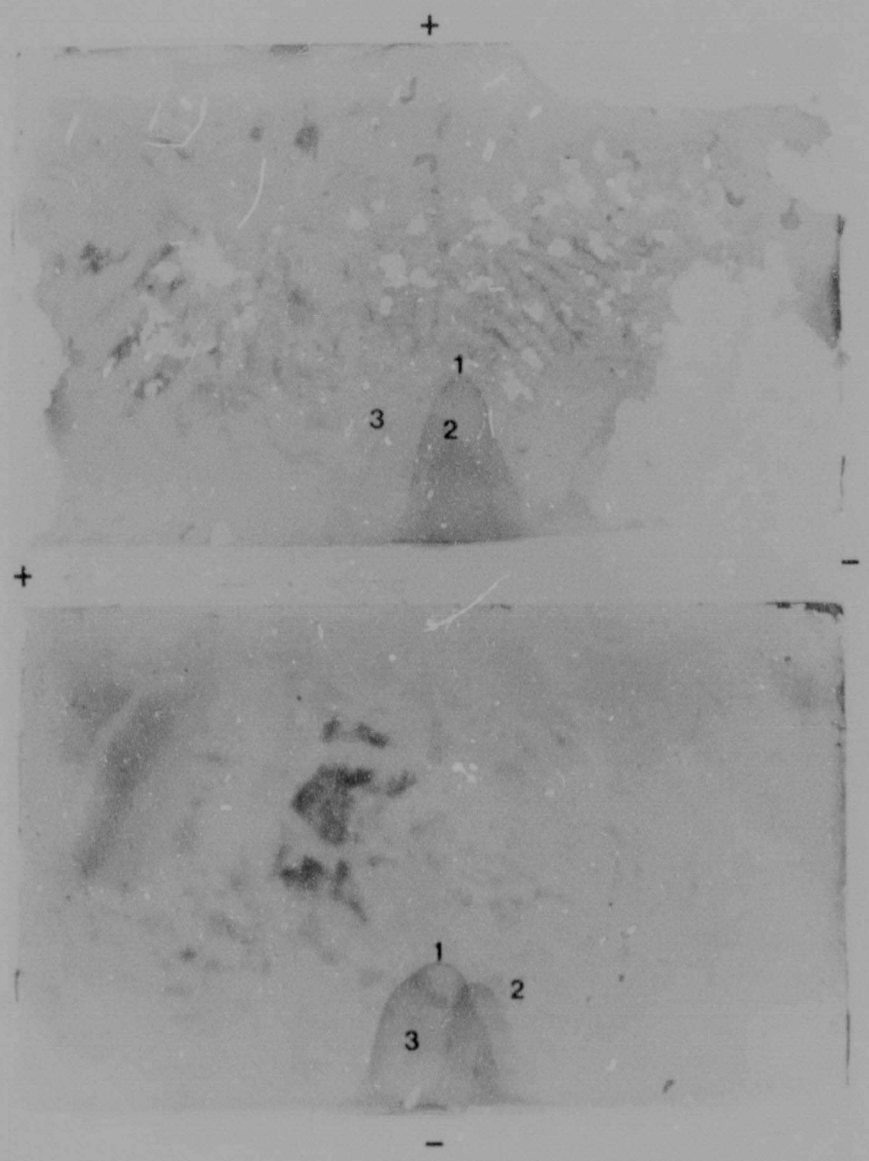

Figure 8

Crossed immunoelectrophoresis using monospecific antibodies to transferrin, haemopexin and α_2-macroglobulin before (top) and after (bottom) labelling transferrin with radioactive iron.

(1) transferrin
(2) α_2-macroglobulin
(3) haemopexin

3.2 Application of Sephadex-acrylamide Gels and comparison with Polyacrylamide Gels

The Sephadex-Acrylamide Gel Electrophoretic method was applied to various body fluids such as plasma, interstitial fluid, lymph, aqueous humor and CSF of various animals and

compared with polyacrylamide gel electrophoresis of the same. At the same time a routine study was undertaken on the plasma of these animals to determine the protein c. centrations (prot. conc.), haematocrits (Hct), albumin-globulin ratios (A/G ratios), etc. and to compare them to known values. Sephadex-acrylamide gel electrophoresis was also applied to study the effects of physical exercise, heat stress and fever conditions on body fluid proteins.

3.2.1 Comparative studies on the body fluids of animals

Statistical analyses of data was done by either Student's t-test or the Paired Student's t-test. The latter was applied in sections 3.2.2.1.1, 3.2.2.1.2 and 3.2.2.2. With the Paired Student's t-test the significance of the mean difference in blood parameters ($\Delta \bar{x}$) was compared to 0. In all the results a two tailed significance test was applied and significance was only accepted at $P < 0,0^{5}$.

3.2.1.1 Human

During PAGE as well as SAGE 2,5 μl of plasma or serum were applied per gel. During CIE 0,4 ml of anti-whole serum per agarose plate (8,5ml agarose buffer) were used. The electrophoretic separation time of the plasma proteins on the acrylamide gel was 20 min at 90 volts and 25 min at 160 volts. For the sephadex-acrylamide gel this was 15 min at 90 volts and 20 min at 160 volts. Haematocrit and plasma protein concentrations were also determined and the results are shown in Table 3.

Table 3

Plasma protein concentrations and haematocrits of human subjects at rest.

	Prot. conc. (g/l)		Hct (%)	
	♀	♂	♀	♂
$\bar{x}$	81,80	75,10	47,6	43,5
SD	0,22	0,79	3,1	4,0
n	19	14	19	14
P	< 0,001		< 0,005	

For comparative purposes the body fluid parameters determined in this study are presented together with comparable values for the same animal species available from the literature in the appropriate sections.

Table 4

Haematocrit and plasma protein concentration of the human.

Protein concentration (g/l)	Hct %	Reference
	44,7	Astrand & Saltin (1964)
70,0±5,0	49,0±2,0	Poortmans (1971)
70,0		Lewis (1974)
72,2±1,4	44,3±1,5	Tibes et al (1974)
73,0	46,8	Noakes & Carter (1976)
	42,2±0,6	Davis et al (1976)
70,4±6,0		Reichel et al (1976)
72,0±9,8		Chalmers et al (1977)
81,8±0,2	47,6±3,1	Results - male
75,1±0,8	43,5±4,0	Results - female

As was shown previously, SAGE of plasma proteins yielded results differing in some respects from those obtained by PAGE. The relative mobilities, and thus band position on the gel, for several of the plasma proteins separated by

PAGE and SAGE, were established by the use of monospecific antibodies during crossed immunoelectrophoresis. Several plasma proteins were identified in this way, as illustrated in Fig. 9.

