Plasma Albumin as an Acceptor of Free Fatty Acids

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The investigations of Dole (1956) and of Gordon & Cherkes (1956) revealed that free fatty acids are normally present in plasma, and are involved in the mobilization of fatty acids from fat depots to other tissues. The free fatty acids are found in association with the albumin of the plasma (Kendall, 1941; Cohn, Hughes & Weare, 1947; Saifer & Goldman, 1961) and, to a much less extent, with high-density lipoproteins (Lindgren & Nichols, 1960). There is evidence that plasma albumin influences lipid metabolism through its interaction with fatty acids, since Gordon & Cherkes (1958), White & Engel (1958*a*) and Reshef, Shafrir & Shapiro (1958) demonstrated that adipose tissue released free fatty acids when incubated in media containing this protein. In the absence of plasma albumin, however, it has been found that fatty acids are not released, but tend to accumulate in the tissue, particularly when hormones that stimulate lipolysis are added to the medium (White & Engel, 1958*a*, *b*; Lopez, White & Engel, 1959; Raben & Hollenberg, 1960; Freinkel, 1961; Rudman, Brown & Malkin, 1963). Hypoalbuminaemia in man was found by Bogdonoff, Linhart & Estes (1961) to be associated with a decrease in the mobilization of free fatty acids induced by noradrenaline.

In the present study various proteins have been compared with respect to their effects in solubilizing fatty acids in aqueous media, and in the release of free fatty acids by adipose tissue *in vitro*. The influence of plasma albumin on fatty acid metabolism in adipose tissue has also been investigated. The results extend the findings of other workers and emphasize the importance of albumin in these effects.

MATERIALS AND METHODS

Fraction V (albumin) of bovine plasma (Armour Pharmaceutical Co., Kankakee, Ill., U.S.A.), casein (Merck and Co., Rahway, N.J., U.S.A.), crystalline egg albumin (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.), soya-bean α-protein (Archer Daniels Midland Co., Cincinnati, Ohio, U.S.A.), and bovine fibrinogen (Pentex Inc., Kankakee, Ill., U.S.A.) were extracted with fat solvent to diminish their contents of residual fatty acids. The dry substances were stirred mechanically in 95% (v/v) ethanol–diethyl ether (3:1, v/v), 10 g./100 ml., at 0° for 15 min. The mixture was filtered on a Buchner funnel, and the cake was washed with anhydrous diethyl ether, then dried *in vacuo*. Extraction decreased the free fatty acids of the bovine plasma albumin to 0·0064 m-equiv./g., or 0·44 mole of fatty acid/mole of albumin (mol.wt. 69 000 according to Scatchard, Batchelder & Brown, 1946). The very low free-fatty acid content of the extracted casein was estimated to be about 0·0032 m-equiv./g. The protein fractions of human blood plasma were prepared according to the method of Cohn *et al.* (1946). Dextran was added to the incubation media as a 6% solution in 0·9% NaCl ('dextran, hydrolysed'; lot 90–1 from Connaught Medical Research Laboratories, Toronto, Canada). For use in testing its effect on the solubility of oleate, dextran was precipitated from this solution by the addition of ethanol, washed repeatedly with ethanol and diethyl ether, and dried *in vacuo*.

Solubilizing effects on fatty acid. To test the solubilizing effects of proteins, peptone (Bactopeptone; Difco Laboratories, Detroit, Mich., U.S.A.) and dextran on oleate, stock

Table 1. *Effects of high-molecular-weight compounds on solution of oleate*

The mixtures were prepared by adding to 2 ml. of a 5% (w/v) solution of the appropriate substance in Krebs–Henseleit medium 1 ml. of oleate (10 m-equiv./l. in 40% ethanol) and 2 ml. of Krebs–Henseleit medium. After 1 hr. at 37° $E_{640\ m\mu}$ was recorded.

