraction with light petroleum. Only low specific activities were obtained for the zero incubation times and these were subtracted from subsequent values.

Determination of specific activities. The light petroleum extracts were evaporated to dryness on planchets of aluminium foil which had been weighed with a microtorsion balance. The planchets were then reweighed. The samples were then counted with an end-window Geiger–Müller tube which gave 6×10^4 counts/min./μc. The self-absorption of the samples (1 mg./cm.^{2}) was considered to be negligible. The specific activity of the unsaponifiable lipid was based upon the weight of this fraction. Specific activities of saponifiable lipid and phospholipid were based upon the weights of the corresponding fatty acids obtained after hydrolysis.

Incubation of inert cells with radioactive lipoproteins. Two freshly drawn samples (50 ml. each) of whole blood were mixed with citric acid–sodium citrate–dextrose, ethylenediaminetetra-acetic acid, streptomycin and penicillin as described above. One portion (A) was stored at 2° and the other (B) was incubated for 4·5 hr. at 34·5° with sodium [*Me*-^{14}C]acetate (30 μc). The plasma was separated by centrifuging and 8 ml. of 0·16 M-sodium acetate added to reduce the specific activity of unmetabolized radioactive acetate by at least 1000-fold. The cells from A were removed by centrifuging, washed with 0·16 M-sodium acetate

* Part 1: James, Lovelock & Webb (1959).

and added to the plasma from B. The 'reconstituted' blood was incubated at 34° and 10 ml. samples were withdrawn at different time intervals for analysis.

RESULTS

In reporting and discussing the results, the following classifications are used. Cells include red cells, white cells and platelets. The β-lipoprotein is principally that first isolated by Oncley, Gurd & Melin (1950). The α-lipoprotein includes all plasma lipids, with the exception of β-lipoprotein, and includes chylomicra and, possibly, as yet unidentified lipoproteins. Neutral fat includes fatty acid glycerides, cholesterol esters and possibly other sterol esters. The unsaponifiable fat includes cholesterol, other sterols and possibly some esters which are resistant to hydrolysis, since prolonged saponification leads to further production of small quantities of fatty acid.

Fig. 1 shows the increase of specific activity of the neutral fat of the cells, α-lipoprotein and β-lipoprotein when whole blood is incubated with radioactive acetate. Figs. 2 and 3 show the analogous results for the phospholipids and unsaponifiable fat respectively.

In other experiments whole blood was incubated with radioactive acetate for 4–5 hr. and then the cells were replaced by non-radioactive washed cells

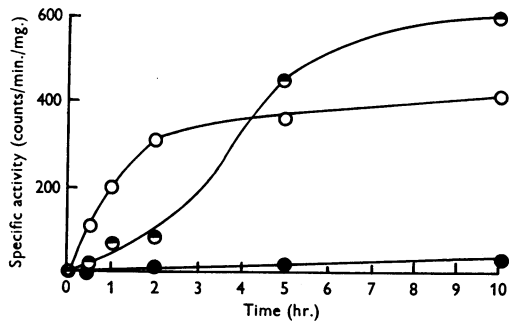

Fig. 1. Labelling of blood neutral fat on incubation with radioactive acetate. Whole blood was incubated with sodium [*Me*-^{14}C]acetate at 34·5°. ○, Neutral fat from cells; ●, neutral fat from β-lipoprotein; ◓, neutral fat from α-lipoprotein.

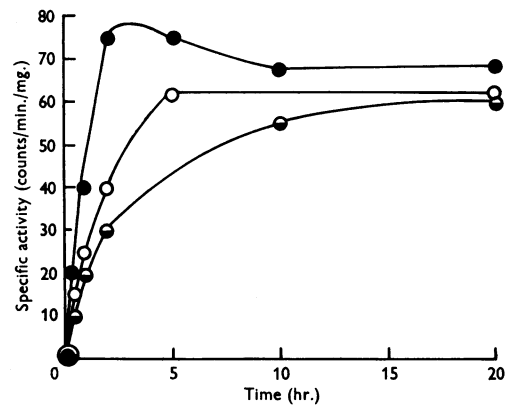

Fig. 2. Labelling of blood phospholipids on incubation with radioactive acetate. Whole blood was incubated with sodium [*Me*-^{14}C]acetate at 34·5°. ○, Phospholipids from cells; ●, phospholipids from β-lipoprotein; ◒, phospholipids from α-lipoprotein.