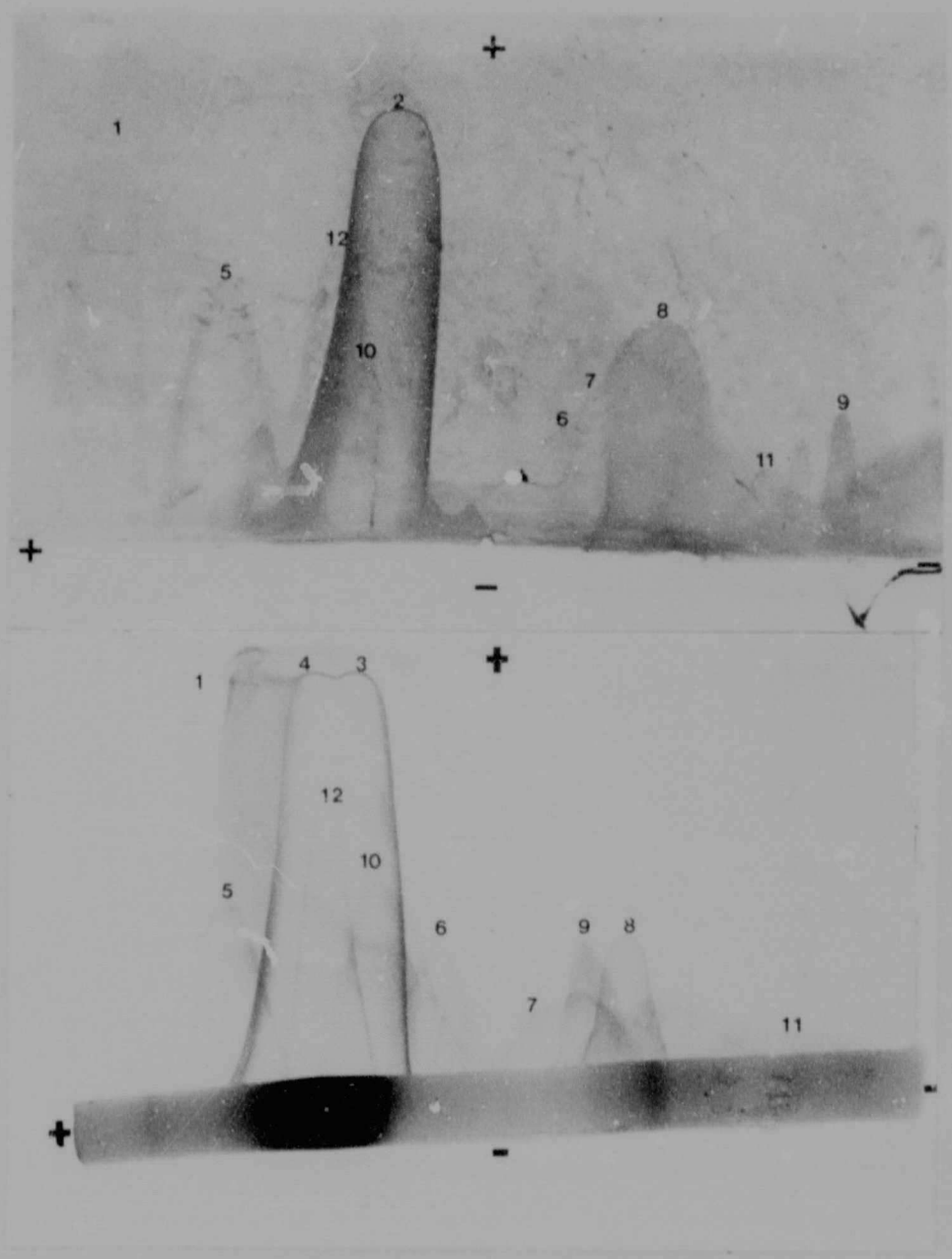

Figure 9

Crossed immunoelectrophoresis of human plasma proteins separated on polyacrylamide gel (top) and sephadex-acrylamide gel (bottom) against anti-whole serum. Individual proteins identified by CIE against the respective monospecific antibodies.

(1) prealbumin
(2) albumin
(3) upper albumin
(4) lower albumin
(5) orosomucoid
(6) ceruloplasmin
(7) haemopexin
(8) transferrin
(9) α_2-macroglobulin
(10) α_1-antitrypsin
(11) immunoglobulins
(12) α_1-lipoprotein

The continuous nature of the double albumin rocket peak, indicating antigenic relationship between the two albumin bands separated on sephadex-acrylamide, was confirmed by crossed immunoelectrophoresis of plasma against monospecific anti-albumin (Fig. 10).

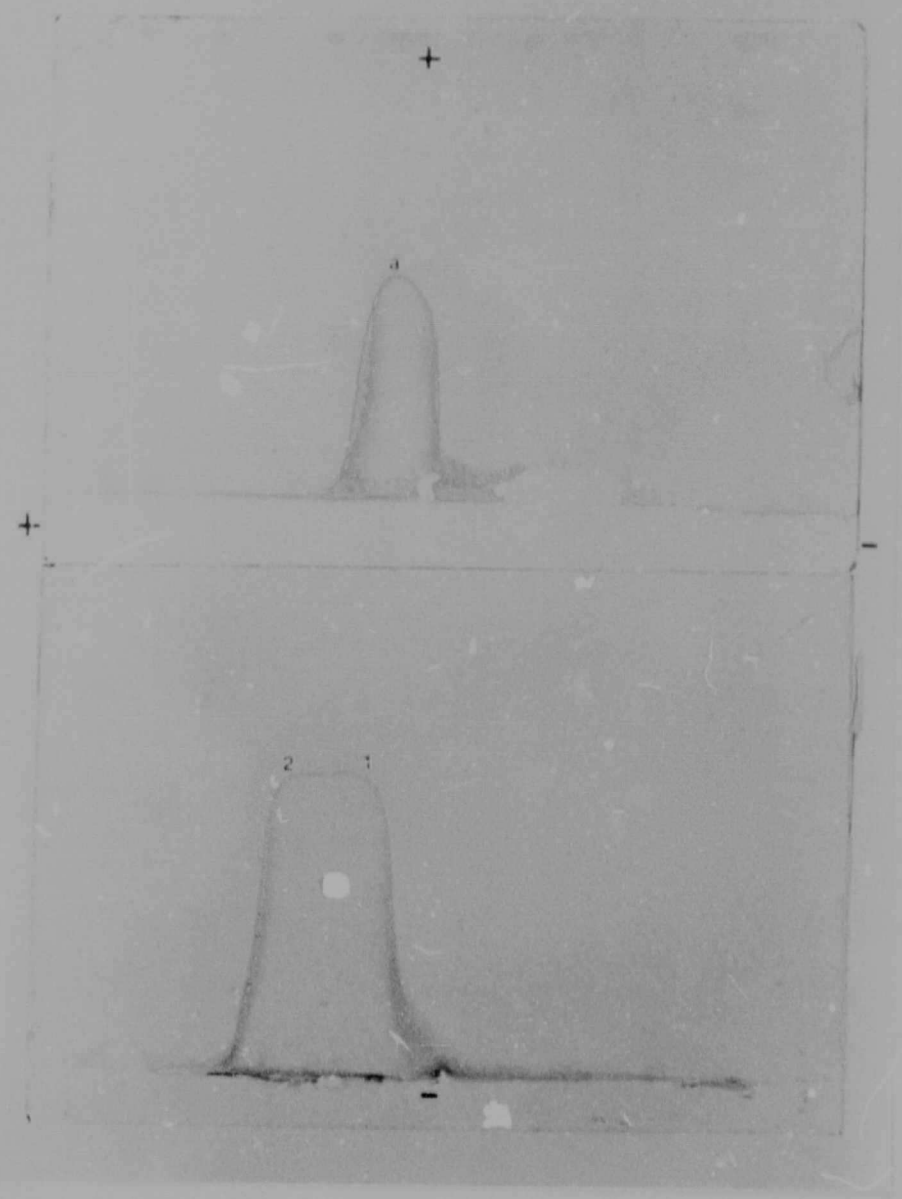

Figure 10

Crossed immunoelectrophoresis against monospecific anti-albumin of human plasma proteins separated on polyacrylamide (top) and sephadex-acrylamide (bottom). (a) albumin (1) upper albumin (2) lower albumin

3.2.1.2 Baboon

For both PAGE and SAGE, 25 µl plasma per gel were applied. Separation times were 23 min at 90 volts and 20 min at 160 volts for the polyacrylamide gels, and 14 min at 90 volts and 20 min at 160 volts for the sephadex-acrylamide gels. In addition to plasma, studies were also conducted on cerebrospinal fluid, aqueous humor and pericardial fluid. Haematocrit and protein concentrations of the body fluids are reflected in Table 5. Due to practical difficulties, such as volume, the same body fluids could not be studied in all the animals concerned. Collection of some body fluids (e.g. lymph) from cattle and sheep, the blood of which were collected from animals slaughtered at a local abbatoir, was also impossible due to prevailing conditions and regulations.

Table 5

Haematocrit and protein concentrations of baboon body fluids.

	Hct %	Prot. conc. (g/l) Plasma	CSF	Aq.h.	Pericard.
$\bar{x}$	44,0	71,60	0,93	2,32	11,50
SD	2,9	10,40	0,85	1,22	
n	7	7	5	5	

CSF - cerebrospinal fluid
Aqh - aqueous humor
Pericard. - pericardial fluid

Separation of the baboon body fluids on polyacrylamide gel (Fig. 11) and subsequent scanning of the gels were carried out in order to determine A/G ratios of the different body fluids. The results are presented in Table 6.

