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A comparative study of the proteins in the body fluids of animals

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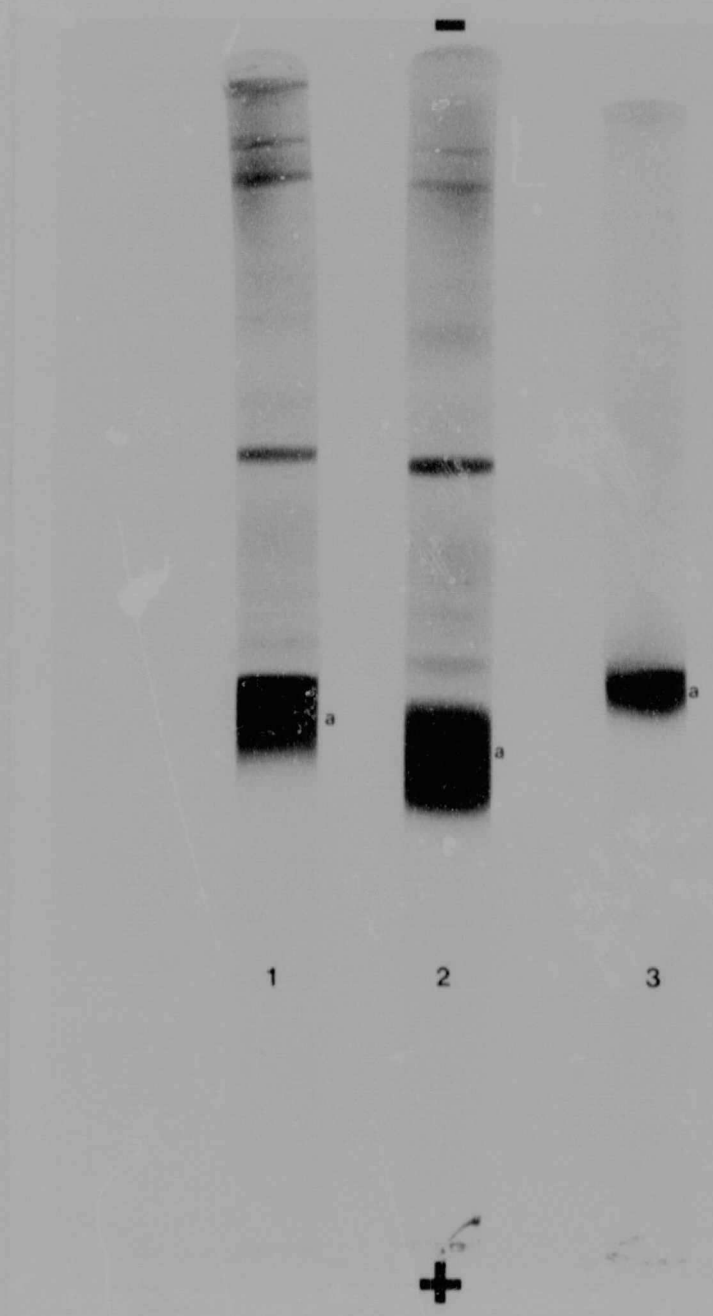


Figure 23

Polyacrylamide gel electrophoresis of rabbit plasma (1), lymph (2) and aqueous humor (3). Albumin (a).

