DFP, eserine and Nu 1250 that were, respectively, 200, 200 and 20 times less than those concentrations just capable of causing a potassium loss.

3. It seems that the permeability of the nerve cell to potassium is dependent on the energy-producing reactions of the cell rather than on the presence of an active acetylcholine–cholinesterase system.

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The Fractionation of Mammalian Serum Proteins with Ether

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A technique for the separation of the protein components of human citrated plasma using aqueous systems containing ether has been described by Kekwick & Mackay (1954). When blood protein fractions from animals have been required the method devised for human plasma was used for the fractionation of their serum proteins. Because of the diversity of the electrophoretic pattern of animals' sera, not only from species to species, but between individuals of the same species, the consistency of the composition of the fractions separated was a little surprising, and as the results to be described show, the method may have a wider application than was at first envisaged.

MATERIALS

Serum. The serum of guinea pigs, rats and rabbits was prepared from the pooled blood of groups of 10–250 animals. These animals were bled by cutting the throat of the animal after it had been stunned. Ox, sheep and monkey blood also represented a pool from several animals, while that of cat, dog and horse was taken from a single animal by cannula from a vein, when necessary under an anaesthetic. The blood was collected in dry bottles, allowed to clot at room temperature, and stored overnight at 4°. Serum was collected after the blood had been centrifuged at 1500 rev./min. for 30 min. at 0°.

Diethyl ether. This was of anaesthetic grade, free from antiperoxidants and was stored in the dark at 4°.

Ethanol. Rectified spirit was diluted with water to 50% (v/v) alcohol.

Acetate–phosphate buffers: Na_2HPO_4, $2H_2O$, 0·5M = 89·04 g./l.; acetic acid, 0·5M. 2·0M acetic acid is diluted 1 vol. to 4 and is standardized by titration. 2·0M acetic acid (114·5 ml. glacial acetic acid/l.) was standardized by diluting to approximately 0·2M and titrating against standard 0·2M-NaOH. (*a*) pH 4·0: 0·5M-HAc: 86 ml./l. = 0·034M; 0·5M-Na_2HPO_4, $2H_2O$: 14 ml./l. = 0·007M; I = 0·05. (*b*) pH 6·0: 0·5M-HAc: 46 ml./l. = 0·023M; 0·5M-Na_2HPO_4, $2H_2O$: 54 ml./l. = 0·027M; I = 0·05. (*c*) pH 7·0: Na_2HPO_4,

$2H_2O$: 46·26 g./l.; NaH_2PO_4: 29·33 g./l.; $I = 1·0$; diluted to the required ionic strength. (*d*) pH 8·0: Na_2HPO_4, $2H_2O$: 53·054 g./l.; KH_2PO_4: 5·962 g./l.; $I = 1·0$; diluted to the required ionic strength.

0·5 M *Sodium bicarbonate*: 42·00 g./l.

0·2 M *Zinc acetate*: $(CH_3COO)_2Zn$, $2H_2O$: 43·9 g./l.

METHODS

The determination of pH. The values of pH were determined with a MacInnes type glass electrode, calibrated against a solution containing 0·09 M-KCl + 0·01 M-HCl which was assumed to have a pH of 2·10. Measurements were made at room temperature with a valve potentiometer, the accuracy being 0·01 pH unit.

Nitrogen determinations. The protein nitrogen was determined by the micro-Kjeldahl technique. The values given are the mean of duplicate determinations and are expressed as mg. N/ml.

Cholesterol determinations. These were made by Martin's (personal communication) modification of the method of Brand & Sperry (1943).

Calculations of ionic strength. These were made as described by Kekwick & Mackay (1954). Volumes of distilled water to be added to adjust the solutions to the required ionic strength were calculated from the known salt concentrations, the ionic strength effects of the proteins being disregarded.