Table 6

Baboon body fluid A/G ratios as determined by PAGE.

	Plasma	pericard.	Aq.h.
$\bar{x}$	1,78	1,96	1,03
SD	0,76	0,04	0,47
n	15	3	3

Similar results to those obtained from human plasma, were obtained by electrophoresis of baboon plasma on polyacrylamide and sephadex-acrylamide respectively (Fig. 12). Differences in electrophoretic banding pattern and the double albumin band on the sephadex-acrylamide gel can clearly be seen.

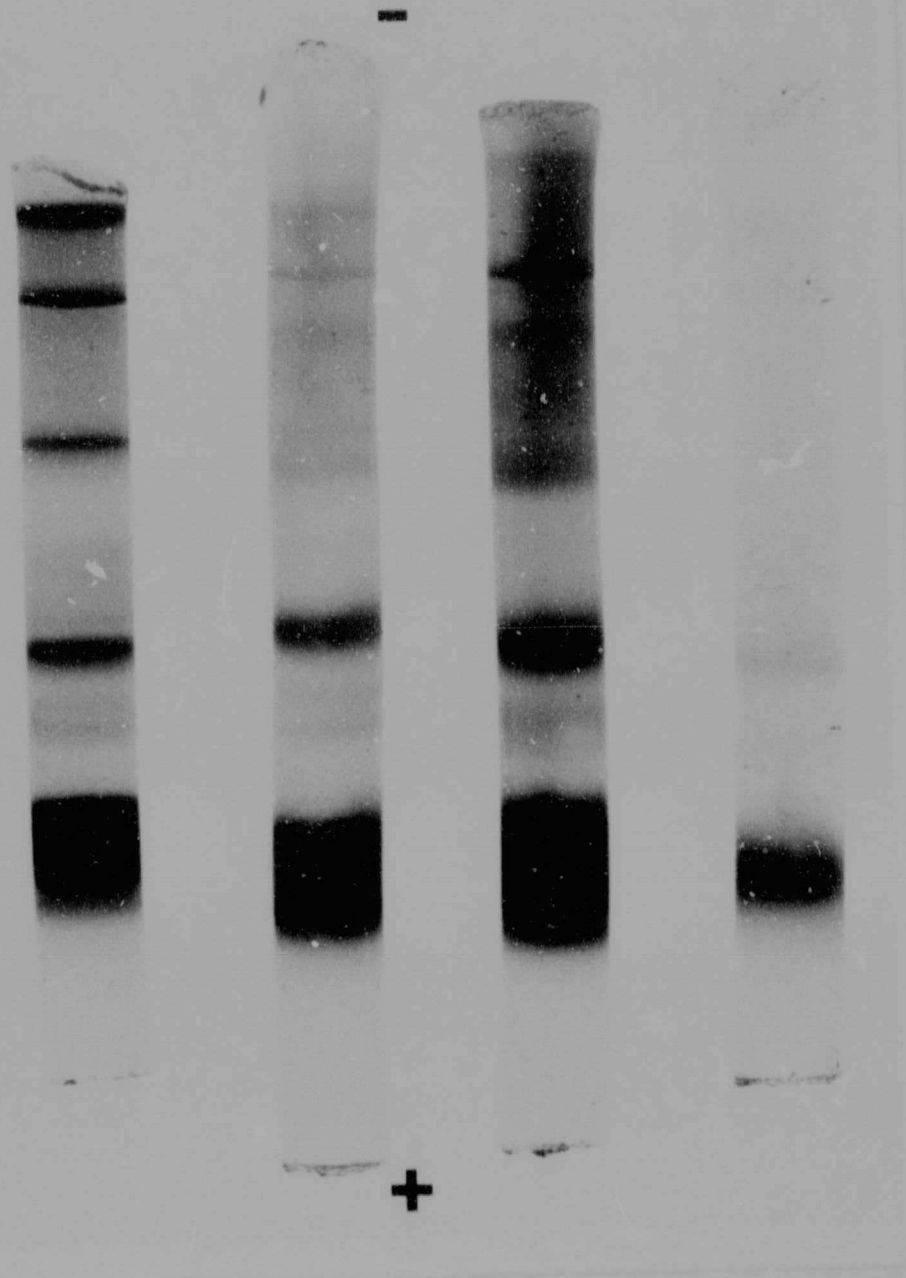

Figure 11

Polyacrylamide gel electrophoresis of baboon plasma, pericardial fluid, aqueous humor and cerebrospinal fluid (L → R).

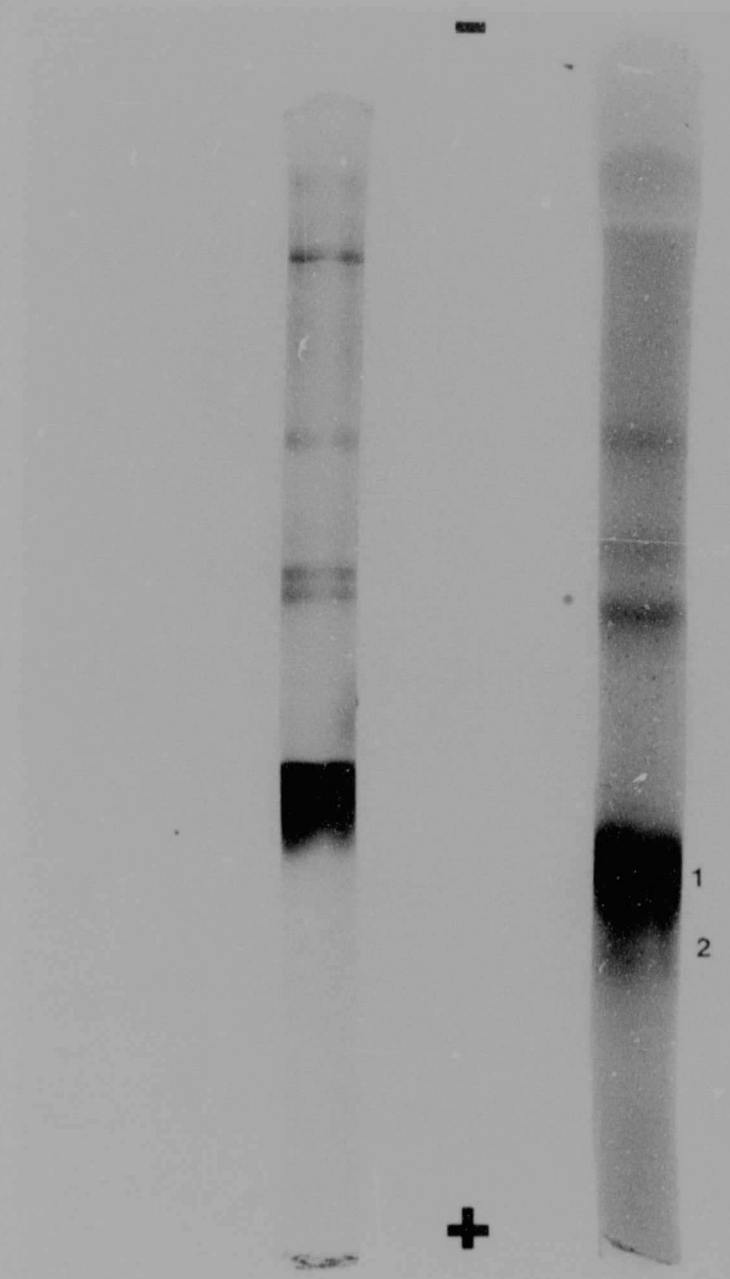

Figure 12

Comparative PAGE (left) and SAGE (right) of baboon plasma proteins. (1) upper albumin (2) lower albumin.

The presence of two albumins as reflected by a double albumin rocket peak, as well as differences in electrophoretic banding of other proteins, depending on the electrophoretic medium used, could also be demonstrated during CIE (Figs. 13 and 14). For this purpose, antibodies against baboon plasma were raised in rabbits. During CIE 0,45 ml of the antibody

preparation were incorporated in each agarose plate (8,5 ml agarose).