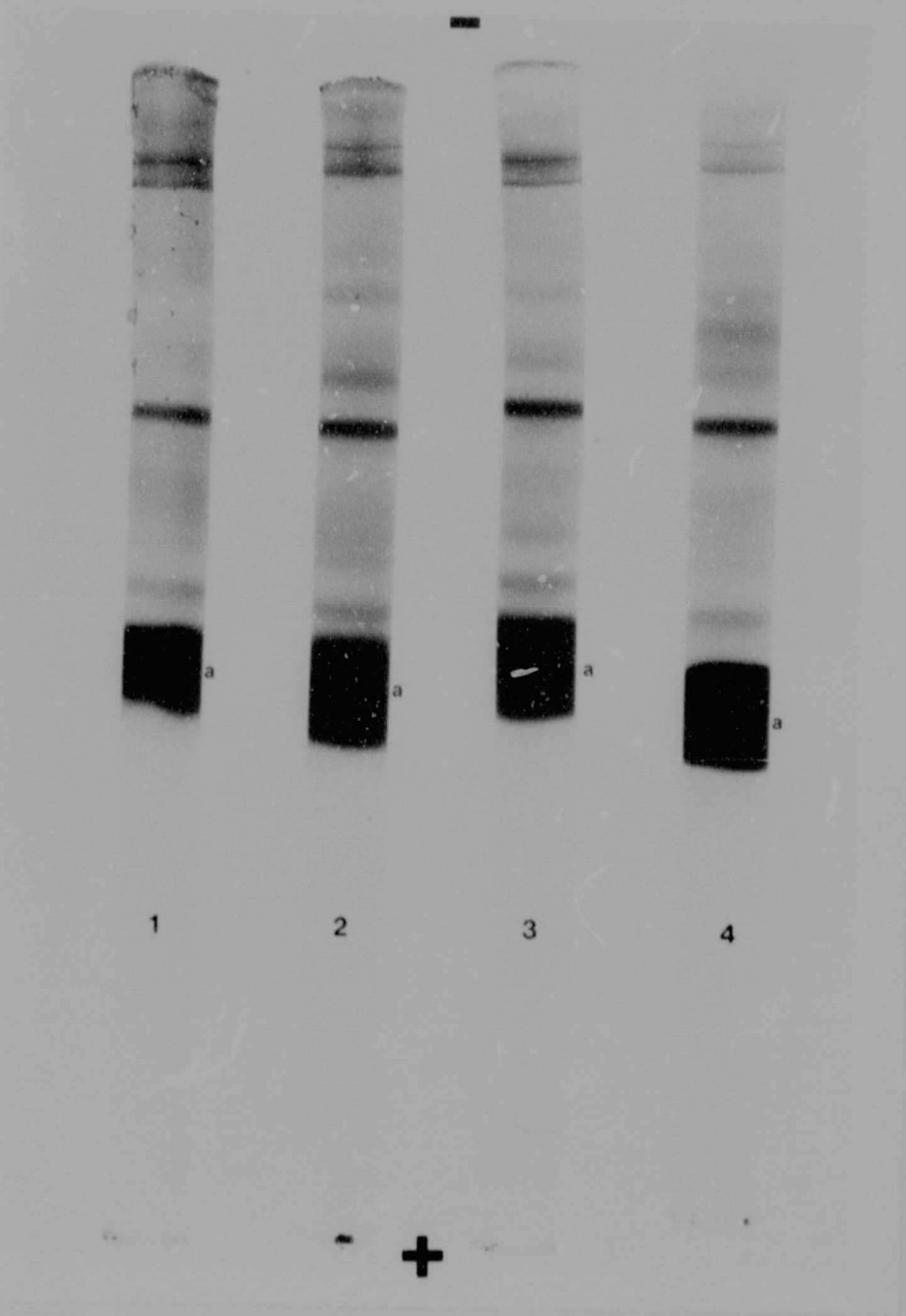


Figure 24

Polyacrylamide gel electrophoresis of rabbit plasma (1), femoral lymph (2), thoracic lymph (3) and femoral lymph obtained during passive movements of the leg (4). Albumin (a).

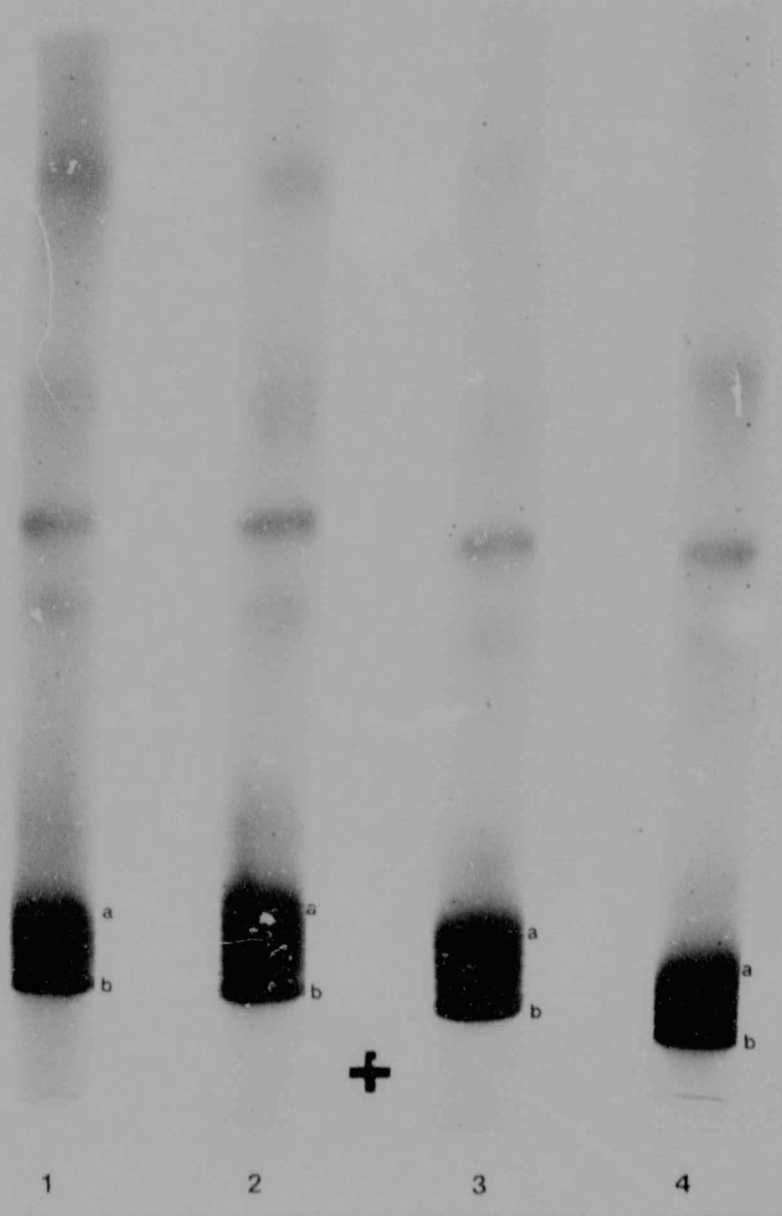


Figure 25

Sephadex-acrylamide gel electrophoresis of rabbit plasma (1), femoral lymph (2), thoracic lymph (3) and femoral lymph obtained during passive movements of the leg (4). Upper albumin (a), lower albumin (b).