Electrophoresis measurements. Samples were dialysed to equilibrium at 2° against phosphate buffer pH 8·0, $I = 0·2$. The measurements were made at a protein concentration of 1·5–2·0 g./100 ml. in the Tiselius apparatus (1937) at 0°, and a potential gradient of 6 v/cm. Optical observations by the diagonal Schlieren method (Philpot, 1938) were photographically recorded with a light of 546 mμ. wavelength.

The recorded photographs were projected on to mm. graph paper with an enlargement of 8 diameters, and tracings were made. The curves were analysed by reflexion across a median line and the areas were measured to determine the quantities of the components present. The results are expressed as the percentage of the total protein in a mixture attributable to any single component. The analytical values quoted are the mean of exposures obtained simultaneously from the ascending and descending limbs.

Paper electrophoresis. Paper electrophoresis was carried out in a Durrum (1950) type apparatus using Whatman no. 1 filter paper. The paper strip 4 × 18 in. was allowed to take up buffer for 3 hr. before the protein was placed on it. Phosphate buffer (pH 8·0, $I = 0·1$) was used in the apparatus, and the separation was made with a current of 3·0 mA for 16 hr. The proteins were subsequently stained with Azochloramine B (Pluckthorn & Gotting, 1951).

Agglutinin assay. Agglutinins were assayed by the coarse titration method described by Kekwick & Mackay (1954).

The removal of zinc. Zinc was removed by the passage of the protein solution through a column of ion-exchange resin, Zeo-Karb 225 in the H phase.

The removal of solvents. Solvents were removed by dialysis at 0°, or by drying from the frozen state.

Precipitation techniques. The quantities of serum used in the individual experiments varied between 30 and 2000 ml. Precipitations were carried out in glass vessels similar to those described by Kekwick & Mackay (1954). The temperature was controlled by immersing the vessel in a tank containing refrigerated aqueous ethylene glycol (25 %, v/v) at a temperature of $-3·5 \pm 0·5°$. Solutions necessary to adjust the pH or ionic strength and solvents were added through a jet, while the contents of the vessel were mechanically stirred to prevent high local concentrations, especially of solvents, around the jet.

The precipitating systems were allowed to equilibrate in the bath at $-3·5°$, or in an ice-water bath at 0°, depending on the ether concentration. Experience showed that even when the volumes involved were small, the mixtures should be allowed to equilibrate for at least 16 hr. if the results were to be reproducible.

When pH alterations were made samples of 2–5 ml. of the bulk solution were titrated electrometrically to determine the amount of buffer, acid or alkali required, and the calculated quantity added to the bulk solution.

The ionic strength was adjusted by dilution with distilled water. When ether was added no allowance was made for volume changes during solution, and the amount of ether added is the stated percentage of the initial volume of the solution at 2°.

Precipitates were removed by centrifuging at the temperature of the precipitation in screw-capped bottles in a bucket centrifuge, the head of which was refrigerated, and the temperature controlled to $\pm 0·5°$. The precipitates were dissolved in phosphate buffer, pH 8·0, in phosphate buffer, pH 7·0, or in the case of albumin, in distilled water. The total nitrogen was estimated, and from this the percentage of the total serum nitrogen precipitated at each stage. In some of the experiments cholesterol determinations, and an assay of a *Salmonella* agglutinin were made on the fractions in order to find the possible location of lipid and antibody globulins respectively.

In the course of the experiments a great number of fractions were prepared, and paper electrophoresis was used in the preliminary survey to choose those fractions to be analysed accurately by the Tiselius method. Where insufficient material was available for a run in the Tiselius apparatus, the purity of the fraction was judged by paper electrophoresis alone.

EXPERIMENTAL

Fractions were separated from serum and from each other in the four stages outlined below. The tables will show that although the separation of the serum components could generally be relied on to follow this scheme, there was some species variation.