Compound tested...	$E_{640\ m\mu}$						
	Bovine plasma albumin	Casein	Fibrinogen	Gelatin	Soya-bean α-protein	Peptone	Dextran
Concn. of oleic acid (m-equiv./l.)							
2	0·042	0·270	0·105	0·225	0·180	0·240	0·238
0	0·032	0·081	0·045	0·028	0·050	0·005	0·025
Difference	0·010	0·189	0·060	0·197	0·130	0·235	0·213

solutions (5%, w/v) of the substances in Krebs–Henseleit medium (Krebs & Henseleit, 1932) were diluted with this medium to the desired concentrations. The stock solution of oleate (10 m-equiv./l.), pH 7·9, was prepared by adding the molar equivalent of N-NaOH to a weighed amount of oleic acid (British Drug Houses Ltd., Poole, Dorset) and diluting with 40% (v/v) ethanol (Table 1) or water (Fig. 1). For tests in which the final concentrations of protein ranged from 0 to 3%, and of oleate ranged from 0 to 4 m-equiv./l. (Fig. 1), 3 ml. of the solution of protein at the desired concentration in Krebs–Henseleit medium was mixed with 2 ml. of the desired concentration of oleate in water. After flushing with oxygen + carbon dioxide (95:5) mixture, the 15 mm. × 150 mm. test tubes were screw-capped and shaken in a water bath at 37° for 1 hr. The solubilizing effect was estimated by recording $E_{640\,m\mu}$, by using a Coleman Junior spectrophotometer (Table 1), or $E_{420\,m\mu}$, by using a Klett photoelectric colorimeter (Fig. 1). The small extinction due to protein alone was subtracted.

Preparation and assay of anterior-pituitary fat-mobilizing extract. Minced bovine anterior pituitary glands were stirred with 4 vol. of water at pH 8 for 2 hr.: the procedures were carried out at 0–2° (Best & Campbell, 1936). The residue obtained on centrifuging was re-extracted with 1 vol. of water. The combined supernatants were adjusted to pH 5 with N-HCl and the precipitate was removed by centrifuging. To the supernatant, 2 vol. of acetone was added. The precipitate was triturated with acetone, the mixture was filtered on a Buchner funnel, and the cake was washed with diethyl ether and dried in a vacuum desiccator. The fat-mobilizing activity of the preparation (APP–60) was assayed by the method of Campbell (1938) in mice kept without food from the time of subcutaneous injection and killed after 7 hr. The mean total lipid of the liver (determined gravimetrically by the method of Best, Lucas, Patterson & Ridout, 1946) in the control group was 590 ± 45 mg./100 g. body wt., and in the groups injected with 1·25 mg. and 5·0 mg. of APP–60 per mouse the corresponding values were 1010 ± 41 and 1320 ± 103 mg./100 g. body wt. respectively.

Corticotrophin. Corticotrophin from pig pituitary glands (Nordic Biochemicals, Montreal, Canada; lot no. A–9501) was prepared by the oxycel procedure (Astwood, Raben, Payne & Grady, 1951). Its adrenocorticotrophic-hormone activity, assayed by the depletion of adrenal ascorbic acid in hypophysectomized rats injected subcutaneously, was 77·4 i.u./mg. (Rerup, 1957).

Incubation of tissues. Adult rats of the Wistar strain were killed by decapitation. Samples (about 150 mg.) were cut by scissors from the epididymal fat tissue and placed in 5 ml. of Krebs–Henseleit bicarbonate medium that had been equilibrated with an oxygen + carbon dioxide (95:5) mixture and contained the desired substances in solution. The Erlenmeyer flasks were flushed with gas, stoppered and incubated in a water bath (Dubnoff, 1948) at 37° for 3 hr. at 90–100 strokes/min. The time elapsed from killing the first animal to the end of placing the tissue samples in 50–60 flasks was about 20 min.

Determination of free fatty acids. The free fatty acids in the medium (duplicate 1 ml. samples) and in the tissue were determined by the method of Dole & Meinertz (1960). Free fatty acids were also determined by using various concentrations of sulphuric acid in the extraction mixture. Concentrations above that specified did not increase the amounts of free fatty acids recovered from media containing