Crossed immunoelectrophoresis of rabbit plasma, lymph, aqueous humor and cerebrospinal fluid after PAGE and SAGE were performed, but clear rocket peaks were not obtained. No explanation(s) could be found

From the foregoing tables it can be seen that although plasma protein concentration was higher than protein concentration of any other body fluid for any given animal, the A/G ratios of the other body fluids were often found to be higher than that of the homologous plasma. A possible reason is that because albumin is one of the smallest and most abundant molecules in plasma, it moves out of plasma into the other body fluids more frequently and with less difficulty than any of the globulins. It would thus appear that the presence of plasma proteins in body fluids other than plasma, is dependant on the concentration and molecular size of the individual plasma protein components.

3.2.2 The Effects of Physical Exercise, Heat Stress and Fever Conditions upon Albumin in Body Fluids

In the second part of this study, the effects of strenuous physical exercise upon humans, of an endogenous fever induced in rabbits with endotoxin and of rats exposed to an environmental temperature of 37°C, were investigated with particular reference to the albumins.

The body fluid parameters studied during physical stress conditions were haematocrit, protein concentrations, albumin/globulin ratios and the heterogeneity of albumin as

demonstrable by electrophoresis on sephadex-acrylamide gel.

3.2.2.1 Physical Exercise

3.2.2.1.1 Controlled laboratory conditions

In this section changes in blood parameters of humans during physical exercise were studied in subjects before (untrained) and after (trained) undergoing a course of physical training (Chamber of Mines of S.A.). By comparing the subjects' blood lactic acid concentration curves it was established that the subjects' physiological ability to perform physical exercise was improved, meaning that the duration of exercising was increased before exhaustion. The state of hydration, water intake, weight loss, and anthropomorphic measurements were not measured (P. Jooste, personal communication). Results obtained on the haematocrit and albumin/globulin ratios of the trained and untrained human subjects before and after a five-hour treadmill exercise are given in Tables 15 and 16.

Table 15

Haematocrit (%) of trained and untrained human subjects before and after a five hour treadmill exercise.

Subject	UNTRAINED		TRAINED	
	Before	After	Before	After
1	49,5	46,5	49,7	48,5
2	48,5	49,2	49,0	47,5
3	53,0	49,2	49,5	49,5
4	53,0	48,2	50,5	49,7
5	54,5	53,2	56,0	53,7
6	51,0	53,0	50,7	54,7
7	51,2	53,0	51,0	49,7
$\Delta\bar{x}$	-0,77		-0,44	
SD	2,5		2,08	
n	7		7	
P	NS		NS	
$\Delta\bar{x}$ *	-0,61			
SD	1,76			
n	7			
P	NS			

* measured between the Hct of trained and untrained human subjects before a five hour treadmill exercise.

Table 16

Albumin/globulin ratios of plasma before and after a five hour treadmill exercise of trained and untrained human subjects.

Subject	UNTRAINED		TRAINED	
	Before	After	Before	After
1	2,12	2,93	2,42	2,80
2	2,25	3,02	2,05	3,00
3	1,47	2,46	1,25	1,29
4	0,84	2,97	1,45	1,63
5	0,95	1,75	1,15	1,43
6	1,31	1,17	1,32	1,63
$\Delta\bar{x}$	0,89		0,36	
SD	0,73		0,31	
n	6		6	
P	$< 0,05$		$<0,05$	
$\Delta\bar{x}$ *	0,12			
SD	0,32			
n	6			
P	NS			

*measured between the A/G of plasma from trained and untrained human subjects before a five hour treadmill exercise.

From Table 15 it can be seen that no significant changes in haematocrit of either the untrained or the trained subjects occurred during physical exercise. No significant difference in resting haematocrit between the trained and untrained subjects could be seen. The changes in the plasma A/G ratios of the untrained subjects before and after the treadmill exercise was found to increase significantly ($P<0,05$). Similarly the differences in the A/G ratios of the trained subjects before and after the five hour treadmill exercise was also found to increase significantly ($P<0,05$). The resting A/G ratios of subjects before and after a physical training course were, however, not significantly different.

Comparative electrophoresis of the plasma protein of untrained and trained human subjects before and after physical exercise yielded the results shown in Figs. 26 and 27.

From Fig. 26 it can be seen that no obvious change in the plasma protein electrophoretograms of either trained or untrained subjects due to physical exercise, could be demonstrated by electrophoresis on acrylamide gels. Electrophoresis on sephadex-acrylamide gels (Fig. 27), indicated a marked increase in the relative concentration of the lower albumin fraction in the untrained subjects after physical exercise, while any such change in the electrophoretograms of the trained subjects, if at all present, could not be detected. The above results of the electrophoretic studies were also quantified by crossed immunoelectrophoresis and comparing the areas enclosed by both relevant albumin peaks. A ratio of total albumin area before exercise to total albumin area after exercise was thus obtained and the results are summarized in Table 17. From these results it can be seen that an increase in relative concentration of the albumin in untrained subjects after exercise could be demonstrated on sephadex-acrylamide gel but not on acrylamide gels. This could have been the result of an increased relative concentration of the lower albumin fraction (Fig. 27). No significant change in relative albumin concentration could be demonstrated for the trained subjects.

Table 17

Ratio of total albumin area before exercise to total albumin area after exercise as determined by CIE (n=6).

Subjects	Acrylamide	Sephadex
Untrained	0,98 ± 0,25	0,68 ± 0,13
Trained	1,02 ± 0,08	1,11 ± 0,21

Similar results were obtained between trained subjects exercising under laboratory conditions and those of trained subjects exercising under field conditions (see section 3.2.2.1.2).