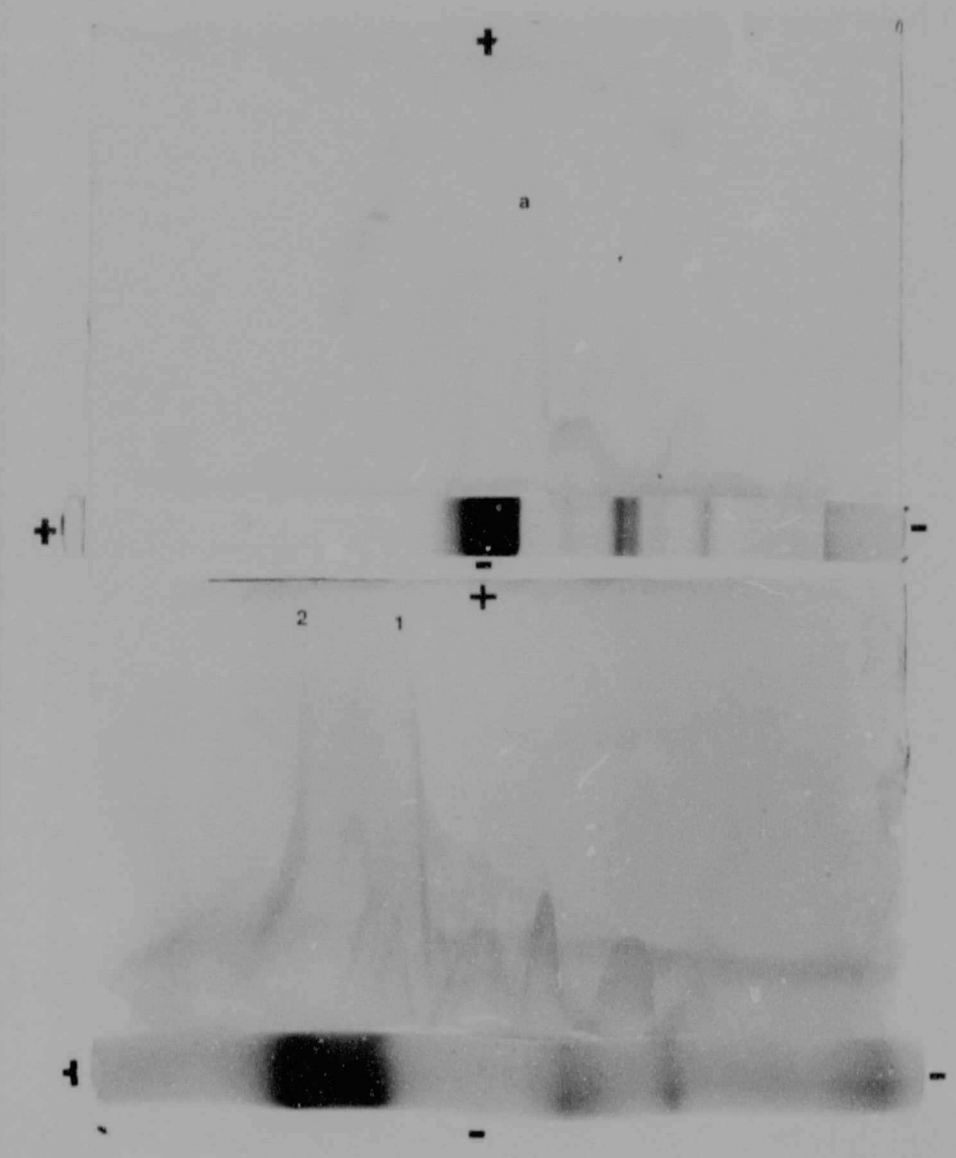

Figure 13

Crossed immunoelectrophoresis of baboon plasma proteins separated on polyacrylamide gel (top) and sephadex-acrylamide gel (bottom) against baboon anti-whole serum. (a) albumin (1) upper albumin (2) lower albumin.

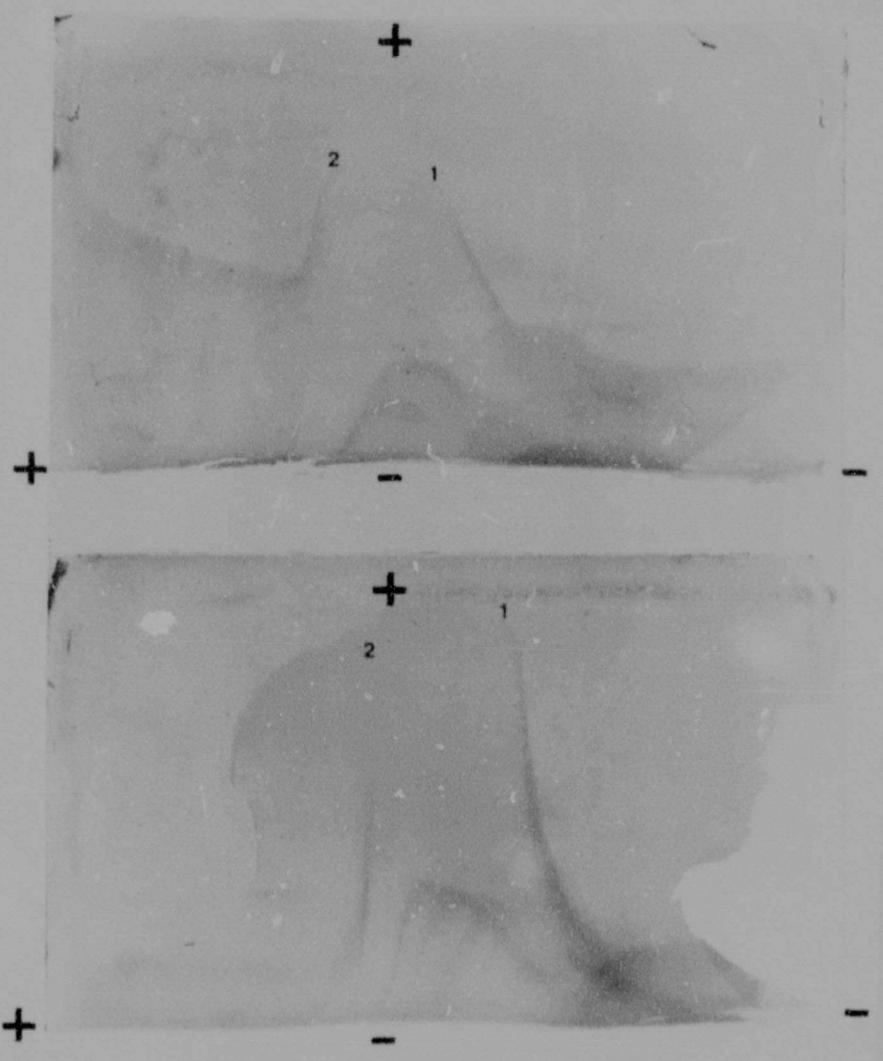

Figure 14

Crossed immunoelectrophoresis of baboon aqueous humor (top) and cerebrospinal fluid (bottom) after SAGE against baboon anti-whole serum. (1) upper albumin (2) lower albumin.

3.2.1.3 Pig

Separation times for the pig plasma proteins were 23 min at 90 volts and 20 min at 160 volts on acrylamide and 14 min at 160 volts on sephadex-acrylamide. For both PAGE and SAGE 2,5 µl of plasma were electrophoresed. Antibodies against pig plasma proteins were prepared in rabbits. During CIE 1,25 µl of plasma were applied to the gel and 0,75 ml of purified antibodies per agarose plate (8,5 ml agarose) were used. Plasma protein concentrations for pig were determined to be 69,3 ± 5,5 g/l (n=11).

Electrophoretic separation of pig plasma proteins on respectively acrylamide and sephadex-acrylamide gel yielded distinctly different results with regard to electrophoretic banding pattern and the heterogeneity of some protein fractions (Fig. 15). These results were also confirmed by CIE of pig plasma proteins against specific anti-whole serum, as shown in Fig. 15.