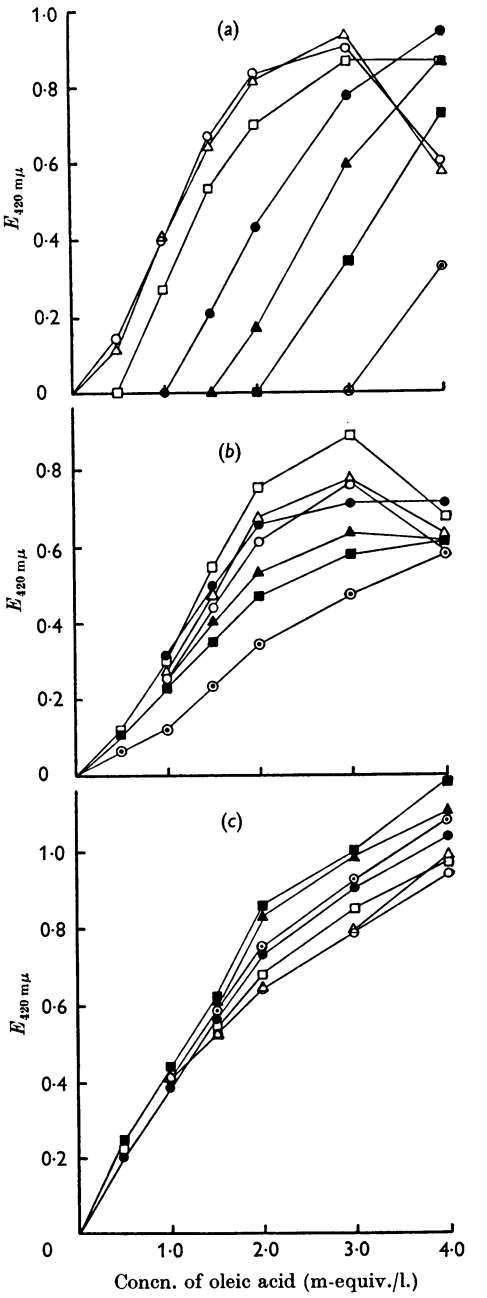

Fig. 1. $E_{420\,m\mu}$ values of mixtures of oleate with bovine plasma albumin (*a*), casein (*b*) or gelatin (*c*) in Krebs–Henseleit medium (diluted to 60%) and incubated at 37° for 1 hr. The final concentrations (w/v) of plasma albumin, casein and gelatin were: 0·1% (○), 0·2% (△), 0·5% (□), 1·0% (●), 1·5% (▲), 2·0% (■) and 3·0% (⊙). The oleate concentrations ranged from 0 to 4 m-equiv./l. Some of the points and intermediary lines have been omitted from the initial portions of (*b*) and (*c*) for ease of reproduction; the lines omitted lie within these shown.

oleic acid, and plasma albumin or casein, indicating that failure of the extraction procedure to break fatty acid–protein bonds was not a factor in the results obtained. The extraction mixture thereafter contained 40 parts of propan-2-ol, 10 parts of heptane and 1 part of 2 N-sulphuric acid.

For the estimation of tissue free fatty acids, the piece of tissue from an incubation flask was placed in 5 ml. of the Dole & Meinertz (1960) extraction mixture in a test tube and squeezed repeatedly with the flattened end of a stirring rod. The determination of free fatty acids in the fluid was then performed as usual.

Dry fat-free residue of adipose tissue. The dry fat-free residue of the adipose tissue was obtained by extracting the fresh tissue samples (about 150 mg. wet wt.), or the tissue remnant after extraction of the free fatty acids, once with acetone, thrice with boiling 95 % (v/v) ethanol and finally with diethyl ether in 13 mm. × 95 mm. flat-bottomed test tubes. During each of these extractions (5 min.) the tissue was squeezed with the flattened end of a stirring rod, and the extracts were discarded. The residues were dried at 110° for 1 hr. and weighed to the nearest 0·01 mg.

The values for the free fatty acids are given as m-equiv./g. of the dry fat-free residue of the adipose tissue. Samples of fresh tissue from fed rats contained about 2 % of dry fat-free residue. The mean total nitrogen of these dried and defatted samples was found by Kjeldahl digestion and distillation to be 14·5 ± 0·22 (range 13·9–15·6) g./100 g. of dry fat-free residue.

RESULTS

Solution of fatty acids in aqueous media. Of the seven substances tested, bovine plasma albumin (fraction V) was the most effective in solubilizing fatty acid in dilute Krebs–Henseleit medium, as indicated by the low extinction value (Table 1). When the amounts of both plasma albumin and oleate were altered, clear solutions were obtained within certain concentration limits (Fig. 1*a*). The point at which turbidity first appeared was specified by a fairly constant oleate:albumin molar ratio over a wide range of albumin concentration. In final concentrations of 5, 10, 15 and 20 g. of albumin/l., the highest values for the oleate:albumin molar ratio in the clear solutions were 6·9, 7·45, 7·45 and 7·42 respectively.