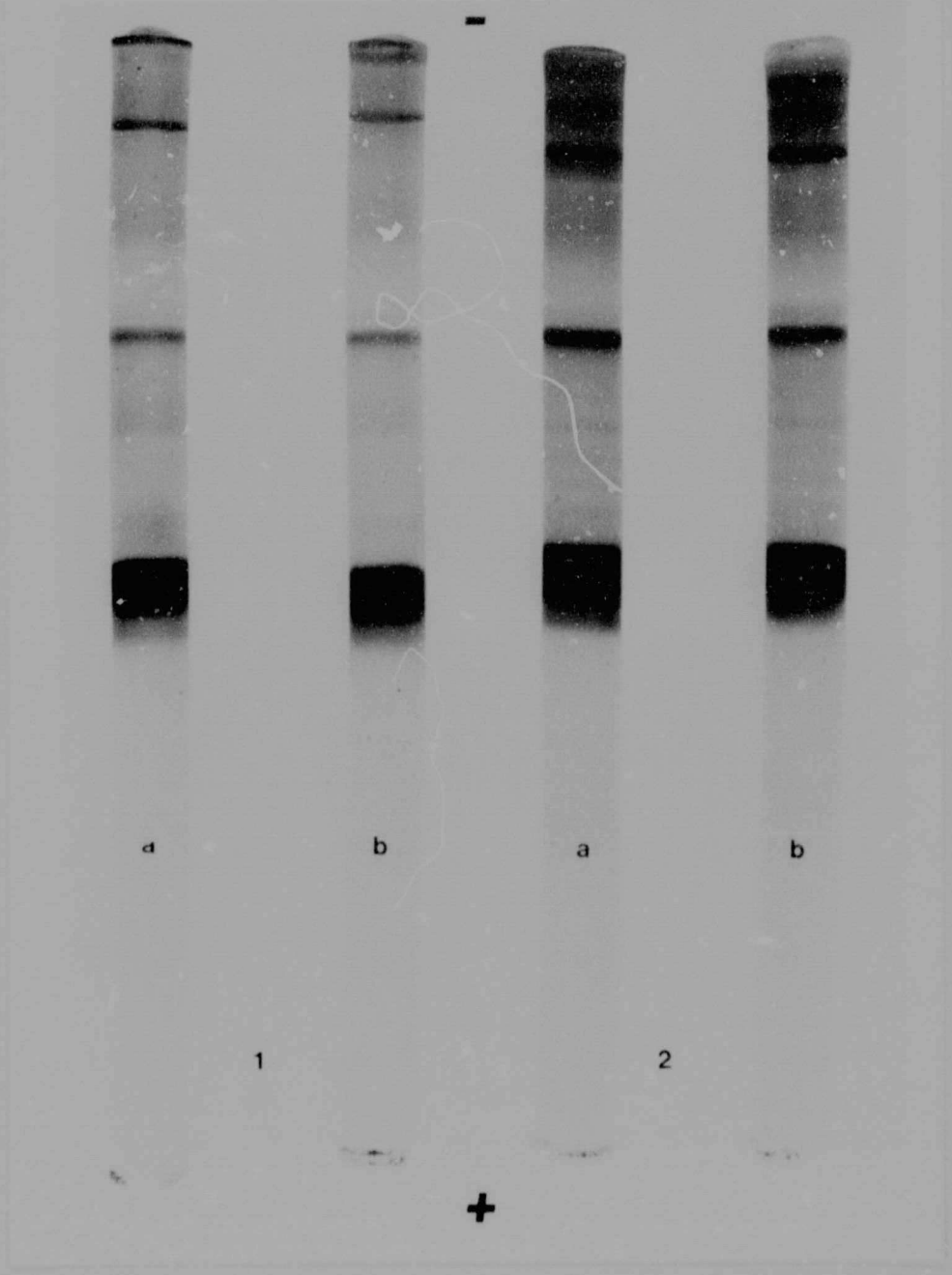


Figure 26

Polyacrylamide gel electrophoresis of human plasma proteins before (a) and after (b) a five hour treadmill exercise of untrained (1) and trained (2) human subjects.