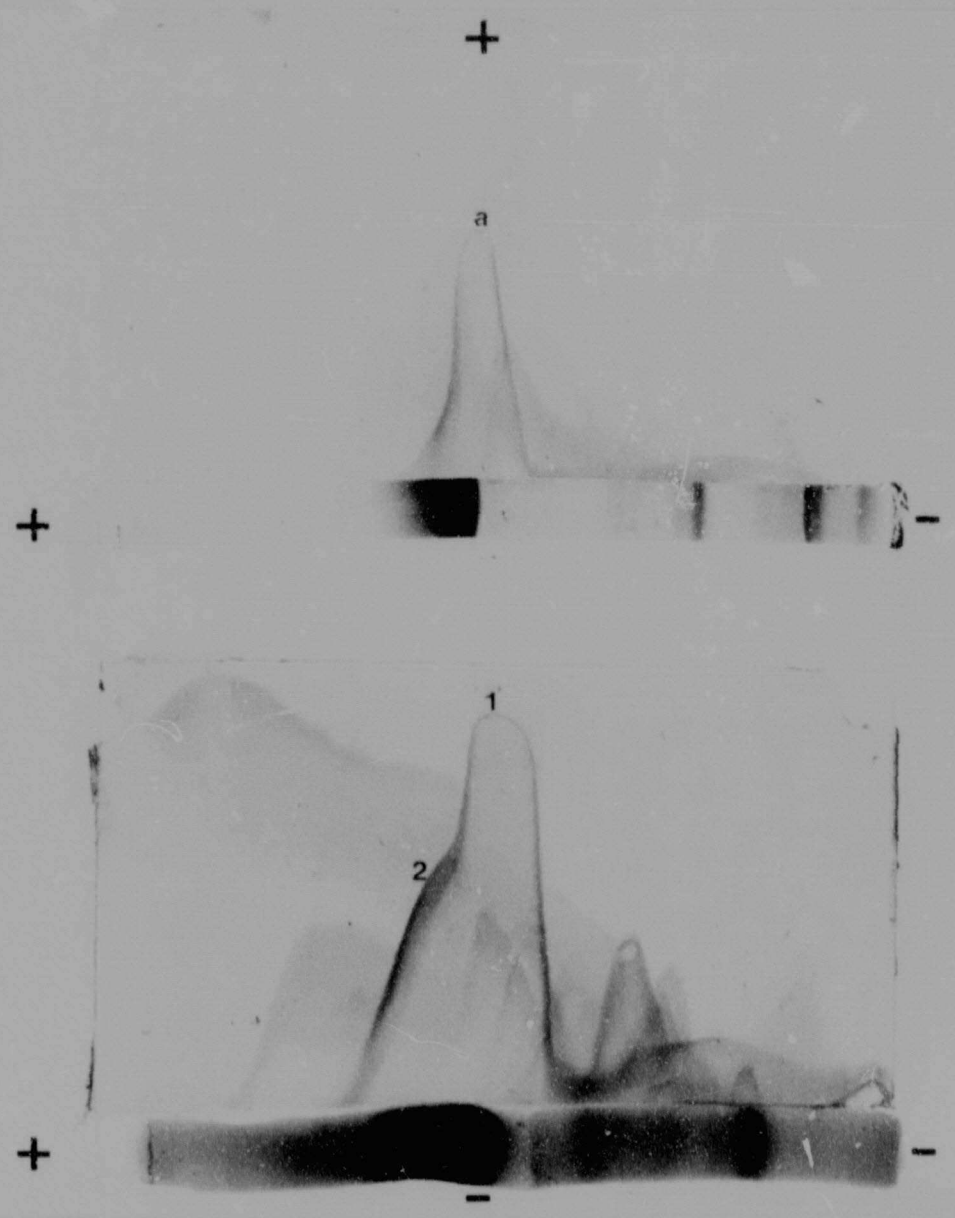

Figure 15

Crossed immunoelectrophoresis of pig plasma proteins after PAGE (top) and SAGE (bottom) against anti-whole serum.
(a) albumin (1) upper albumin (2) lower albumin.

3.2.1.4 Bovine

Each gel was loaded with 2,5 µl plasma during both PAGE and SAGE. The load during CIE was 1,25 µl plasma per gel and 1,5 ml purified antibodies per agarose plate (8,5 ml agarose) were used. Antibodies against bovine plasma proteins were raised in rabbits. Electrophoretic separation on acrylamide gels was done for 26 min at 90 volts and 17 min at 160 volts. Equivalent times for the sephadex-acrylamide gels were 17 min at 90 volts and 17 min at 160 volts.

Haematocrit and plasma protein concentrations of bovine blood were determined (Table 7).

Table 7

Bovine haematocrit and plasma protein concentration.

	Hct (%)	Plasma prot. conc. (g/l)
$\bar{x}$	42,5	79,70
SD	0,6	0,76
n	8	8

The plasma protein concentration (79,7 ± 0,8 g/l) measured in this study compares favourably to the 75,0 ± 5,8 g/l reported by Kawamura et al (1974). On the other hand Pappenheimer and Soto-Rivera (1948) reported a lower plasma protein concentration (41,0 g/l).

It could also be shown with bovine plasma that SAGE resulted in separation of plasma albumin into more than one subfraction, as seen in Fig. 16. These results were also verified by CIE against specific antibodies raised in rabbits (Fig. 17).

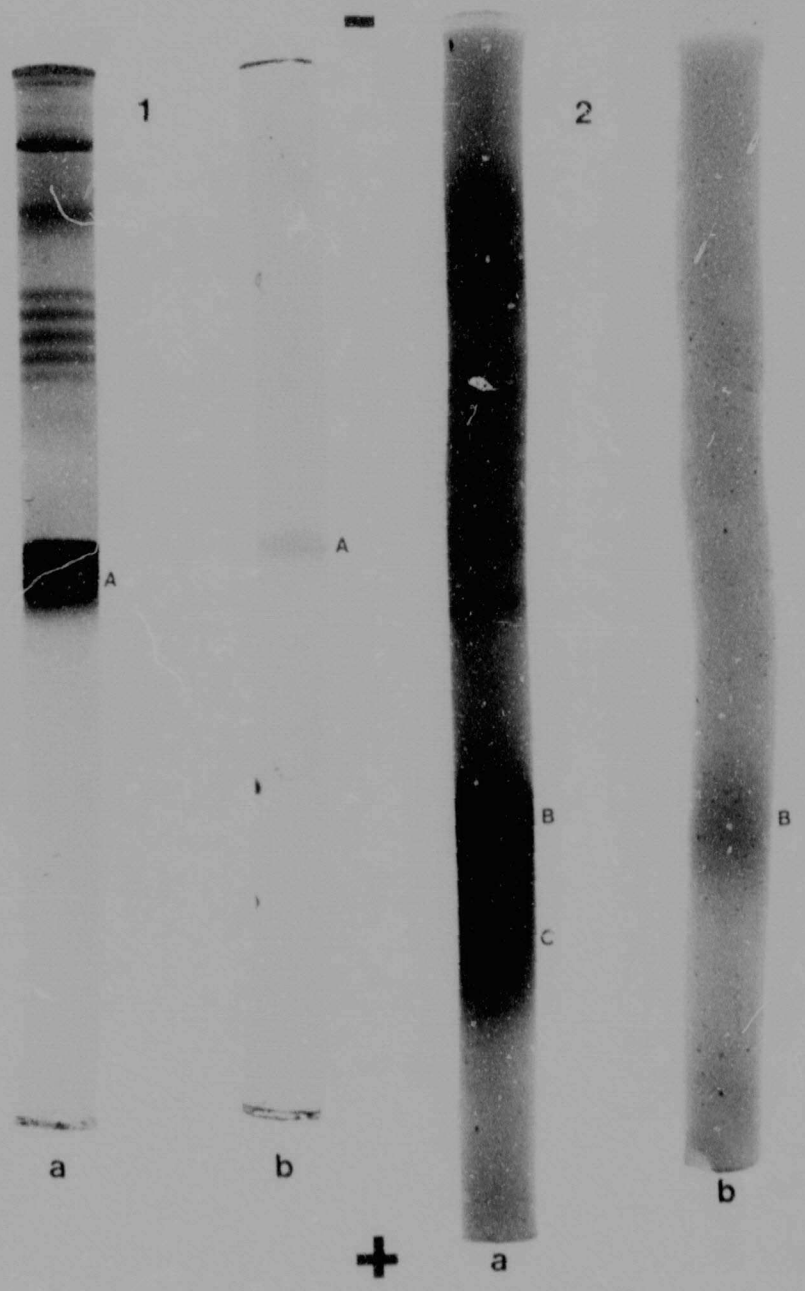

Figure 16

Electrophoretic separation of bovine plasma (a) and aqueous humor (b) on acrylamide (1) and sephadex-acrylamide (2) gels. (A) albumin (B) upper albumin (C) lower albumin.