Turbidity occurred with all concentrations of oleate in the presence of casein or gelatin, and increased with each increment in oleate concentration (Figs. 1*b* and 1*c*). Since the initial portions of the curves relating extinction ($E_{420\,m\mu}$) to oleate concentration (m-equiv./l.) are approximately linear, the slopes were estimated. The values of the slopes for mixtures of oleate (0–2 m-equiv./l.) with casein or with gelatin (0·1–3 %, w/v) are of comparable magnitude (ranges 0·17–0·40 and 0·38–0·42 respectively); with high oleate:plasma albumin molar ratios, above 7·4, the slopes are also similar (range 0·33–0·47). Inflexions of the curves for 0·1 and 0·2 % of albumin, and for 0·1, 0·2, 0·5 and 1·5 % of casein, occurred at an oleate concentration of 3 m-equiv./l.

In similar tests with dextran–oleate mixtures, coarse granular precipitates occurred at all concentrations of dextran and oleate.

Acceptors of free fatty acids produced by adipose tissue. Effects of various substances (proteins, peptone and dextran) on adipose tissue *in vitro* are shown in Table 2. In the presence of plasma albumin, free fatty acids were released during incubation. In early work, the release was found to be higher with unextracted plasma albumin than with that extracted for 1 hr. at room temperature (Expt. 1 in Table 2). With plasma albumin extracted for a shorter period at 0°, as described in the Materials and Methods section, the difference became much less, and this method was used subsequently. The fat-mobilizing preparation and corticotrophin, at dosages that produced a maximal effect, and with plasma albumin in the medium, caused a two- to four-fold increase in release, and accumulation of free fatty acids in the tissue amounting to two- to three-fold the initial concentration.

After incubation of adipose tissue, no free fatty acids or negligible amounts were found in media containing fibrinogen, casein, gelatin (Expts. 1, 2 and 3 in Table 2), egg albumin, peptone or dextran (Expt. 2 in Table 2), or no added substance (Expt. 3 in Table 2). The concentrations of free fatty acids in the tissue were higher under these conditions than with plasma albumin in the medium; the increases ranged from 30 to 150 %. The fat-mobilizing preparation or corticotrophin added to the Krebs–Henseleit medium or to the media containing substances other than plasma albumin caused the greatest accumulation of free fatty acids in the tissue, with one exception, namely the incubation medium containing egg albumin. Despite the high content of free fatty acids in the tissue under these conditions no significant amounts of free fatty acids were found in the media.

Effect of the addition of glucose to the medium. When adipose tissue from fed rats was incubated in media containing glucose (2 mg./ml.) the output of free fatty acids was less than under the previous conditions (starved rats, no glucose). Corticotrophin increased the free fatty acids in the medium almost eightfold, and raised the concentration in the tissue slightly. Incubation with corticotrophin, but without plasma albumin, in the medium again resulted in high accumulation of free fatty acids in adipose tissue, but no release (Table 3).

Effects of various concentrations of plasma albumin. As the concentration of plasma albumin in the medium was raised from 0 to 5 % (w/v), the amounts of free fatty acids released by adipose tissue *in vitro* increased, whereas the amounts in

Table 2. *Effect of acceptors on the release of free fatty acids from adipose tissue* in vitro

Epididymal adipose tissue samples (about 150 mg.) from rats of 250–290 g. body wt., kept without food for 18–24 hr., were incubated for 3 hr. at 37° in 5 ml. of Krebs–Henseleit medium containing the appropriate substance (20 mg./ml.) tested as an acceptor of fatty acids, and the anterior pituitary preparation (APP–60) or corticotrophin A–9501 (CT) as shown. Bovine plasma albumin not extracted previously is indicated (U). Initial concentrations of free fatty acids in adipose tissue are given in parentheses. The results in Expts. 1, 2 and 3 are the means ± S.E.M. from three, three and six incubations respectively. —, Not determined.