(1) *The separation of serum into globulins and albumin.* The sera used in the experiments had the protein concentrations and electrophoretic composition shown in Table 1. It was assumed that they were isotonic with 0·15 M-NaCl for the purpose of calculating the ionic strength of the mixtures. The pH was adjusted to $5·50 \pm 0·02$ with 0·5 M acetic acid, and the serum diluted to 4 times the original volume with distilled water ($I = 0·037$). The protein solution was then slowly cooled to $-3·5°$, and 18·5 vol. % ether added. The centrifuged precipitate (G 1) was suspended in a volume of distilled water equal to 40 % of the original serum volume and dissolved by the addition of pH 4·0 buffer. The precipitate should dissolve completely between pH 4·50 and 4·60. The volume of buffer required is shown in Table 2. These values, and similar values given in other tables, are the

mean of a number of titrations, and should be used only as an indication of the quantities required. Unless sufficient pH 4·0 buffer is added to dissolve the G 1 completely, the separation of γ-globulin from α- and β-globulins is impaired. Table 2 also shows the percentage of the total protein precipitated under the conditions described above, and the electrophoretic composition of the fraction from different animals.

(2) *The precipitation of α- and β-globulins from dissolved G 1.* α- and β-globulins (G 2) were precipitated from dissolved G 1 under the following conditions: pH 5·0±0·01, $I=0{\cdot}010$, ether 10 vol. %, $T=0°$. Samples of the G 1 solution were titrated with pH 6·0 buffer and the volume required to bring the pH of the bulk solution to pH 5·0±0·01 was calculated (see Table 3). To adjust the buffer protein mixture to an ionic strength $I=0{\cdot}01$ at pH 5·0 the total volume of the

Table 1. *The protein nitrogen and electrophoretic analyses of mammalian sera*

Animal	No. of batches	Protein N (mg./ml.)	Electrophoretic analysis (% total protein)			
				Globulins		
			Albumin	α	β	γ
Guinea pig	6	8·3	65	12	12	11
Rat	4	11·3	63	13	18	6
Rabbit	2	11·1	68	6	13	13
Dog	2	8·7	60	11	19	10
Cat	2	9·0	38	21	11	29
Sheep	4	10·8	53	18	8	21
Ox	1	11·4	49	16	8	27
Horse	1	11·1	36	20	10	34
Monkey	1	11·7	58	5	17	20

Table 2. *The precipitation of globulins (G 1) from serum*

Ether 18·5 vol. %; $I=0{\cdot}037$; $T=-3{\cdot}5°$; pH = 5·50±0·02.

Animal	Acetic acid 0·5 M (ml./10 ml. serum)	G 1 nitrogen (% initial N)	pH 4·0 buffer for solution (% serum volume)	Electrophoretic analysis (% total protein)			
					Globulins		
				Albumin	α	β	γ
Guinea pig	0·97	29	30	6	17	29	48
					69		
Rat	1·00	40	55	0			33
Rabbit	0·90	26	35	1	20	47	32
Dog	0·70	25	30	14	42	19	24
Cat	1·10	47	50	17	6	25	51
Sheep	0·88	34	50	13	23	12	52
						45	
Ox	1·10	41	40	6	39		
Horse	1·00	42	55	0	18	32	50
Monkey	0·85	36	85	2	34	22	42

Table 3. *The precipitation of α- and β-globulins (G 2) from G 1*

Ether 10 vol. %; $I=0{\cdot}010$; $T=0°$; pH 5·0±0·02.