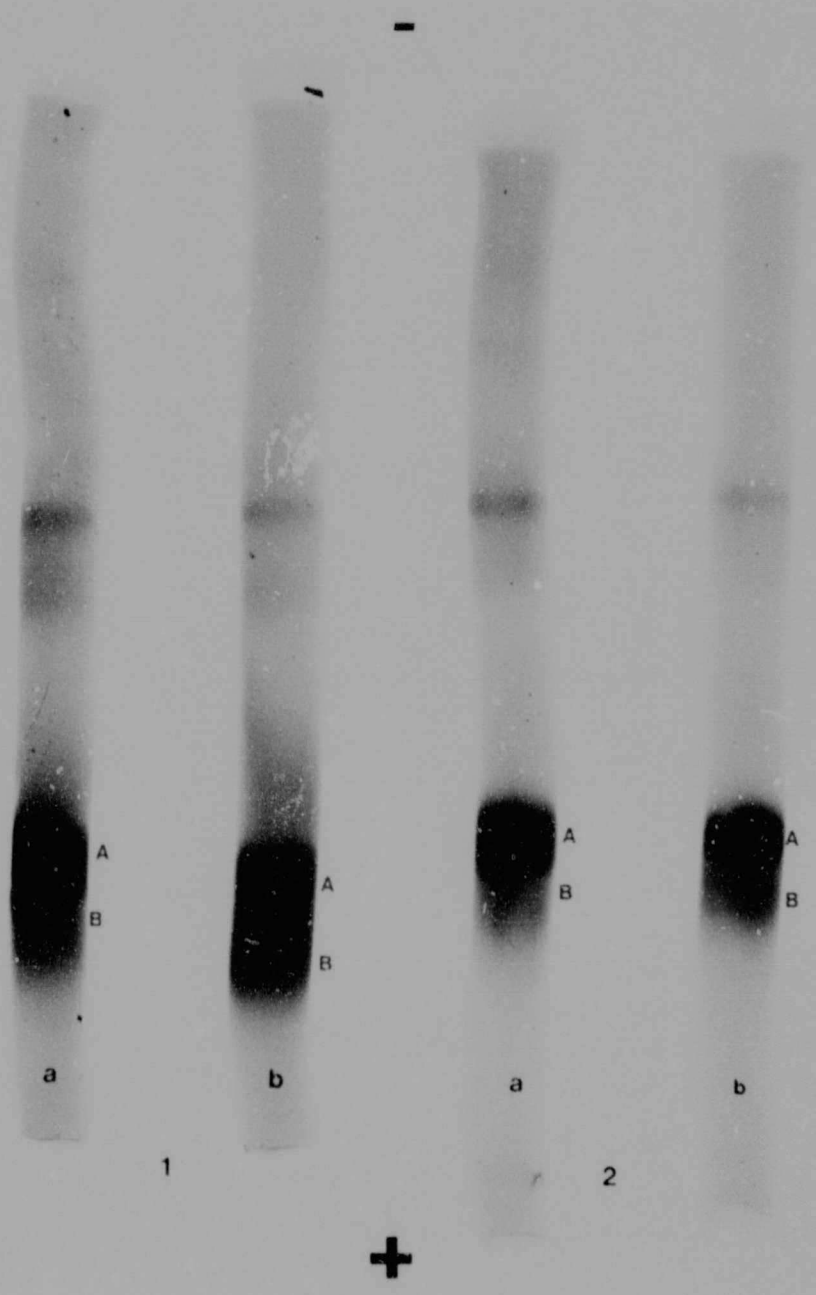


Figure 27

Sephadex-acrylamide gel electrophoresis of human plasma proteins before (a) and after (b) a five hour treadmill exercise of untrained (1) and trained (2) human subjects. Upper albumin (A), lower albumin (B).

3.2.2.1.2 Field Conditions

The effects of physical stress under field conditions on blood parameters were studied in long distance athletes. The level of physical training of each subject was not determined, but as samples were collected from subjects participating on a competitive basis, it was assumed that each subject was in a state of physical training.

Blood samples from fourty-two runners were collected and the data was arranged according to the distances which were run (Tables 18-22). In all cases, except the Veterans' marathon (minimum age 45 y, Table 19), the participants were not representative of any specific age group.

Table 18

Haematocrit and plasma protein concentration of human subjects before and after a 96 km run.

Athlete	Hct (%)		Prot. conc. (g/l)	
	Before	After	Before	After
1	48,2	49,5	103,0	96,0
2	56,0	52,7	-	95,0
3	53,5	50,5	95,0	94,0
4	53,2	50,5	87,5	87,5
5	55,0	50,5	103,5	84,0
6	52,0	47,2	96,0	80,0
7	46,5	47,7	72,0	84,0
8	51,5	56,0	-	97,0
$\Delta\bar{x}$	-1,41		-5,25	
SD	3,34		11,53	
n	8		6	
P	NS		NS	

Table 19

Haematocrit and plasma protein concentration of human subjects over 45 years of age before and after a 42 km run.

Athlete	Hct (%)		Prot. conc. (g/l)	
	Before	After	Before	After
9	46,0	46,0	80,0	79,0
10	52,0	47,0	70,0	74,0
11	44,0	30,5	79,0	79,0
12	48,0	44,0	71,0	85,0
13	47,0	44,0	-	-
14	47,0	47,0	-	-
15	49,0	40,0	-	-
16	51,0	49,5	-	-
17	45,0	40,0	-	-
18	44,0	43,0	-	-
19	47,0	43,0	-	-
20	49,0	47,0	82,0	76,0
21	53,0	49,0	79,0	79,0
22	49,0	43,0	57,0	50,0
$\Delta\bar{x}$	-4,14		0,57	
SD	3,65		7,02	
n	14		7	
P	< 0,001		NS	

Table 20

Haematocrit and plasma protein concentration of human subjects (all age groups) before and after a 42 km run.

Athlete	Hct (%)		Prot. conc. (g/l)	
	Before	After	Before	After
23	49,0	44,5	76,0	71,0
24	48,5	44,0	67,0	76,0
25	44,0	46,0	-	-
26	45,0	41,5	77,0	87,0
27	54,0	50,5	82,0	82,0
28	50,0	47,0	75,0	66,0
29	47,0	44,0	76,0	82,0
30	49,0	44,5	94,0	84,0
31	49,0	44,5	-	-
$\bar{\Delta x}$	-3,22		0,14	
SD	2,06		8,40	
n	9		7	
p	< 0,005		NS	

Table 21

Haematocrit of human subjects before and after a 32 km run.

