significant because, in similar experiments with artificial ferrihaemoglobins, denatured protein was precipitated, making it impossible to judge whether a reaction had occurred.

Rossi-Fanelli & Antonini (1957) have shown that deuterohaemin can also combine with apomyoglobin to give an active oxymyoglobin, but the oxygen affinity was much higher, though the Bohr effect was similar to that of the native myoglobin. Clearly, as with haemoglobin, variation of the side chains influences the reactivity towards oxygen and it would appear that protohaem represents the most suitable prosthetic group for the physiological activity of haemoglobin and myoglobin.

SUMMARY

1. Artificial myoglobins have been prepared from aetiohaemin III, dimethylprotohaemin IX and dimethylmesohaemin IX.

2. The spectra for the ferric, ferro and carbon monoxide complexes of these myoglobins are given.

3. Fluoride complexes of ferridimethylmesomyoglobin can be formed at pH 6·2 and 8·0, providing further evidence that combination through the haematin iron had taken place.

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Fatty Acids of Human Blood

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Peters & Man (1943) studied the fatty acid composition of human blood. Brun (1939) and Kirk (1938) observed that esterified and free cholesterol in blood cell was different from that in plasma in healthy individuals. Studies by Chevallier, Manuel & Rouillard (1951) and Evans, Waldron, Oleksyshyn & Riemenschneider (1956) have indicated that the partition of the unsaturated fatty acids in blood cells was quite different from that in plasma. Alkali-isomerization spectrophotometric methods (Hammond & Lundberg, 1955; Luddy, Barford & Riemenschneider, 1958) have been employed to determine the polyunsaturated fatty acids of human blood plasma, the component lipids of human plasma and atheroma. Very few workers have, however, analysed the component fatty acids of human blood cells, plasma and whole blood. This paper describes an investigation into the component lipids and fatty acids of whole blood, plasma and blood cells of healthy men, alkali-isomerization spectrophotometric methods, iodine values of fatty acids and low-temperature crystallization techniques being utilized.

EXPERIMENTAL

From each of the normal five subjects in the post-absorptive state (8–10 hr.) about 250 ml. of blood was drawn via the antecubital vein into heparinized syringes. The blood was transferred to a sterile bottle to which had been added 50 ml. of a solution containing 3·0% of sodium citrate, 0·85% of sodium chloride and 2·0% of sodium sulphathiazole. Afterwards the whole solution was lyophilized and the bottle kept evacuated.

The ages of the above-mentioned five subjects were from 30 to 35 years. Their calorific and fat intakes per day per man were 2500–2640 and 55–65 g. respectively.

Another seven normal healthy men selected were from 33 to 40 years of age. Their calorific and fat intakes per day per man were 2268–2400 and 43–71 g. respectively. From each of the seven subjects in the post-absorptive state, about 40 ml. of blood was taken in a bulb containing sodium oxalate. Plasma and cells were separated as described by Evans *et al.* (1956).

Extraction of the lipid material. About 60 g. of lyophilized blood was treated with 600 ml. of ethanol at 60°. Ethanol was then distilled *in vacuo*. To the residue about 600 ml. of ethanol–ether (3:1) was added and warmed to 60°. It was kept overnight and then filtered. The treatment with ethanol–ether (3:1) was repeated until the lipids were completely extracted. To the whole ethanol–ether extract, 2 ml. of ethanolic 0·1% quinol was added and the mixture was evaporated on the water bath. Quinol was then washed out with hot water. The extract was treated with light petroleum (40–60°) and the lipids were recovered. The lipids from blood cells and plasma were also extracted in the same way as described above. The extracted lipid material was made to a volume of 100 ml. with light petroleum (40–60°) and portions were then taken for further analysis.

Analyses of lipids. Free and total cholesterol, total phospholipids and total fatty acids were estimated according to methods previously reported (Patil & Magar, 1959). The saturated fatty acid content was determined by oxidation of the fatty acids (Crombie, Comber & Boatman, 1955) in whole blood, and with blood cells and plasma it was calculated as the difference between the total fatty acids and the sum of oleic acid and polyunsaturated fatty acids. Wijs' iodine value was found by the semi-micro method of Sims & Stone (1956). Low-temperature crystallization of mixed fatty acids was carried out at −60° from acetone and at −25° from ether, according to the scheme of Gupta & Hilditch (1951). Polyunsaturated fatty acids were estimated by the alkali-isomerization spectrophotometric method of Herb & Riemenschneider (1953). It was assumed that the pentaenoic acids consisted of 50% of docosapentaenoic acid and 50% of eicosapentaenoic acid.

RESULTS AND DISCUSSION

Lipids and component fatty acids of plasma and blood cells. The levels of total lipids, total cholesterol and total fatty acids in blood cells were lower than those of plasma but the level of total phospholipids was high compared with that of plasma (Table 1). Ester cholesterol was much higher than free cholesterol in plasma. On the contrary, free cholesterol was higher than ester cholesterol in blood cells. This results in the level of free cholesterol in blood cells being higher than that of plasma, whereas the level of ester cholesterol was very low in blood cells as compared with that of plasma. This observation supports the findings of Erickson *et al.* (1937) and Brun (1939).