Animal	pH 6·0 buffer (ml./10 ml. G 1)	G 2 nitrogen (% initial N)	Electrophoretic analysis (% total protein)			
				Globulins		
			Albumin	α	β	γ
Guinea pig	4·0	16	0·6	49·4	50	0
Rat	4·5	22	0	63	37	0
Rabbit	3·6	10	0	55	45	0
Dog	5·0	14	10	45	44	0
Cat	4·0	19	11	47	14	28
Sheep	4·3	15	2·0	34	64	0
Ox	5·0	9·0	0·6	66	33·4	0
Horse	5·0	10	0	36	41	23
Monkey	5·0	18	4	63	33	0

G 2 system is the volume of pH 4·0 buffer × 2·5 + the volume of pH 6·0 buffer × 4·82 (Kekwick & Mackay, 1954). The quantity of distilled water to be added is a volume equal to the total volume of the G 2 system minus the sum of the G 1 volume, the pH 6·0 buffer to be added and the ether to be added. Ether is 10 % of the calculated total G 2 volume. The precipitation is carried out by adding the pH 6·0 buffer to bring the pH to 5·00 ± 0·01, then the distilled water to dilute to an ionic strength of 0·01, cooling the system to 0° and then adding ether. The mixture was allowed to stand overnight at 0°, the precipitate removed by centrifuging at 0° and dissolved in pH 8·0 buffer. The G 2 fractions gave densely turbid solutions which could be cleared for electrophoresis by extraction with ether at −25° (McFarlane, 1942).

Table 3 shows the amounts of protein precipitated at this stage, and the electrophoretic composition of the fraction.

(3) *The precipitation of γ-globulins from the G 2 supernatant.* The pH of the G2 supernatant was adjusted to 6·70 ± 0·05 by the addition of 0·5 M-$NaHCO_3$ (see Table 4), and while the temperature of the solution was lowered to −3·5°, the ether concentration was raised to 18·5 vol. %. The precipitate (G 3) was allowed to settle, and was then removed by centrifuging at −3·5°. Electrophoretic analyses of the fraction are shown in Table 4.

dissolved in 0·15 M-NaCl or in phosphate buffer. Table 5 shows the degree of purification and the amount of protein recovered from the initial G 3 solution.

(4) *The precipitation of albumin from the G 1 supernatant.* The supernatant of the precipitated G 1, containing the bulk of the albumin, was held at −3·5° to prevent the ether coming out of solution. Acetic acid was added to bring the pH to 4·90 ± 0·10 when, in most animals, a precipitate formed which was removed by centrifuging. This precipitate contained a variable amount of the residual globulins and their removal assisted in the production of a pure albumin. To the supernatant was added half its volume of 50 % (v/v) ethanol, which brought about the precipitation of protein as shown in Table 6.

Alternatively, albumin could be precipitated from the globulin supernatant by the addition of a solution of 0·2 M zinc acetate at pH 4·9 ± 0·10. Depending on the animal, the final concentration of zinc acetate required was 0·02–0·04 M. The albumin precipitate was dissolved in distilled water, or a suitable buffer and the zinc removed by passing the solution through an ion-exchange resin. The electrophoretic analysis of the albumin prepared by these means is shown in Table 7.

The purification of albumin. It may be seen from Tables 6 and 7 that there is considerable variation in the purity of the albumin solutions. In some cases albumin could be purified by reprecipitation from aqueous solution with ether. The albumin solutions were diluted to a protein concentration of approximately 2 g./100 ml. with distilled water, and were titrated with 0·5 M-$NaHCO_3$ or with 0·5 M acetic acid to a pH of 4·90 ± 0·10. The ether concentration was brought to 13 vol. % at a temperature of −3·5°, and a precipitate AP 1 was collected after the system had been allowed to equilibrate at this temperature. The precipitate, in addition to the globulin contaminants associated with the crude albumin, contained also a variable amount of albumin. The ether concentration of the supernatant was then raised to 18·5 vol. % and a second precipitate (AP 2) was obtained. If to the supernatant of this precipitate zinc acetate to a final concentration of 0·04 M, or an equal volume of 50 vol. % ethanol was added a third precipitate was generally obtained. If the two previous precipitations had removed all the globulin from solution, e.g. as with guinea pig serum, this precipitate (AP 3) was pure albumin. The amounts of the initial nitrogen recovered at each stage, and the amount of albumin in the different precipitates is shown in Table 8.