Athlete	Hct (%) Before	Hct (%) After
32	46,0	47,0
33	50,0	48,0
34	47,0	44,0
35	46,0	44,0
$\Delta \bar{x}$	-1,5	
SD	1,73	
n	4	
P	NS	

Table 22

Haematocrit of human subjects before and after a 16 km run.

Athlete	Hct (%) Before	Hct (%) After
36	48,0	43,0
37	49,5	49,0
38	46,5	46,0
39	42,5	44,5
40	51,0	47,0
41	43,0	42,0
42	48,0	52,0
$\Delta \bar{x}$	-0,64	
SD	3,15	
n	7	
P	NS	

In Tables 18-20 no significant differences in protein concentration could be demonstrated. The decreases in Hct from before to after the 42 km races were found to be significant ($P<0,001$ Table 19; $P<0,005$ Table 20). No significant changes in Hct were observed for athletes running distances of 96 km (Table 18), 32 km (Table 21) and 16 km (Table 22).

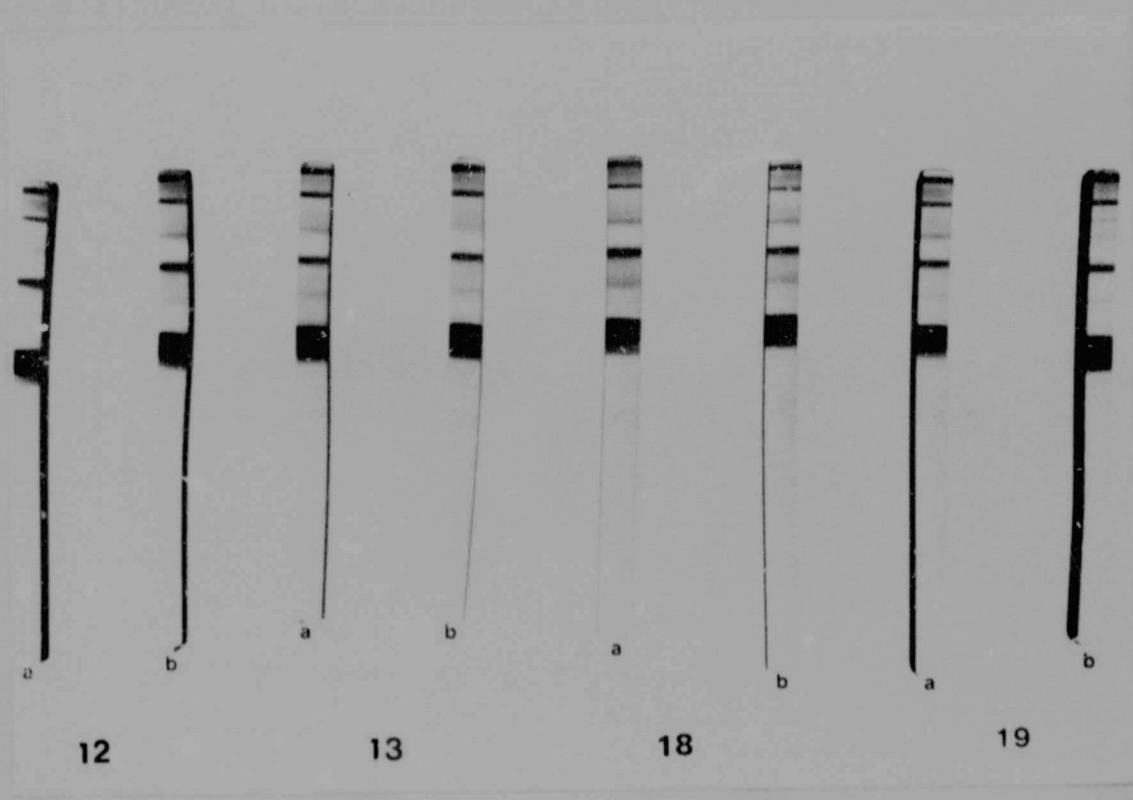


Figure 28

Separation of plasma proteins on acrylamide gel, before (a) and after (b) a 42 km run (corresponding to subjects 12, 13, 18 and 19 in Table 19).

During SAGE it was observed that the albumin of subjects taking part in the long distance races migrated further than the albumin of subjects not accustomed to regular physical exercise, under the same separating conditions.

In agreement with the studies carried out under controlled laboratory conditions (section 3.2.2.1.1), it was found that most subjects did not display albumin heterogeneity either before or after exercise, with a few cases where heterogeneity of the albumin fraction could be demonstrated before but not after exercise (Fig. 29). These results were verified by doing CIE (Figs. 30 and 31) which indicated that the lower albumin, if present, either shifted to the position of the upper albumin, or disappeared altogether during exercise or that the upper albumin migrated faster to cover the lower albumin.