	Medium				
		Pituitary preparation		Concn. of free fatty acids (m-equiv./g. of dry fat-free residue)	
Expt. no.	Substance tested	Type	Concn. (μg./ml.)	Tissue	Medium
1	Plasma albumin (U)	APP–60	0	—	0·48 ± 0·03
			20	—	0·95 ± 0·27
	Plasma albumin	APP–60	0	—	0·20 ± 0·02
			20	—	0·46 ± 0·11
	Fibrinogen	APP–60	0	—	0·09 ± 0·08
			20	—	0·03 ± 0·02
	Casein	APP–60	0	—	0·00
			20	—	0·02 ± 0·02
	Gelatin	APP–60	0	—	0·05 ± 0·02
			20	—	0·05 ± 0·02
2				(0·57 ± 0·06)	
	Plasma albumin	CT	0	0·24 ± 0·07	0·41 ± 0·04
			2	1·43 ± 0·08	1·60 ± 0·11
	Casein	CT	0	0·61 ± 0·36	0·00
			2	2·44 ± 0·16	0·00
	Gelatin	CT	0	0·33 ± 0·10	0·00
			2	2·82 ± 0·21	0·00
	Egg albumin	CT	0	0·31 ± 0·11	0·00
			2	0·44 ± 0·09	0·00
	Dextran	CT	0	0·33 ± 0·05	0·00
			2	1·71 ± 0·22	0·00
	Peptone	CT	0	0·33 ± 0·05	0·00
			2	2·47 ± 0·69	0·00
3				(0·73 ± 0·07)	
	None	CT	0	1·06 ± 0·17	0·00
			2	2·64 ± 0·14	0·00
	Plasma albumin	CT	0	0·41 ± 0·03	0·59 ± 0·12
			2	1·16 ± 0·08	1·38 ± 0·16
	Casein	CT	0	0·63 ± 0·09	0·00
			2	1·92 ± 0·14	0·00

Table 3. *Effects of plasma albumin on adipose tissue* in vitro

Epididymal adipose tissue samples (about 150 mg.) from adult fed rats were incubated for 3 hr. at 37° in 5 ml. of Krebs–Henseleit medium containing 2 mg. of glucose/ml. Bovine plasma albumin and corticotrophin were added as indicated. The results are given as the means ± S.E.M. from four incubations. The initial free fatty acid content of unincubated tissue was 0·29 ± 0·04 m-equiv./g. of dry fat-free residue.

Concn. of plasma albumin (mg./ml.)	Concn. of corticotrophin (μg./ml.)	Concn. of free fatty acids (m-equiv./g. of dry fat-free residue)	
		Tissue	Medium
0	0	0·53 ± 0·04	0·00
0	2	1·42 ± 0·29	0·00
20	0	0·21 ± 0·01	0·11 ± 0·01
20	2	0·32 ± 0·06	0·85 ± 0·15

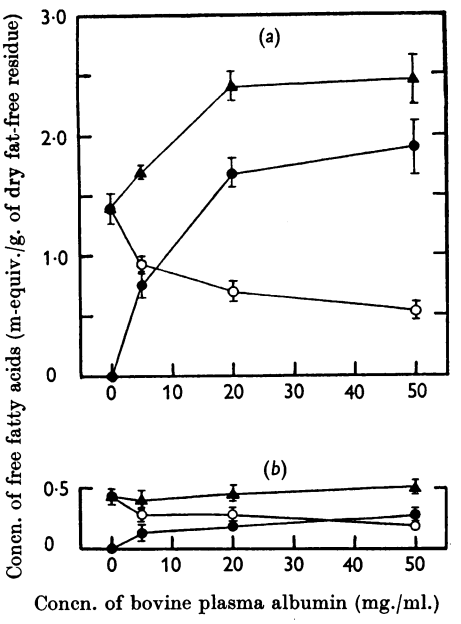

Fig. 2. Effects of plasma albumin concentration on the free fatty acids of the tissue (○), the medium (●), and these combined (▲), after incubation of epididymal adipose tissue from fed rats for 3 hr. at 37° in Krebs–Henseleit medium (*a*) containing 2 μg. of corticotrophin/ml., or (*b*) without corticotrophin. Before incubation the concentration of free fatty acids in the tissue was 0·22 m-equiv./g. of dry fat-free residue, and in the medium was below the limit of detection. The vertical lines indicate S.E.M.

the tissue decreased (Fig. 2*b*). In corresponding systems stimulated by the addition of corticotrophin, the output of free fatty acids increased greatly as the concentration of albumin was raised (Fig. 2*a*). This rise was most marked with up to 2 % albumin, and in 5 % albumin was eightfold the control unstimulated concentration. The greatest accumulation of free fatty acids in the corticotrophin-stimulated tissue occurred in the absence of albumin, however, and diminished progressively as the concentration of albumin was raised. After incubation with corticotrophin, the relationship of the logarithm of the free fatty acid concentration in the tissue to the albumin concentration in the medium was approximately linear. The total free fatty acids (medium plus tissue) in the system stimulated by corticotrophin increased with increasing concentration of albumin in the medium.