Table 4. *The precipitation of γ-globulin (G 3) from G 2 supernatant*

Ether 18·5 vol. %; $T = -3·5°$; pH 6·70 ± 0·05.

Animal	0·5 M-$NaHCO_3$ (ml./10 ml. G 2 S)	G 3 nitrogen (% initial N)	Electrophoretic analysis (% total protein)			
				Globulins		
			Albumin	α	β	γ
Guinea pig	0·14	7·0	9	8	17	66
Rat	0·14	5·4	6	0	0	94
Rabbit	0·15	7·0	4	0	0	96
Dog	0·15	5·0	4	0	8	88
Cat	0·14	13·0	10	0	6	84
Sheep	0·16	9·0	3	0	11	86
Ox	0·14	8·0	0	9	0	93
Horse	0·15	10	0	0	20	80
Monkey	0·15	10	0	0	7	93

Table 5. *The purification of γ-globulin*

Ether 18·5 vol. %; $I = 0·05$; $T = -3·5°$; pH 7·0.

Animal	γ-Globulin in G 3 (% total protein)	γ-Globulin in G 4 (% total protein)	G 4 nitrogen (% G 3 nitrogen)
Guinea pig	66	86	63
Rat	94	100	55
Rabbit	96	100	26
Cat	84	100	65
Dog	88	100	40
Sheep	86	94	42
Ox	93	100	82
Horse	80	100	—

From this fraction in some animals, it was possible to prepare electrophoretically pure γ-globulin. The precipitated G 3 was dissolved in pH 7·0 buffer, $I = 0·05$, and diluted with this buffer to a protein concentration between 0·5 and 0·7 g./100 ml. Ether was added to a concentration of 18·5 vol. % at a temperature of −3·5°. The precipitate was

Table 6. *The precipitation of protein from G1S by ethanol*

Ether 12 vol. %; ethanol 17 vol. %; $T = -5°$; pH 4·8–5·0.

Animal	Acetic acid 0·5M (ml./10 ml. G1S)	AP nitrogen (% G1S nitrogen)	Electrophoretic analysis (% total protein)			
			Albumin	Globulins		
				α	β	γ
Guinea pig	0·20	55	91	9	0	0
Rabbit	0·18	78	90	10		0
Rat	0·16	29	92	5	3	0
Dog	0·20	—	89	0	11	0
Cat	0·17	63	76	10		14
Sheep	0·27	56	82	9	0	9
Ox	0·24	61	75	1	14	10
Monkey	0·20	84	75	1	13	11

Table 7. *The precipitation of protein from G1S by addition of zinc acetate*

Ether 18·5 vol. %; $T = -3·5°$; pH 4·8–5·0.

Animal	Acetic acid 0·5M (ml./10 ml. G1S)	Zinc acetate concn. (M)	AP nitrogen (% G1S N)	Electrophoretic analysis (% total protein)			
				Albumin	Globulins		
					α	β	γ
Guinea pig	0·20	0·02	74	92	8	0	0
Rabbit	0·18	0·02	83	89	0	11	0
Rat	0·14	0·04	58	91	9	0	0
Dog	0·20	0·02	62	86	7	7	0
Cat	0·14	0·02	70	79	10		11
Sheep	0·27	0·02	92	89	11		0
Ox	0·24	0·04	71	82	2	7	9
Horse	0·24	0·02	—	71	29		0
Monkey	0·20	0·02	87	89	1	10	0

Table 8. *The purification of albumin from AP*

Protein concentration of AP = 2 g./100 ml. pH 4·90 ± 0·10.