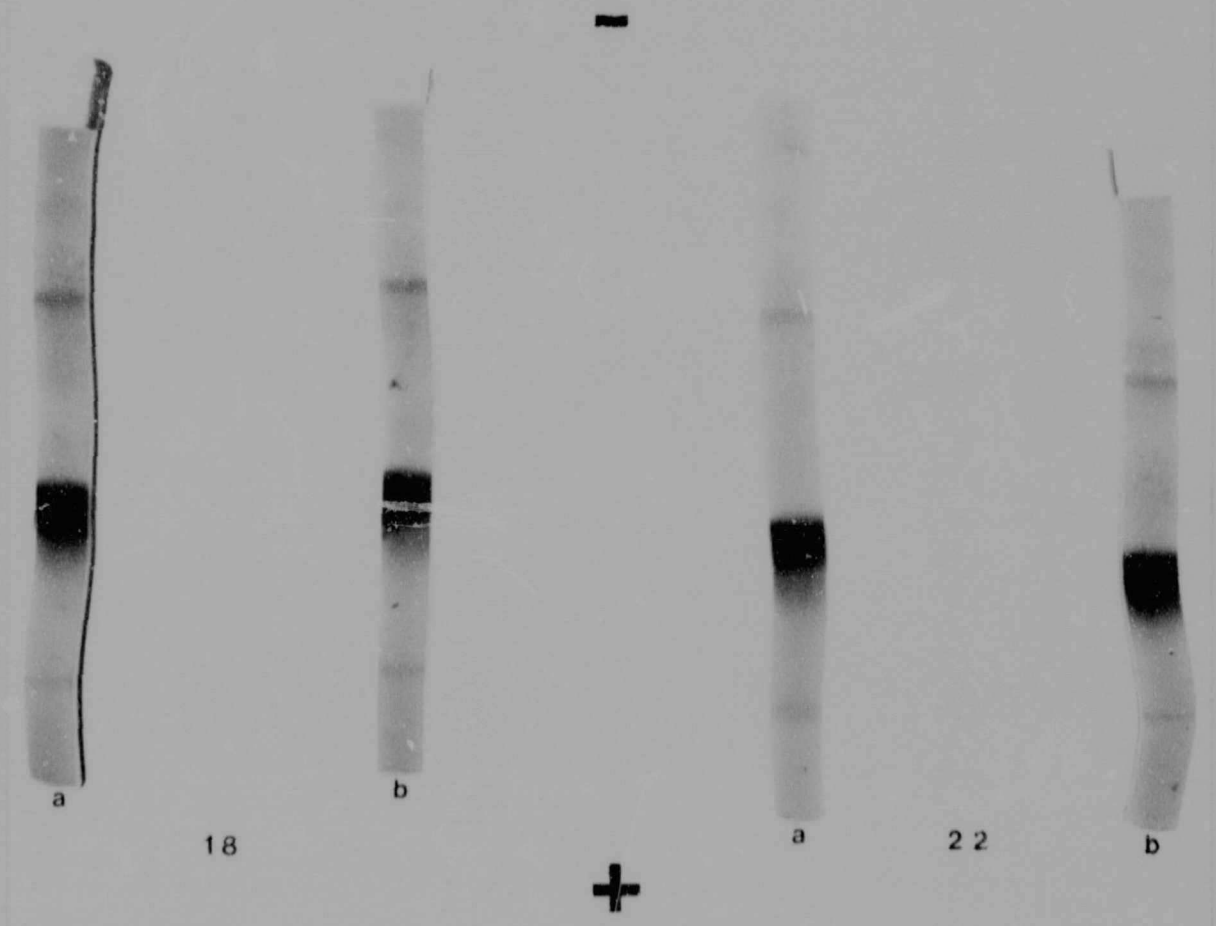


Figure 29

Sephadex-acrylamide gel electrophoresis of plasma samples (subjects 18 and 22) collected before (a) and after (b) a 42 km run.