Effect of plasma protein fractions from human blood. Fractions II, IV_1, IV_4 and V of human blood plasma contained, respectively, 0·1, 1·5, 0·8 and 1·8 m-equiv. of free fatty acids/100 g. of protein. Incubation of 2 % (w/v) solutions of these fractions in Krebs–Henseleit buffer for 3 hr. at 37° with and without corticotrophin (2 μg./ml.) did not alter these values appreciably.

Fatty acids were released from adipose tissue that was incubated with fraction IV_1, IV_4 or V in solution, and corticotrophin increased the amount released under these conditions (Fig. 3). The output was highest in the systems containing fraction V (albumin) of human or bovine origin, the former being the more effective. When albumin and corticotrophin were present in the medium, the total concentration of free fatty acids (tissue plus medium) rose to the highest levels. Fraction II did not permit transfer of free fatty acids from the tissue incubated with or without corticotrophin (Fig. 3).

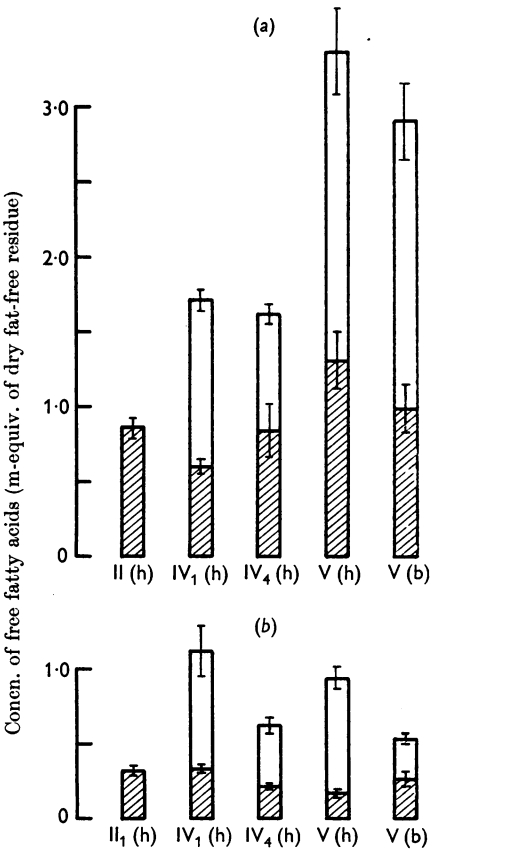

Fig. 3. Effects of protein fractions II, IV_1, IV_4 and V from human (h) or bovine (b) plasma on the free fatty acids of epididymal adipose tissue (▨), and of the media (□), after incubation for 3 hr. at 37° with (*a*) 2 μg. of corticotrophin/ml. of medium, or (*b*) without corticotrophin. The protein concentration was 2 % (w/v) in Krebs–Henseleit medium. The initial content of free fatty acids in the tissue was 0·21 m-equiv./g. of dry fat-free residue. The vertical lines indicate S.E.M.

DISCUSSION

Following the investigations of Luck and his colleagues (Ballou, Boyer & Luck, 1945; Boyer, Ballou & Luck, 1947; Duggan & Luck, 1948) on the interaction of short-chain fatty acids with proteins, Cogin (1951) observed that the electrophoretic mobility of plasma albumin was increased by the addition of elaidic acid until there were 8 moles of fatty acid/mole of albumin, at which point turbidity appeared. Robinson & French (1953) found that albumin was effective in the chylomicron 'clearing reaction' in plasma, up to an excess of 5·7–6·2 moles of fatty acid/mole of albumin. Plasma albumin was also found, by Gordon, Boyle, Brown, Cherkes & Anfinsen (1953), to be necessary for the clearing of a vegetable-oil emulsion; added fatty acids produced complete inhibition of clearing at the estimated value of 7–8 moles of fatty acid/mole of protein. In human serum albumin, Goodman (1958) found 7 binding sites of high affinity (2 of these of very high affinity) for long-chain fatty acids. In the present study, plasma albumin was found to be uniquely effective, in comparison with the other substances studied, in solubilizing a long-chain fatty acid in an aqueous medium. Since the solubilizing action was observed up to the limiting value of 7·8 moles of fatty acid/mole of albumin, which is comparable with the values cited above, the effect appears to be dependent on binding.