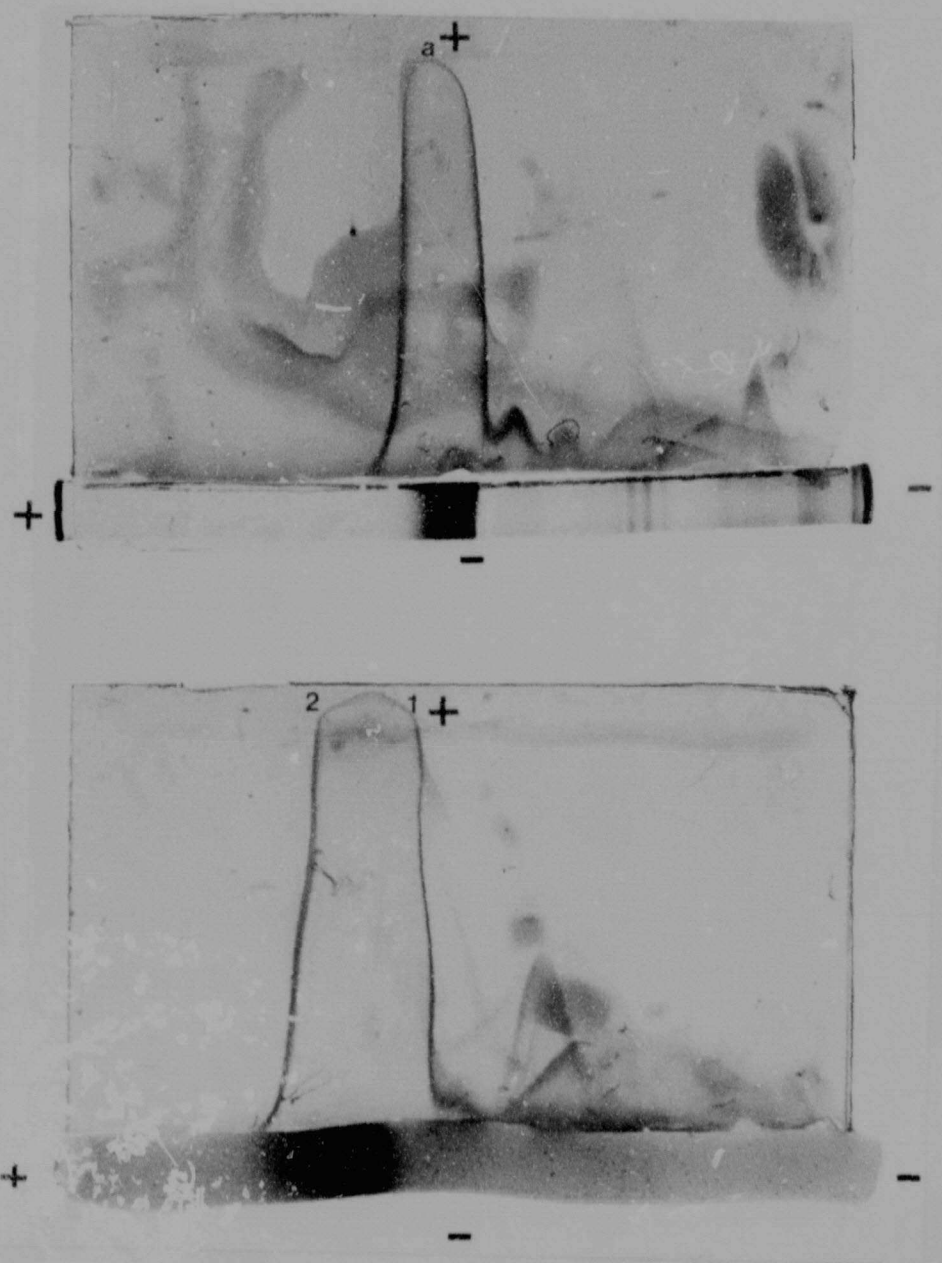

Figure 17

Crossed immunoelectrophoresis of bovine plasma proteins after PAGE (top) and SAGE (bottom) against anti-whole serum. (a) albumin (1) upper albumin (2) lower albumin.

3.2.1.5 Sheep

Plasma A/G ratios as well as haematocrit were determined for the sheep (Table 8). PAGE and SAGE of sheep plasma proteins, illustrated in Fig. 18, yielded similar results to those obtained with plasma from other animal species. Separation times and plasma load per gel were the same as described for bovine plasma. Due to insufficient quantities of sheep plasma, specific antibodies to sheep plasma proteins were not prepared.

Table 8

Haematocrit and plasma A/G ratios of the sheep.

	Hct %	A/G plasma
$\bar{x}$	30,0	1,35
SD	1,8	0,37
n	4	5

Plasma A/G ratio reported (1,35 ± 0,37) was found to be greater than the A/G ratio (0,44 ± 0,01) reported by Vreim et al (1976b). The reason(s) for this is not yet clear.

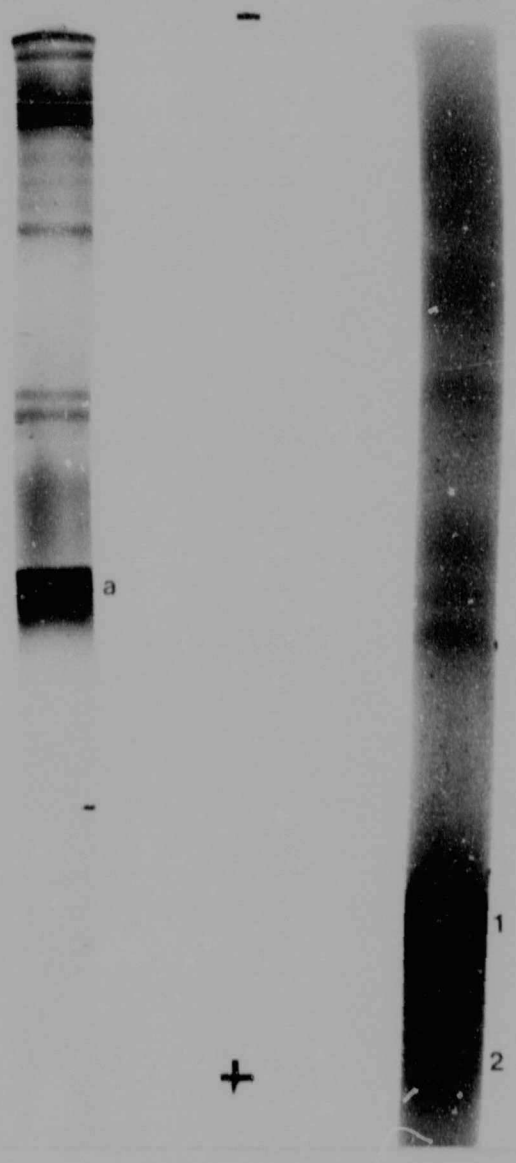

Figure 18
Polyacrylamide gel electrophoresis (left) and SAGE (right) of sheep plasma proteins. (a) albumin (1) upper albumin (2) lower albumin.

3.2.1.6 Horse

Separation times for the horse plasma protein were 23 min at 90 volts and 20 min at 160 volts on acrylamide and 14 min at 90 volts and 20 min at 160 volts on sephadex-acrylamide. For both PAGE and SAGE 2,5 µl of plasma per gel were applied. During CIE 0,9 ml purified antibodies per agarose plate (8,5 ml agarose) were used. Antibodies to horse plasma proteins were raised in rabbits. Plasma protein concentrations and A/G ratios were also determined, the results of which are given in Table 9.

