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# *IN VITRO* LIPID SYNTHESIS IN FOWL BLOOD

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SUMMARY

1. Fowl whole blood has been shown to incorporate labelled acetate into both cellular and plasma lipids.

2. Long chain saturated and unsaturated acids are shown to be synthesized and then incorporated into triglycerides, phospholipids and cholesterol esters.

3. "Sterol"-like unsaponifiable substances are also synthesized from acetate.

4. Comparison of the synthetic ability of leucocytes, young erythrocytes and old erythrocytes show that all cells contribute to the synthesis, the most active being the leucocyte and young erythrocyte preparations.

## INTRODUCTION

Previous papers have described lipid synthesis in human blood[1], the exchange of these lipids between cells and lipoproteins[2] and the relative contributions to this lipid synthesis of the different cell types[3]. The latter work demonstrated that in human blood the leucocytes carry out the major part of the synthesis.

As a corollary to these studies with the non-nucleated human erythrocyte we have turned our attention to the nucleated fowl cell. Results are presented in this paper on the *in vitro* incorporation of labelled acetate into lipids in both whole blood and fractions enriched in old erythrocytes, young erythrocytes and leucocytes.

## MATERIALS AND METHODS

All equipment used up to extraction of the lipid was sterile and siliconized. Blood was drawn into acid–citrate–dextrose and either used directly for the incubation as described below or centrifuged. The plasma was removed and the cells resuspended in a dense medium prepared by dissolving 27 % (w/v) frozen-dried fowl plasma in distilled water, followed by dialysis against several changes of 0.8% NaCl in the cold. Centrifugation of the suspension at 1000 × *g* for 10 min at 4° produced an upper layer rich in leucocytes, which was removed (fraction L), and a lower layer containing very few leucocytes. The erythrocyte layer was subdivided into an upper fraction (Y.E.) and a lower fraction (O.E.). Experiments on the ratio between radioactivity and haemoglobin on similarly prepared fractions after $^{59}Fe$ administration had shown that the upper fraction (Y.E.) contained a relatively high proportion of young cells. The cells in all fractions were resuspended in the original plasma. Total cell counts and differential counts were made on all fractions using in each case samples of 1000 cells.

To the samples were added 1 $\mu$C/ml Na-[Me-$^{14}$C]acetate, streptomycin-penicillin (2:1 by weight, 2 mg/10 ml blood) and sodium ethylene diamine-tetraacetate (0.1 ml of 0.1 % solution/10 ml blood). After equilibration with 5 % $CO_2$, incubation was carried out in large stoppered roller tubes at 37° for 6 h, after which the preparations were centrifuged. Plasma samples were removed, cells washed once in a large volume of saline, and both plasma and cell samples were extracted with 20 volumes of ethanol–ether for 12 h at room temperature.

The ethanol–ether solutions were then filtered and evaporated to dryness *in vacuo*. In some experiments the lipid residue was extracted twice with acetone; the insoluble material is referred to as phospholipid and the soluble portion as neutral fat. This neutral fat fraction was then further fractionated by chromatography using a liquid-solid system as described previously[1]. The methods of saponification, methylation and separation by gas liquid chromatography have been published earlier[1].

## RESULTS

As was demonstrated with human blood[1] both cellular and plasma lipids were found to be extensively labelled in two experiments after incubating whole fowl blood with radioactive acetate (Table I).

Separation of the fatty acids from these fractions and determination of specific

activities showed the pattern of labelling to be similar to that in human blood (Table II). Both the saturated acids up to $C_{18}$ and the monoenoic, dienoic and trienoic $C_{18}$ and the trienoic and tetraenoic $C_{20}$ acids were labelled, indicating considerable biosynthesis. The fatty acid composition of these lipids is given in Table III.

In another experiment the lipid classes from the cells and the plasma were separated by chromatography on silica gel. The specific activities of the fatty acids

TABLE I

SPECIFIC ACTIVITY OF NEUTRAL FAT AND PHOSPHOLIPID AFTER INCUBATION OF WHOLE FOWL BLOOD WITH [Me-$^{14}$C]ACETATE

| *Expt.* | *Neutral fat* | | *Phospholipid* | |
|---|---|---|---|---|
| | *Mixed cells* | *Plasma* | *Mixed cells* | *Plasma* |
| I | 940* | 418 | 876 | 1975 |
| II | 1417 | 783 | 550 | 837 |

* Counts/min/mg.