Iodine values of the fatty acids of plasma and blood cells varied from 102·3 to 106·2 and from 92 to 104·0 respectively. Saturated fatty acids comprised one-third of the total fatty acids in plasma and saturated fatty acids of blood cells constituted about half of the total fatty acids (Table 2). The unsaturated fatty acid fraction of plasma constituted 64–70% of the total fatty acids; oleic acid was the main component. In blood cells, the total unsaturated fatty acids contained about 50%, oleic acid being the major component. The level of oleic acid in blood cells was somewhat lower than that of plasma (Table 2). In the polyunsaturated fatty acids group, dienoic acid was the major component in plasma, whereas tetraenoic acid was the major component in blood cells. The level of dienoic acid in blood cells was quite low as compared with that of plasma (Table 2). The levels of pentaenoic acid and hexaenoic acid in blood cells and plasma were low, although there was more of both acids in the blood cells. Trienoic acid was present in small quantities in plasma, whereas it was absent from blood cells. Evans *et al.* (1956) were also unable to show the presence of trienoic acid in blood cells.

Low-temperature crystallization of fatty acids of lyophilized whole blood. Iodine values of whole-blood lipids varied from 73 to 75·9 and its mixed fatty acids from 102·0 to 112·0. By low-temperature crystallization at −60° and −25° in acetone and ether respectively, the mixed fatty acids were resolved into four fractions, differing in unsaturation (Table 3). The compositions of the fractions were studied by oxidation of the fatty acids, alkali-isomerization and determination of the iodine values. The fraction D contained predominantly higher polyunsaturated fatty acids. The fraction C, which contained saturated fatty acids, oleic acid and unsaturated fatty acids, was again recrystallized at −25° in ether. In the fraction B (soluble at −25°), the percentage of saturated fatty acids was decreased and the percentage of

Table 1. *Component lipids of plasma and blood cells of seven men*

Average values, ±S.D., are given.

Component lipid	Plasma (mg./100 ml. of plasma)	Blood cells (mg./100 ml. of packed cells)
Total lipid	485±11·6	397·7±12·6
Phospholipid	168±12·0	230·8±6·6
Free cholesterol	33±5·4	96·3±7·1
Ester cholesterol	127±9·4	21·3±3·0
Total cholesterol	160±7·7	117·6±6·6
Total fatty acids	343±7·4	238·7±8·0

Table 2. *Component fatty acids in plasma and blood cells of seven men*

Average values of percentage of total fatty acids, ±S.D., are given.

Component acids	Plasma	Blood cells
Saturated fatty acids	31·43±2·89	50·2 ±1·44
Oleic acid*	33·3 ±2·25	21·2 ±2·11
Dienoic acid	22·0 ±1·49	6·73±0·85
Trienoic acid	2·35±0·15	—
Tetraenoic acid	6·38±0·35	14·3 ±0·54
Pentaenoic acid	1·19±0·07	3·01±0·17
Hexaenoic acid	2·46±0·10	4·46±0·64

* Contained small amounts of palmitoleic acid.

Table 3. *Low-temperature crystallization of fatty acids of human whole blood*

A, Major group of saturated fatty acids; B, major group of monoethenoid fatty acids; C, saturated fatty acids, oleic acid and unsaturated fatty acid; D, major group of polyunsaturated fatty acids.

	Fractions	Weight (g.)	Percentage (w/w)	Iodine value
A	Insoluble at −25°	0·32±0·07	40·4±7·6	65·5±10·1
B	Soluble at −25°	0·22±0·06	29·7±8·7	119·5± 9·0
C	Insoluble at −60°	0·54±0·07	—	88·8± 5·5
D	Soluble at −60°	0·23±0·06	29·9±7·2	156·6±10·7

Table 4. *Component lipids in lyophilized human whole blood*

	Average ± S.D.
Blood lyophilized (g.)	60·7 ±2.2
Total lipids (g.)	1·29±0·06
Iodine value of lipids	74·7 ±1·2
Total fatty acids (g.)	0·77±0·03
Iodine value of fatty acids	109·0 ±2·3
	Concn. (g./100 g. of whole blood) ± S.D.
Free cholesterol	0·19±0·01
Ester cholesterol	0·67±0·01
Total cholesterol	0·85±0·01
Phospholipids	0·96±0·04

Table 5. *Component fatty acids of human whole blood*

Acids	Percentage of total fatty acids ± S.D.
Saturated	35·87±1·84
Oleic*	30·91±1·16
Dienoic	18·95±1·32
Trienoic	1·59±0·12
Tetraenoic	8·38±0·77
Pentaenoic	1·56±0·16
Hexaenoic	2·82±0·34

* Included small amounts of palmitoleic acid.

monoethenoid and other unsaturated fatty acids was increased. Fraction A (insoluble at −25°) consisted of large amounts of saturated fatty acids.

Lipids and component fatty acids of whole blood. Table 4 shows the range of variation in the percentage of cholesterol and phospholipids and total fatty acids in five samples of lyophilized whole blood. The cholesterol was chiefly in the ester form. The weight of phospholipids in whole blood was more than the total amount of free and ester cholesterol.

The percentage of oleic acid was highest in the unsaturated fatty acid group (30·9 %), although the oleic acid contained a little palmitoleic acid which was not separately determined (Table 5). The total amount of polyunsaturated fatty acids comprised about one-third of the total fatty acids. In the polyunsaturated fatty acids, the dienoic acid was predominant although tetraenoic acid was also present in high concentrations. Trienoic acid, pentaenoic acid and hexaenoic acid were present in only small quantities. The levels of dienoic acid, trienoic acid and oleic acid were slightly lower than those of the plasma, whereas the levels of tetraenoic acid, pentaenoic acid, hexaenoic acid and saturated fatty acids were higher than those of plasma.

SUMMARY

1. The composition of lipids from plasma, blood cells and whole blood has been studied.

2. Saturated fatty acids and tetraenoic acids of blood cells were higher than those of plasma. The levels of oleic acid and dienoic acid of plasma were high compared with those of blood cells.

3. Oleic acid and polyunsaturated fatty acids of whole blood comprised each about one-third part of total fatty acids. The dienoic acid was the highest in the polyunsaturated fatty acids group.

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