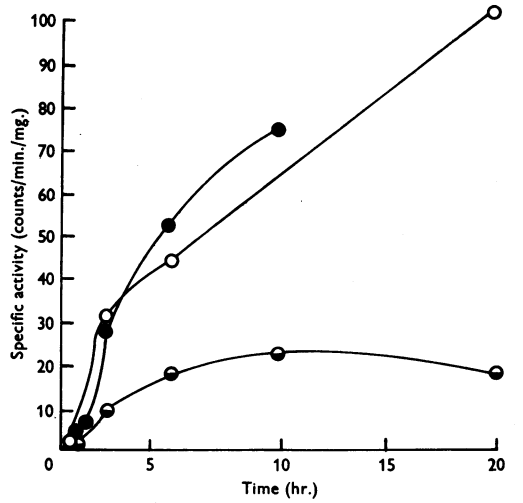

Fig. 3. Labelling of blood unsaponifiable lipid on incubation with radioactive acetate. Whole blood was incubated with sodium [*Me*-^{14}C]acetate at 34·5°. ○, Unsaponifiable lipid from cells; ●, unsaponifiable lipid from β-lipoprotein; ◒, unsaponifiable lipid from α-lipoprotein.

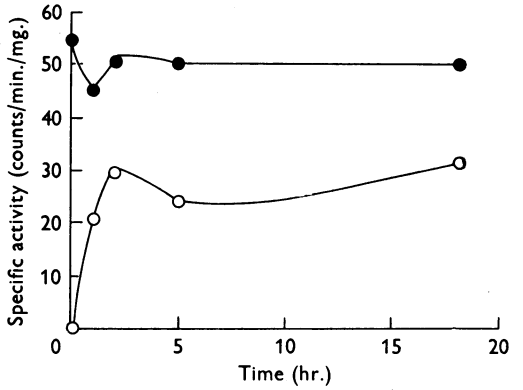

Fig. 4. Exchange of neutral fat on incubation of labelled plasma with unlabelled blood cells. Whole blood was incubated with sodium [*Me*-^{14}C]acetate at 34·5° for 4·5 hr. The cells were separated and replaced by non-radioactive cells. ○, Neutral fat from cells; ●, neutral fat from plasma.

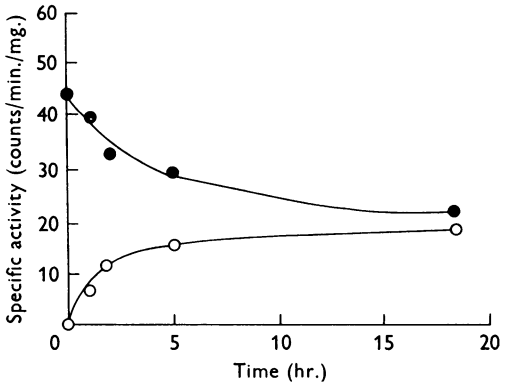

Fig. 5. Exchange of phospholipid on incubation of labelled plasma with unlabelled blood cells. Whole blood was incubated with sodium [*Me*-^{14}C]acetate at 34·5° for 4·5 hr. The cells were separated and replaced by non-radioactive cells. ○, Phospholipid from cells; ●, phospholipid from plasma.

from the same donor. The subsquent changes in specific activities of the neutral fat of the plasma and new cells are shown in Fig. 4. The corresponding changes in the phospholipids are shown in Fig. 5.

DISCUSSION

When whole blood is incubated *in vitro* with sodium [*Me*-^{14}C]acetate radioactivity is incorporated into the lipids of both the cells and the plasma lipoproteins. When plasma alone is incubated with active acetate very little radioactivity is found in the plasma lipids; this is possibly attributable to the metabolic activity of platelets remaining after the separation of the other cells. It follows that the experimental results include two general phenomena, firstly the incorporation of activity from radioactive acetate into their lipids by the red and white cells and secondly the exchange of active and inactive lipids between the cells and their plasma. In the whole animal the exchange of lipids from the plasma occurs not only with the cells but also with the other organs, particularly the liver (Hahn & Hevesy, 1939). Although this implies that the blood *in vitro* is not in a steady state so far as lipid metabolism is concerned, the isolation of the blood is nevertheless essential in distinguishing the contribution of the cells to the lipid metabolism of the whole animal.

The results of the incubation experiments indicate at first a linear rise of lipid specific activity with time for the cells, followed by a levelling off. The levelling off of specific activity could be due to the establishment of an equilibrium distribution of isotopic carbon, but it is more probably attributable to the depletion of essential metabolites in the blood or perhaps also to the accumulation of inhibiting substances. The phospholipid and the unsaponifiable lipid were observed to exchange freely with all classes of plasma lipoproteins. Neutral-fat activity was found, however, only in the α-lipoprotein-containing fraction. The free exchange of phospholipid and unsaponifiable lipid but not of neutral fat between the β-lipoprotein and the cells is consistent with a cell-like structure for β-lipoprotein, in which a central core of neutral fat is surrounded by an impermeable membrane of phospholipid, unsaponifiable lipid and protein.

Quantitative aspects. Of the added isotopic carbon 3–5 % was recovered in the lipid fractions at the time the steady level was reached. This result is particularly striking when it is recalled that 95 % of the dry weight of the cell fraction consists of haemoglobin from the red cells (Wintrobe, 1951), which is metabolically innert.