The total amount of free fatty acids obtained during incubation of adipose tissue depends on the net difference between generation of fatty acids by lipolysis and esterification (Engel & White, 1960; Bally, Cahill, LeBoeuf & Renold, 1960; Raben & Hollenberg, 1960; Vaughan, 1961). The relation between rates of release and of uptake of free fatty acids by the tissue, affecting distribution between medium and tissue, may influence these reactions. Despite the complex nature of the situation, the simplest explanation of the correlation between albumin concentration and the total amount of free fatty acids in the incubation system is that the albumin aided transfer to the medium, thereby decreasing free fatty acid concentrations in the tissue and permitting lipolysis to proceed further.

The findings of Engel & White (1960), Raben & Hollenberg (1960), Freinkel (1961) and Rudman *et al.* (1963) that, in the absence of serum albumin, free fatty acids accumulate in adipose tissue but are not transferred to the medium are confirmed. A specific role of plasma or serum albumin in permitting the release of free fatty acids from adipose tissue is indicated, since it was the most effective of the plasma protein fractions tested, and since fibrinogen, fraction II and proteins not derived from plasma were ineffective. The activities of plasma fractions IV_1 and IV_4 in the release of free fatty acids from adipose tissue may be partly due to contamination by albumin.

Output of free fatty acids from adipose tissue *in vitro* involves at least two processes: generation of free fatty acids, chiefly by lipolysis, and transfer across the cell membrane, which requires a special acceptor in the exterior fluid. The results suggest that in the body plasma albumin is a necessary factor in the transfer process, in addition to acting as a carrier of free fatty acids.

SUMMARY

1. Plasma albumin was more effective in solubilizing oleate in an aqueous medium than any of five other proteins or dextran. The solubilizing effect occurred up to an oleate:plasma albumin molar ratio of 7·4, or a total free fatty acids:plasma albumin molar ratio of 7·8.

2. Fraction V (albumin), and to a smaller extent fractions IV_1 and IV_4 of human plasma, permitted the transfer of free fatty acids from adipose tissue *in vitro* to the medium, but casein, fibrinogen, fraction II of plasma, egg albumin, gelatin, peptone and dextran did not.

3. Corticotrophin increased the concentration of free fatty acids in the incubated tissue, but their release was dependent on the presence of plasma fractions V, IV_1 or IV_4.

4. Increasing concentrations of plasma albumin in the medium increased the output of free fatty acids from adipose tissue *in vitro*, decreased the accumulation of free fatty acids in the tissue and increased the total amount of free fatty acids in tissue plus medium.

5. It is concluded that for the release of free fatty acids from adipose tissue a substance with special properties as an acceptor of fatty acids is an absolute requirement, and that plasma albumin performs this function most effectively.

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Chemical Studies on Haemoglobins A_1 and A_0

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The heterogeneity of adult human haemoglobin is well established on the basis of alkali denaturation studies (Brinkman & Jonxis, 1935), starch-block electrophoresis (Kunkel & Wallenius, 1955), and solubility techniques with variable solvents (Roche, Derrien & Roques, 1952) and variable solute tests (Allison & Tombs, 1957). Chromatography on ion exchangers (Morrison & Cook, 1955; Huisman & Prins, 1955) confirmed these observations. Schneck & Schroeder (1961) were the first to investigate the minor components with great care and to work out a correlation between the fractions isolated by chromatography and by starch-block electrophoresis. The haemoglobin of a normal adult individual was separated into four major fractions (Huisman & Meyering, 1960) by chromatography on CM-cellulose. These fractions were termed, in the order of their appearance from the column, HbA_1, HbA_0, HbF and HbA_2. In addition, small amounts of methaemoglobin reductase and unidentified proteins were present. A_1 is the most rapidly moving electrophoretic component and can be resolved on CM-cellulose into three components, namely A_1^A, A_1^B and A_1^C. This component was termed A_3 by Schneck & Schroeder (1961). A_0 is the major component (about 80 % of total), which was termed A_1 by Schneck & Schroeder (1961). On electrophoresis, it moves at a position intermediate between the most-slowly-moving component, A_2 (by both systems of nomenclature), and the most-rapidly-moving component. Foetal haemoglobin F can be resolved into two components, F_1 and F_0 (Huisman & Meyering, 1960). F_1 contains an acetyl group at the *N*-terminal end of one of its polypeptide chains (Schroeder, Cua & Fenninger, 1962). No differences in oxygen affinities were observed with HbA_1,

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