TABLE II

SPECIFIC ACTIVITIES OF INDIVIDUAL FATTY ACIDS FROM NEUTRAL FAT AND PHOSPHOLIPID OF COMBINED RED AND WHITE CELLS, AND PLASMA

| | *Neutral fat* | | *Phospholipid* | |
|---|---|---|---|---|
| | *Cells* | *Plasma* | *Cells* | *Plasma* |
| Myristic | N.D.* | 1253** | N.D. | N.D. |
| Palmitic | 556 | 535 | 240 | 143 |
| Stearic | 297 | 311 | 132 | 45 |
| Oleic | 125 | 9.5 | 35 | 50 |
| Linoleic + linolenic | 23.4 | 8.4 | 14 | 30 |
| Arachidonic | N.D. | 127 | 72 | 44 |
| $C_{20}$ triene | N.D. | N.D. | 286 | 278 |

* N.D., not determined.
** Counts/min/mg.

TABLE III

FATTY ACID COMPOSITION* OF COMBINED RED AND WHITE CELLS AND PLASMA, NEUTRAL FAT AND PHOSPHOLIPID (FROM TABLE II)

| | *Neutral fat* | | *Phospholipid* | |
|---|---|---|---|---|
| | *Mixed cells* | *Plasma* | *Mixed cells* | *Plasma* |
| Myristic | trace | 0.6 | trace | trace |
| Palmitic | 20.6 | 20.7 | 19.3 | 23.7 |
| Stearic | 21.2 | 9.7 | 18.8 | 23.4 |
| Oleic | 18.7 | 31.2 | 13.9 | 16.7 |
| Linoleic | 38.3 | 30.6 | 37.5 | 22.8 |
| Arachidonic | N.D.** | 4.2 | 5.6 | 8.7 |
| $C_{20}$ triene | N.D. | trace | 1.5 | 4.0 |

* Major components only.
** N.D., not determined.

and non-saponifiable substances in each fraction were determined after saponification and the results are given in Table IV. Unlike human blood the fatty acids and non-saponifiable material of the cholesterol esters were labelled. Maximum labelling was found (as in human blood) in the triglyceride fraction. The mixed fraction of sterol and diglyceride contained a large amount of labelled sterol (97 % of the fraction). In general fowl blood resembles human blood in its ability to synthesize long chain saturated and unsaturated acids from acetate, to incorporate these acids into phospholipids and triglycerides and to secrete these complex lipids into the plasma. The main difference between the two systems lies in the ability of the fowl red and white cells to incorporate fatty acids into cholesterol esters and also to synthesize more unsaponifiable "sterol"-like substances.

*Relative contribution of the different cell types*

The results of incubating labelled acetate with fractions enriched in leucocytes, young erythrocytes and old erythrocytes are given in Table V. It is evident that the highest incorporation of acetate into lipid has taken place in the leucocyte-rich fraction. Thus in whole fowl blood, as in human blood, leucocytes must make a substantial contribution to total lipid synthesis. However, the rate of incorporation of label into both unsaponifiable lipid and fatty acids in the erythrocyte-rich fractions, even after correction for the leucocyte count in the red cell preparation, is still large, so that the substantial proportion of the synthesis observed must be due to erythrocytes.

An indication of the relative rates of synthesis by the several-cell types can be obtained by calculating the incorporation in each fraction due to each of the cell types present and dividing the result by the corresponding cell count. The figures in Table VI summarize the results of such a calculation. They suggest that on average leucocytes synthesize both fatty acids and non-saponifiable substances at least 300 times as fast as young erythrocytes and they in turn synthesize these substances at least 3 times as fast as older erythrocytes. Another experiment gave similar figures for various cell fractions and relative rates of synthesis.

Specific activities of the long chain fatty acids isolated from the total lipids of the leucocyte-rich, young erythrocyte-rich and old erythrocyte-rich fractions are given in Table VII. The activity of most of the individual acids increases in the order: old erythrocyte, young erythrocyte, leucocyte, in agreement with the specific activity of the total lipid.