Fraction	AP1		AP2		AP3	
Ether (vol. %) ...	13		18·5		18·5	
ZnAc (M)	—		—		0·04	
Animal	N % AP	Alb %	N % AP	Alb %	N % AP	Alb %
Guinea pig	9·3	44	44	97	22	100
Rabbit	2·4	42	43	98	16	100
Dog	20	82	35	88	21	98
Cat	25	—	54	86	21	98
Sheep	30	85	38	100	—	—
Ox	10	36	73	86	13	86
Rat	66	90	12	94	12	70

N % AP = % total AP nitrogen recovered.
Alb % = albumin, % total protein.

DISCUSSION

Analysis of the fractions obtained in these experiments, by essentially the method developed for the separation of human plasma proteins, has shown that it is possible to isolate from a variety of mammalian sera, fractions each having a preponderance of a defined electrophoretic component. From some of these fractions further precipitation on the lines already described (Kekwick & Mackay, 1954) gave an electrophoretically pure material. Other fractions are still being investigated with a view to preparing pure α- and β-globulins.

The electrophoretic analyses shown in Table 1 are in broad agreement with other workers (Moore, 1945; Svenssen, 1946; Kekwick & Record, 1941; Bradish, Henderson & Brooksby, 1954). These figures are given not as a study of the electrophoretic composition of allegedly normal sera, but as an example of the type of starting material which must inevitably be used unless the animals have been especially selected.

Tables 2, 6 and 7 show that the bulk of the globulins are precipitated in the fraction G1 and this allows a separation of components having certain well-defined properties, for example the separation of some proteolytic enzymes from their inhibitors (Wilhelm, Miles & Mackay, 1955).

The globulins may then be separated from one another by the precipitation of α- and β-globulins in G2. Except in cat and horse, the fraction G2 was apparently free of γ-globulins. γ-Globulins could then be precipitated from the G2 supernatant in the fraction G3, from which further precipitation provided electrophoretically pure γ-globulin, except in guinea pig and sheep sera.

Table 9. *Cholesterol contents of sera and fractions*

Cholesterol = mg./g. N.

Animal	Serum	Fraction G1	G2	G3	AP
Guinea pig	56	156	206	0	>5
Rat	90	247	216	26	>5
Rabbit	42	141	237	73	>5
Ox	122	—	163	34	—
Sheep	51	108	184	55	4·6

A series of cholesterol estimations indicated that the lipoproteins were precipitated in the fraction G2 (Table 9). This fraction is the subject of work reported elsewhere (Wilhelm *et al.* 1955).

In human serum the *Salmonella* H agglutinin is found to be associated with the γ-globulins, defined as the slowest moving component in the electrophoretic field (Enders, 1944) and has been used as a means of detecting γ-globulins in mixtures (Kekwick & Mackay, 1954). The technique was therefore applied to animal serum fractions. It was found that, although by using a range of *Salmonella*, an agglutinin to one or more could be detected in most of the sera, the agglutinin was not associated exclusively with the fraction G3 as had been the case in human plasma. In horse, guinea pig and rabbit serum agglutinin was found in the fractions G2 and G3 (see also Kekwick & Record, 1941), but in rat, ox, sheep and dog serum the agglutinin was associated with the fraction G2 (Table 10). No agglutinin was found in the cat or monkey sera, but only one pool of each was assayed. The tests carried out were not exhaustive since a study of natural agglutinins was not the primary object of the work. The results show, however, that the concentration of γ-globulins, as defined above, will not necessarily simultaneously concentrate antibody from animals which have acquired a natural immunity. These antibody globulins may be precipitated in the complex fraction G2 which also contains α- and β-globulins. The situation may be altered in animals which have been experimentally immunized. This is an important factor in deciding which fraction to isolate in order to concentrate antibody, and may provide a means of separating natural from immune antibody (cf. Bradish *et al.* 1954).

