FATTY ACID COMPONENTS OF OVINE ERYTHROCYTE LIPIDS*

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As part of a study of comparative lipid metabolism, the distribution of fatty acids has been investigated in a number of mammalian tissues (Horgan and Masters 1963; Masters 1964*a*, 1964*b*, 1964*c*; Connellan and Masters 1965), a major aim of these studies being to facilitate correlation between lipid composition and function. In this context, it is widely recognized that membranes play a fundamental role in cellular metabolism, and that lipid is an essential component of these biomembranes (Stein and Danielli 1956). The study of structure–function relationships in this situation, however, has been hindered by the difficulty of isolating specific membranes without contamination by other lipids. The mature mammalian erythrocyte possesses advantageous characteristics for this type of investigation because of the lack of subcellular particles, and the resultant presence of only a single membrane type (Kögl *et al.* 1960). As an extension of previous investigations, then, the fatty acid composition of ovine erythrocytes has been determined.

Experimental Procedures

Blood was obtained by the venipuncture of Merino ewes and collected in tubes containing heparin as the anticoagulant. Cellular elements were separated from the plasma by centrifuging for 15 min at 1000 *g*, and then washed three times in aqueous 0·9% sodium chloride. After each centrifugation, the upper layer of cells (containing leucocytes and thrombocytes) was removed and discarded. The lipids were then extracted from the erythrocytes and the corresponding plasma specimens, fractionated on silicic acid columns, and the fatty acids determined by gas chromatography.

These techniques have been fully described previously (Horgan and Masters 1963).

Results

All results given represent the mean values of analyses from three separate Merino ewes.

The relative percentage composition of the main lipid fractions in the erythrocytes was: cholesterol esters 30·1%; triglyceride 3·9%; free fatty acids, mono-, and diglycerides 10·8%; and phospholipid 55·1%. In Table 1 the fatty acid composition of these lipid fractions is listed. The main features are that palmitic acid is present in greatest concentration in the cholesterol ester fraction, while stearic acid is highest in the phospholipid and triglyceride fractions. Oleic acid is always present in higher concentration than stearic acid, and is the major component of the phospholipid, triglyceride, and free fatty acid fractions. The phospholipid and free fatty acid fractions are the most highly unsaturated fractions in respect of the C_{18} acids.

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There are considerable quantities of the saturated C_{20}, C_{21}, and C_{22} acids present, particularly in the cholesterol ester fraction, but little unsaturation is evident at this chain length.

Analyses for the corresponding plasma specimens were in general agreement with previously published findings for this source (Horgan and Masters 1963).

TABLE 1

FATTY ACID COMPOSITION OF OVINE ERYTHROCYTE LIPIDS

Values for each component refer to the percentage by weight of the total fatty acid methyl esters in each fraction. "Trace" means that the amount of fatty acid present is less than 0·5%

Fatty Acid	Cholesterol Ester Fraction	Triglyceride Fraction	Free Fatty Acid Fraction	Phospholipid Fraction
12 : 0	0·7	0·5	Trace	Trace
14(br.)*	0·5	Trace	Trace	Trace
14 : 0	2·1	1·1	1·1	0·6
14 : 1	0·7	Trace	Trace	Trace
15(br.)*	Trace	Trace	Trace	Trace
15 : 0	1·2	0·8	0.6	Trace
15 : 1	Trace	Trace	Trace	Trace
16(br.)*	1·4	Trace	Trace	Trace
16 : 0	22·2	18·9	11·5	13·0
16 : 1	3·8	2·7	2·2	1·3
17(br.)*	Trace	Trace	Trace	Trace
17 : 0	1·1	1·1	0·5	0·7
16 : 2	0·2	Trace	0·5	Trace
18(br.)*	Trace	Trace	Trace	Trace
18 : 0	14·5	23·4	8·9	18·7
18 : 1	17·4	36·9	43·1	45·8
18 : 2	8·0	3·1	17·2	10·3
18 : 2?	Trace	0·6	Trace	Trace
18 : 3	2·1	0·6	2·7	1·0
20 : 0	4·4	1·3	1·2	1·7
20 : 1	Trace	Trace	1·0	0·9
20 : 2	0·6	Trace	Trace	Trace
21 : 0	7·7	5·9	5·3	3·1
20 : 4	2·7	Trace	1·8	1·3
22 : 0	8·1	1·7	0·8	Trace

* br. = singly methyl branched chain.

Discussion

The separation of erythrocyte lipids into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions has not been recorded in the literature, although a number of workers have studied the structure–function interrelationships of erythrocyte membranes by means of the identification of individual phospholipids (Dawson, Hemington, and Lindsay 1960; Hanahan, Watts, and Pappajohn 1960) and fatty acid analyses of the membrane lipid (James, Lovelock, and Webb 1959; Veerkamp, Mulder, and Van Deenen 1962). Results in this paper emphasize that

both phospholipids and cholesterol esters are major lipid fractions of the ovine erythrocyte.

The results of Kögl *et al.* (1960) on the major fatty acid components of the membrane lipid from a series of mammalian erythrocytes showed that the fatty acid composition varied between species in a manner which was parallel to the permeability properties of these membranes. Although they did not fractionate the erythrocyte lipid, an overall comparison of their results with the results presented in this paper shows that the analyses are in general agreement. The main disparity is that the values in this paper for stearic acid are somewhat higher, and the oleic acid percentages lower. These differences appear to improve the correlation with haemolysis time and permeability values.

The high oleic acid content of ovine erythrocytes (Table 1; Kögl *et al.* 1960) is unusual when the saturated character of most ruminant lipids are compared (Horgan and Masters 1963; Masters 1964*c*), and in view of the low oleic acid content in erythrocytes from monogastric animals (Veerkamp *et al.* 1962). Examination of the results shows that this acid is located predominantly in the phospholipid fraction (Table 1), which contains the highest level of this acid in all the major ovine tissues (Horgan and Masters 1963; Masters 1964*c*). Possibly this is a factor contributing to the peculiar properties of the ruminant erythrocyte membrane (Rapaport and Guest 1941; Dawson *et al.* 1960).

Further noteworthy features of ovine erythrocyte lipids are the comparatively high content of long-chain (C_{20}, C_{21}, C_{22}) saturated fatty acids, and the low values for polyunsaturated acids. Oleic acid concentration in ovine erythrocytes is far higher than in plasma phospholipid, while the C_{18} acids of the erythrocyte cholesterol ester fraction are considerably more saturated than those in the corresponding plasma fraction.

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