## DISCUSSION

The experiments quoted suggest that nucleated fowl erythrocytes—in contrast to non-nucleated human erythrocytes—retain some capacity for synthesis of both unsaponifiable lipid and fatty acids. The rate of synthesis by erythrocytes is, however, much less than that by leucocytes. The capacity of erythrocytes for synthesizing lipid is paralleled by their ability to incorporate labelled glycine into haemoglobin. This synthesis takes place throughout the life-span of nucleated avian erythrocytes[4], but not in mature non-nucleated erythrocytes[5]. In view of the known part played by acetyl-coenzyme A in lipid synthesis, it may be relevant that in avian erythrocytes,

## TABLE IV

SPECIFIC ACTIVITIES OF TOTAL FATTY ACID AND NON-SAPONIFIABLE MATERIALS IN LIPID COMPONENTS OF COMBINED RED AND WHITE CELLS AND PLASMA

Specific activities from combined extracts of Expts. 1, 2.

| | *Hydrocarbons + cholesterol esters* | | *Largely triglycerides* | | *Sterol + diglycerides* | | *Unknown eluted with ether* | | *Unknown eluted with acetone* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Mixed cells* | *Plasma* | *Mixed cells* | *Plasma* | *Mixed cells* | *Plasma* | *Mixed cells* | *Plasma* | *Mixed cells* | *Plasma* |
| Acids | 594 | 1.9 | 3490 | 1970 | 109 | 520 | 1115 | 474 | 227 | 186 |
| Non-saponifiable substances | 427 | 3.8 | 186 | 29 | 146 | 210 | 268 | 58 | 16 | 12.6 |
| Percentage non-saponifiable substances in fraction | 22.6 | 80 | 15 | 6.8 | 96 | 98 | 49 | 30 | 25 | 25 |

## TABLE V

CELL POPULATION AND LIPID LABELLING

| *Fraction* | *Erythrocyte count* | *Leucocyte count* | *Fatty acid specific activity (counts/min/mg)* | *Fatty acid product of specific activity and weight in mg total activity (counts/min)* | *Unsaponifiable lipid specific activity (counts/min/mg)* | *Unsaponifiable lipid product of specific activity and weight in mg lipid total activity (counts/min)* |
|---|---|---|---|---|---|---|
| L. cells | $0.25 \cdot 10^6$ | $12.8 \cdot 10^3$ | 830 | 79,650 | 510 | 1221 |
| L. plasma | $0.25 \cdot 10^6$ | $12.8 \cdot 10^3$ | 729 | 56,880 | 351 | 596 |
| Y.E. cells | $2.28 \cdot 10^6$ | $0.9 \cdot 10^3$ | 632 | 41,020 | 502 | 1208 |
| Y.E. plasma | $2.28 \cdot 10^6$ | $0.9 \cdot 10^3$ | 370 | 44,400 | 300 | 300 |
| O.E. cells | $4.37 \cdot 10^6$ | $0.2 \cdot 10^3$ | 364 | 10,680 | 155 | 631 |
| O.E. plasma | $4.37 \cdot 10^6$ | $0.2 \cdot 10^3$ | 592 | 34,400 | 223 | 534 |

TABLE VI

RELATIVE SYNTHETIC ACTIVITY OF DIFFERENT CELL TYPES AFTER CORRECTION FOR MIXED CELL POPULATION

| *Cell type* | *Counts in fatty acid/min/cell* | *Relative synthetic activity for fatty acids* | *Counts in non-saponifiable materials/min/cell* | *Relative synthetic activity for non-saponifiable substances* |
|---|---|---|---|---|
| Leucocyte | 10.5 | 1000 | 0.14 | 560 |
| Young erythrocyte | $33.2 \cdot 10^{-3}$ | 3.4 | $0.5 \cdot 10^{-3}$ | 2.0 |
| Old erythrocyte | $9.8 \cdot 10^{-3}$ | 1.0 | $0.25 \cdot 10^{-3}$ | 1.0 |

TABLE VII

SPECIFIC ACTIVITIES OF FATTY ACIDS AND METHYL ESTERS ISOLATED FROM DIFFERENT CELL TYPES

Activity in counts/min/mg.

| *Acid* | *Leucocytes* | *Young erythrocytes* | *Old erythrocytes* |
|---|---|---|---|
| $nC_{16}$ | 1420 | 1000 | 81 |
| $nC_{18}$ | 900 | 926 | 159 |
| Oleic | 120 | 70 | 9.0 |
| Linoleic | 34 | 40 | 15.0 |
| Arachidonic | 54 | 50 | 162 |
| $C_{20}$ triene | 86 | 30 | N.D. |

unlike mammalian erythrocytes, there is evidence of the presence of a tricarboxylic acid cycle[6].

Because the cell population in whole blood is roughly 1 leucocyte to 300 young erythrocytes and 1000 mature erythrocytes, each cell type contributes approximately equally to the total lipid synthesis in whole blood.

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