Table 10. Salmonella *agglutinin titre of sera and fractions*

Animal	*Salmonella*	Agglutinin titre (reciprocal of dilution) Serum	G2	G3
Guinea pig	SL23 (*enteritidis*)	32	128	64
Rat		32	128	0
Rabbit		8	16	4
Ox		8	32	0
Dog	SL664	4	16	0
Horse	5727 *Ab. bov.*	16	16	128
Sheep	SL902	16	320	0

From the proteins remaining after the removal of the globulins, albumin may be precipitated, and the crude albumin from most animals is capable of some degree of purification. Albumins from different animal species showed some differences in solubility in aqueous media in the presence of solvents and metal ions. This is shown in the amounts of albumin precipitated both from the globulin supernatant, and in the amounts recovered at each stage of the purification. Their greatest use is probably as a vehicle for radioactive isotopes, and for this purpose the crude albumin may be as satisfactory, and more economical, than the pure material required for immunological studies.

SUMMARY

1. The method of Kekwick & Mackay (1954) for the fractionation of human plasma proteins was used successfully for the separation of the serum proteins of guinea pig, rat, rabbit, cat, dog, monkey, ox, horse and sheep.

2. By variations in pH, ionic strength, temperature and solvent concentration, three fractions consisting essentially of a mixture of α- and β-globulins (G 2); γ-globulin (G 3) and albumins (AP) were isolated and concentrated. From rat, rabbit, cat, dog, ox and horse serum electrophoretically pure γ-globulin could be prepared from the fraction G 3. From the fraction AP electrophoretically pure albumin was prepared from guinea pig, rabbit and sheep; and 98 % pure albumin from dog and cat.

3. Tests on the fractions showed that cholesterol was largely concentrated in the fraction G 2. The natural antibodies (*Salmonella* agglutinin) were concentrated in G 2 in rat, ox, sheep and dog serum; and in G 2 and G 3 in horse, guinea pig and rabbit serum. It is suggested that the crude albumin might find a use as a vehicle for radioactive isotopes.

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Studies on Ali-esterases and Other Lipid-hydrolysing Enzymes

2. THE EFFECT OF HEPARIN INJECTION ON ALIMENTARY LIPAEMIA AND ON THE PLASMA ESTERASES*

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Although heparin does not affect the turbidity of lipaemic plasma when added *in vitro*, Hahn (1943) and Weld (1944) showed that a visible alimentary lipaemia in dogs disappears within a few minutes after the intravenous injection of small amounts of heparin. The post-heparin plasma contains a factor which is capable of clearing lipaemic plasma *in vitro* (Anderson & Fawcett, 1950); this factor has the properties of a protein and could therefore be an enzyme (Anfinsen, Boyle & Brown, 1952; Spitzer, 1952; Levy & Swank, 1954).

The clearing factor causes a decrease in the size of the large lipoprotein globules, apparently by hydrolysing the triglycerides to produce free fatty acids and glycerol (Graham *et al.* 1951; Swank & Wilmot, 1951; Anfinsen *et al.* 1952; Shore, Nichols & Freeman, 1953; Korn, 1954). It appears to be inhibited by small amounts of protamine (Brown, 1952; Spitzer, 1953) and by large amounts of sodium chloride (Brown, Boyle & Anfinsen, 1953), to be activated by heparin and to act specifically on lipoprotein (Korn, 1954). Pancreatic lipase also exhibits clearing activity *in vitro* and *in vivo* (Spitzer, 1952, 1953), but the clearing factor can be found in the post-heparin plasma of pancreatectomized dogs (Fasoli, Glassman, Magid & Foa, 1954).

It is reported that the clearing factor can be separated from plasma 'esterase' by fractional precipitation with ethanol (Brown *et al.* 1953) and that it does not hydrolyse β-naphthyl laurate (Brown, Baker & Kauffman, 1954). However, Levy & Swank (1954) found that the rate of hydrolysis of tributyrin by dog plasma increased 50–100 % within a few minutes after intravenous injection of heparin, while the esterase activity of normal dog plasma was not affected by incubation with heparin *in vitro*. On injecting small amounts of protamine after injecting the heparin, the activity

* Part 1: Myers & Mendel (1953).