Table 9

Plasma protein concentration and albumin/globulin ratio of the horse.

	Prot. conc. (g/l)	A/G
$\bar{x}$	75,60	1,37
SD	2,76	0,23
n	7	7

Kirk et al (1975) reported similar plasma protein concentrations (75,3 ± 1,6 g/l), but once again a lower A/G ratio (0,58) was reported by Sallman and Moore (1948).

The albumin of horse plasma could be separated into more than one fraction on both acrylamide gel and sephadex-acrylamide gel, as can be seen from Fig. 19. With CIE (Fig. 20) it could be shown that the albumin fraction as separated on acrylamide gel consisted of a main rocket peak with tailing to the anodal side, in which two distinct lesser albumin components could be identified. The albumin fraction as separated on sephadex-acrylamide, however, consisted of two components only.

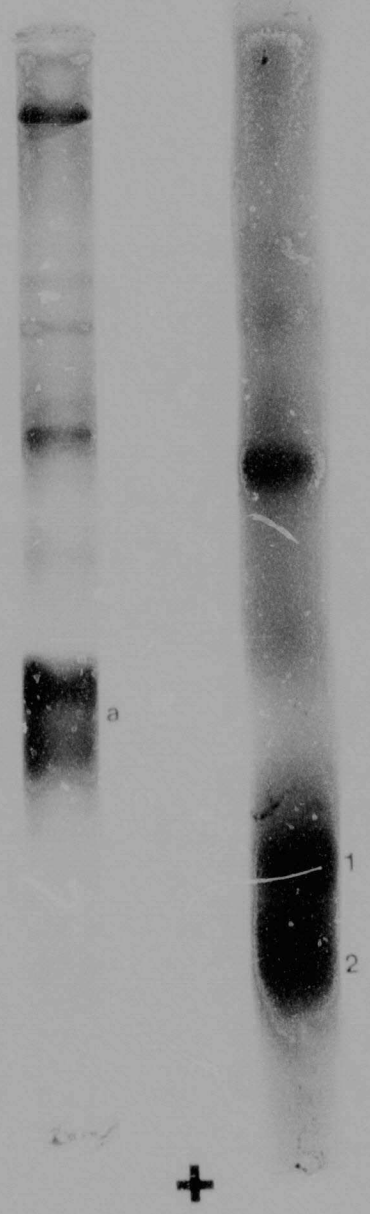

Figure 19

Polyacrylamide gel electrophoresis (left) and SAGE (right) of horse plasma proteins. (a) albumins (1) upper albumin (2) lower albumin.

Figure 20

Crossed immunoelectrophoresis of horse plasma proteins after PAGE (top) and SAGE (bottom) against anti-whole serum.

(a) albumin (1) upper albumin (2) lower albumin.

3.2.1.7 Dog

Plasma load per gel for the dog was 4 µl with electrophoretic separation, due to a relatively low plasma protein concentration (see Table 10). For CIE, antibodies against dog plasma proteins were prepared in rabbits and 0,7 ml of the purified antibody preparation per agarose plate (8,5 ml agarose) were used, the plasma load per gel being 1,25 µl. Separation times were the same as those described for the horse. Plasma protein concentrations were also determined (Table 10).

Table 10

Plasma protein concentration of the dog together with comparable values from the literature.

Protein concentration (g/l)	Reference
50,6 ± 0,8 (n=6)	Results
62,5	Field et al (1934)
56,5 ± 2,6	Courtice & Morris (1955)
65	Gibson & Gaar (1970)
72,8 ± 2,6	Gibson & Segal (1975)
49,0 ± 6,0	Vreim & Staub (1976)
59,0 ± 4,3	Vreim et al (1976a)
57,0 ± 6,0	Schad & Brechtelsbauer (1977a)

Results obtained by electrophoresis of dog plasma proteins on acrylamide gel and sephadex-acrylamide gel are shown in Figure 21 and CIE of the proteins separated respectively on the two gel media against specific antibodies are presented in Figure 21.

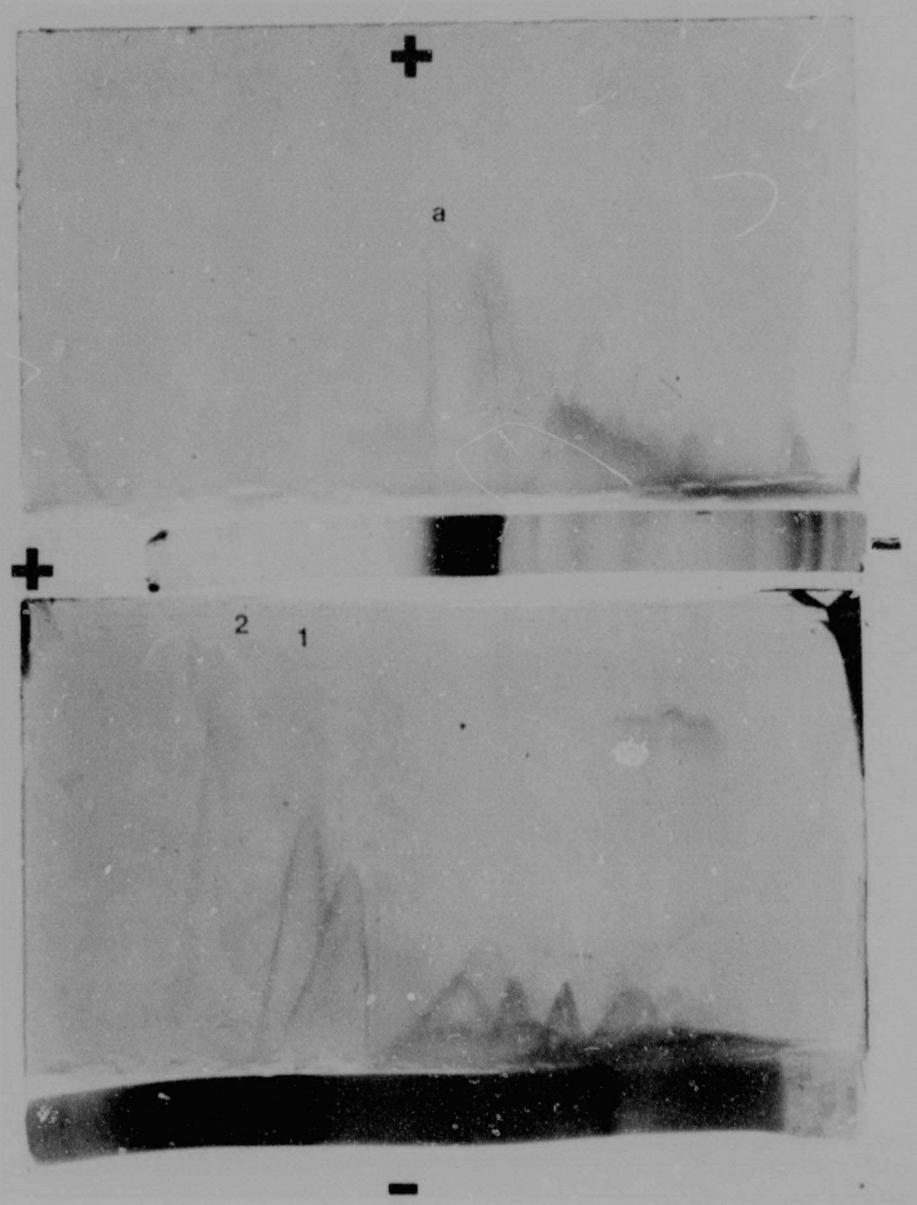

Figure 21

Crossed immunoelectrophoresis of dog plasma proteins after PAGE (top) and SAGE (bottom) against anti-whole serum.
(a) albumin (1) upper albumin (2) lower albumin.

3.2.1.8 Cat

During electrophoretic studies of cat plasma proteins the plasma load per gel as well as the respective electrophoretic separation times were the same as those described for the baboon. For CIE the plasma load per gel was 1,25 µl and 0,5 ml of purified antibodies per agarose plate (8,5 ml agarose) were used. Specific antibodies against cat plasma proteins were raised in rabbits and purified as described in the method section. Plasma protein concentrations were also determined, as shown in Table 11.