Neither the gross lipid components nor their fatty acid moieties are single entities in a chemical sense; in these circumstances the calculation of turnover and exchange rates from the experimental results is made uncertain by the dilution of radioactive substances by inert material simultaneously extracted. Nevertheless, certain approximate values for the turnover rates of the various lipid classes can be deduced.

Exchange of phospholipids. In Fig. 5 is illustrated the exchange of phospholipid when cells are suspended in plasma containing ^{14}C-labelled phospholipid. The growth and decay of phospholipid specific activity between the cells and the plasma are complementary. This experiment would suggest that the plasma phospholipid is in simple diffusion equilibrium with the same phospholipid within or at the surface of the cells. In two experiments of the type illustrated in Fig. 5, the half-life for the exchange process was 6 hr. In a separate experiment plasma phospholipids were found to be at a level of 1·3 μmoles of phosphorus/ml. of whole blood. Taking this value and 800 for the average molecular weight, the exchange of phospholipid was 0·09 mg./ml. of whole blood/hr.

The synthesis of radioactive phospholipid by the cells and its transfer to the plasma lipoproteins is illustrated in Fig. 2. The specific activity of the plasma phospholipids starts to rise immediately at the beginning of the experiment and continues to rise at a rate closely similar to those of the cellular phospholipids. The principal phospholipid of the plasma is phosphatidylcholine (77 %), whereas the cells contain only 31 % of this material (Hack, 1947). Experiments which will be reported in a later paper indicate that the phospholipid metabolism of the cells is complex, and a wide variety of phospholipid species are synthesized. In these circumstances it is hardly possible to deduce from

the experimental data any numerical values of the metabolic turnover of phospholipid by the cells. Since the increase in specific activity of the plasma lipoproteins is comparable with that of the cells, it would appear that the exchange by diffusion is rapid compared with the turnover by metabolism.

Exchange of neutral fat. Unlike the exchange of phospholipid, that of neutral fat does not appear to be determined principally by simple diffusion equilibrium. Fig. 1 shows the exchange of neutral fat between the cells and the plasma-lipoprotein fractions during an incubation experiment. The specific activity of the celullar neutral fat is seen to rise rapidly to a level approximately seven times as high as that of the phospholipid. The plasma β-lipoprotein receives little or none of this activity, even after incubation for 10 hr. After a lag period the α-lipoprotein commences to acquire radioactivity so that after 4 hr. the specific activity reaches the same level as that of the cells. The more rapid rise of activity in the cellular neutral fat than in the lipoprotein fraction would suggest that the synthesis of active neutral fat is proceeding faster in the cells than is the exchange of this lipid by diffusion. This notion is confirmed by the experiment (Fig. 4) in which cells were incubated in plasma containing ^{14}C-labelled neutral fat. No simple complementary exchange of activity occurs as with the phospholipid. Instead, the activity of the cells rises rapidly at first and then remains at a more or less steady level. Apart from a slight initial fall, little change in the plasma neutral-fat activity takes place. The values for the plasma neutral-fat specific activity here shown arise from both the active α-lipoprotein neutral fat and also the greater bulk of inert β-lipoprotein neutral-fat material. The specific activity of the exchangeable neutral fat is therefore probably three or more times that shown in Fig. 4. Failure to achieve equilibrium even after 20 hr. suggests that the exchange of neutral fat is not one of simple complementary diffusion. By making certain assumptions a value for the rate of release of neutral fat by the cells can be derived from the information shown in Fig. 1. These assumptions are first that the pool size of the α-lipoprotein neutral fat is 1 mg./ml. of plasma and second that the observed specific activity of the fat of the cells is the same as that of the fat they release. On this basis the observed rate of rise of the activity of the α-lipoprotein neutral fat requires the release of 0·16 mg. of fat/ml./hr. by the cells.

Exchange of unsaponifiable lipid. The increase of specific activity of the cellular unsaponifiable lipids and the transfer of activity to the plasma lipoproteins (Fig. 3) was similar to that observed with the phospholipids. The principal unsaponifiable lipid of all three fractions is, of course, cholesterol, and in separate experiments only a small proportion of radioactivity was found in the cholesterol when it was precipitated with digitonin. Since 90% of the weight of the unsaponifiable lipid was cholesterol the remaining residue must have possessed a very high specific activity, and it is possible that this activity was associated with other sterols, or their intermediates.

SUMMARY

1. The incorporations *in vitro* of radioactivity from sodium [*Me*-^{14}C]acetate into the lipids of whole blood were measured.

2. Lipids from the cells, plasma β-lipoprotein and the α-lipoproteins were fractionated into unsaponifiable lipid, saponifiable lipid and phospholipid.

3. Exchanges of both 'unsaponifiable lipid' and phospholipid between cells and plasma α- and β-lipoprotein were observed.

4. There was no exchange of saponifiable lipid between the cells and β-lipoprotein. Exchange was observed between the cells and the other plasma lipids.

5. Approximate exchange rates were calculated for saponifiable lipid and phospholipid.

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