Table 11

Plasma protein concentration of the cat with comparable values from the literature.

Protein concentration (g/l)	Reference
63,0 ± 5,3 (n=5)	Results
53,0	Pappenheimer & Soto-Rivera (1948)
70,9 ± 1,4	Courtice & Morris (1955)
62,0	Jacobsson & Kjellmer (1964)
55,0	Lewis & Westcott (1968)
63,5 ± 4,8	Schultze et al (1972)

As was the case with the other animal species studied, electrophoretic separation on acrylamide gels and sephadex-acrylamide gels respectively yielded results differing with respect to both electrophoretic banding patterns and heterogeneity of some protein fractions. These differences could be demonstrated on the stained gels as well as by CIE (Fig. 22).

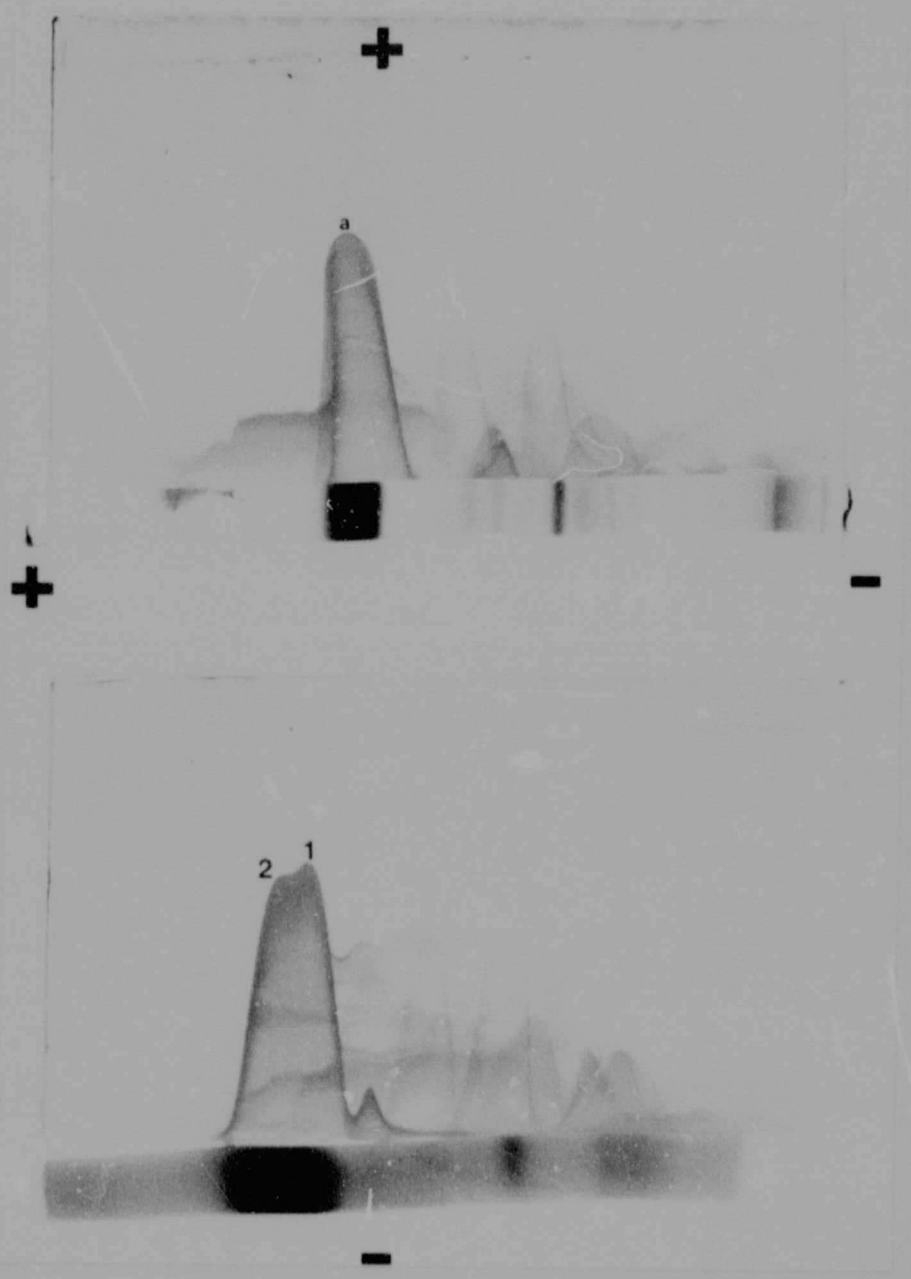

Figure 22

Crossed immunoelectrophoresis of cat plasma proteins after PAGE (top) and SAGE (bottom) against anti-whole serum. (a) albumin (1) upper albumin (2) lower albumin.

3.2.1.9 Rabbit

Several body fluids were sampled from rabbits for this study (see method section). The body fluids were plasma, cerebrospinal fluid, aqueous humor, lymph, pericardial fluid and abdominal fluid. Protein concentrations of each body fluid as well as blood haematocrit were determined (Table 12).

Albumin/globulin ratios for plasma, CSF, pericardial and abdominal fluid were determined as summarised in Table 13.

Table 12

Haematocrit and body fluid protein concentrations of the rabbit.

	Hct %	Protein concentration (g/l)					
		Plasma	CSF	Aq.h.	Lymph	Pf.	Af.
$\bar{x}$	42,0	61,75	2,60	2,29	41,67	16,50	33,32
SD	3,6	8,07	1,64	1,11	6,66	2,64	1,47
n	6	8	5	7	6	3	3

CSF = cerebrospinal fluid, Aq.h. = aqueous humor,
Pf. = pericardial fluid, Af. = abdominal fluid.

Table 13

Albumin/globulin ratios of different rabbit body fluids.

	Plasma	CSF	Pf	Af
$\bar{x}$	1,93	1,10	1,09	1,73
SD	0,49	0,28	0,43	0,61
n	12	4	4	4

CSF = cerebrospinal fluid, Pf. = pericardial fluid,
Af. = abdominal fluid.

Most authors have reported plasma protein concentrations varying between 49,2 $\pm$ 0,8 and 66,3 $\pm$ 5,8 g/l which compare favourably to the value reported in this study (61,8 $\pm$ 8,1 g/l). Lymph protein concentrations reported in the literature are less concentrated than measured in this study (Table 12).

Table 14

Haematocrit and body fluid protein concentrations and albumin/globulin ratios of the rabbit.

Body Fluid	Protein concen. (g/l)	Hct %	A/G	Reference
Plasma			1,27	Sallman & Moore (1948)
	49,2+0,8		1,36	Benson et al (1955)
	65,0			Davson (1967)
	57,0+2,0			Lewis (1969)
	62,0			Cserr et al (1972)
	59,0+1,5		1,98	Haljamae et al (1974)
	60,0+1,0	36		Jones (1975)
	66,3+5,8		1,40	Rutili & Arfors (1977)
	62,0			Tibes et al (1977)
	61,8+8,1	42	1,93	Results
Lymph	26,6+0,8			Benson et al (1955)
	29,1+1,0			Lewis (1969)
	24,7+1,5			Bach & Lewis (1973)
	26,6+0,4			Rutili & Arfors (1977)
	30,0			Tibes et al (1977)
	41,7+6,7			Results
CSF	0,3			Davson (1967)
	2,9			Cserr et al (1972)
	2,6+1,6		1,10	Results

Electrophoretic studies on acrylamide gels and sephadex-acrylamide gels respectively, yielded similar results to those obtained with the body fluids of other animal species studied. Protein loads per gel were as follows: 2,5 µl plasma, 5 µl lymph, 8 µl abdominal fluid, 10 µl pericardial fluid, 20-40 µl aqueous humor and 20-100 µl cerebrospinal fluid. Separation times were 25 min at 90 volts and 20 min at 160 volts for PAGE and 20 min at 90 volts and 20 min at 160 volts for SAGE. The electrophoretic banding patterns of rabbit body fluid proteins separated during PAGE are illustrated in Figs. 23 and 24, and those separated during SAGE in Fig. 25.