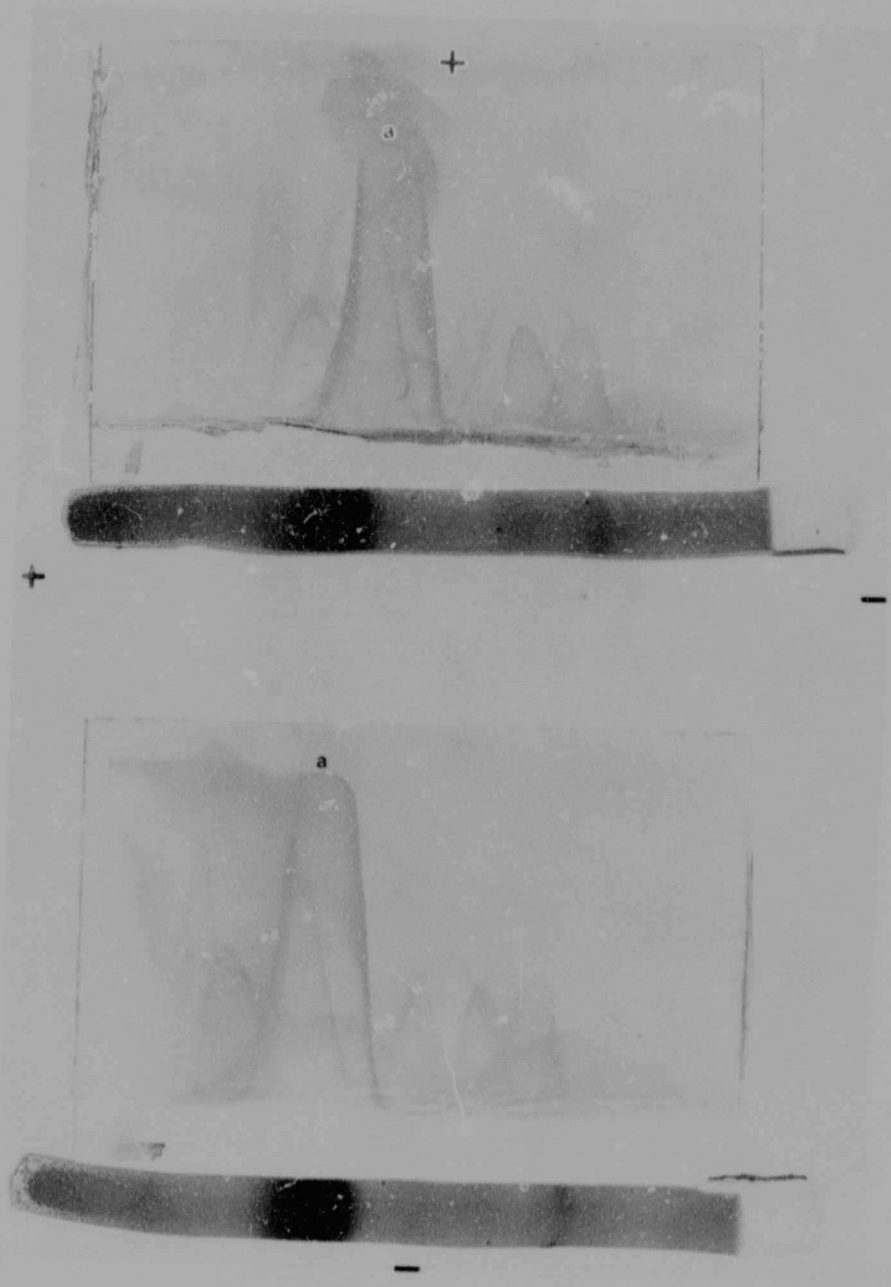


Figure 30

Crossed immunoelectrophoresis of human plasma devoid of albumin heterogeneity, before (top) and after (bottom) a 12 km run. (a) albumin.

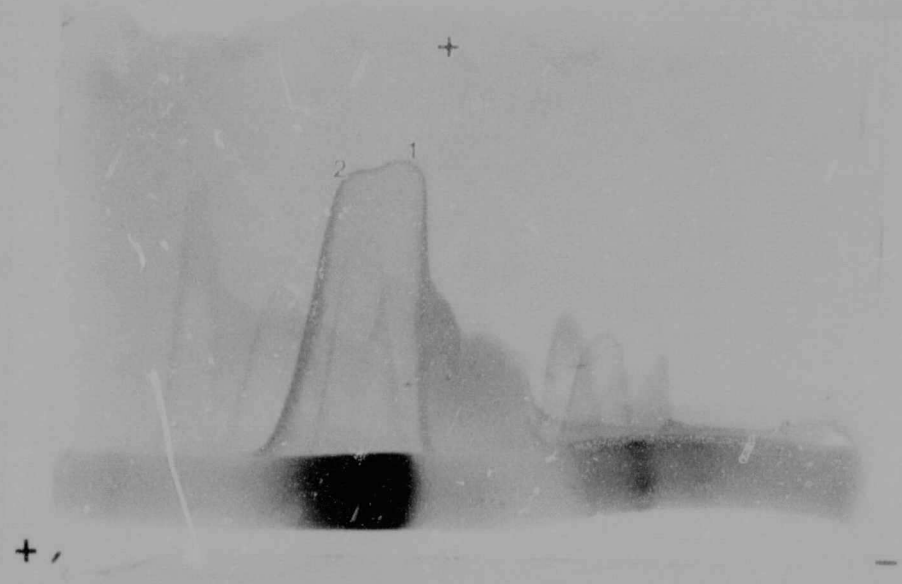


Figure 31

Crossed immunoelectrophoresis of human plasma displaying albumin heterogeneity, collected before (top) and after (bottom) a 42 km run. Electrophoretic medium SAGE. (1) upper albumin (2) lower albumin.

It was attempted to determine the nature of possible changes in plasma albumin during exercise by SAGE of plasma labeled with radioactive tryptophan. Ratios of albumin subfractions before and after a 42 km run were determined from the radioactive counts of the gel sections representing the upper and lower albumin subfractions respectively. From the results thus obtained (Table 23) it can be seen that a slight de-

crease in total albumin was found after the exercise (ratio 1,19 ± 0,08). This decrease in total albumin was found to be entirely due to a decrease in the lower albumin component (ratio 1,56 ± 0,23) as the upper albumin was unchanged after exercise (ratio 1,00 ± 0,04). This is further supported by the increased upper albumin: lower albumin ratio after exercise (2,11 ± 0,29) as compared to the same ratio before exercise (1,35 ± 0,31).

Table 23

Ratios of plasma albumin subfractions before and after a 42 km run as determined by SAGE. Albumin labeled with ^{14}C-Tryptophan (n=5).

	Ratio
Total albumin before: total albumin after	1,19 ± 0,08
Upper albumin before: upper albumin after	1,00 ± 0,04
Lower albumin before: lower albumin after	1,56 ± 0,23
Upper albumin before: lower albumin before	1,35 ± 0,31
Upper albumin after : lower albumin after	2,11 ± 0,29

3.2.2.2 Fever conditions

Fever was induced in six New Zealand white rabbits by the intravenous administration of bacterial endotoxin (see section 2.6.2). A maximum body temperature (rectal) of 40,0 ± 0,05°C (n=6) was achieved in this way. Plasma samples obtained from blood drawn at normal body temperature before administration of endotoxin and at maximum fever, were studied comparatively.

Results obtained from this study of some blood parameters during the period of fever stress are shown in Fig. 32 and Tables 24 to 26. From these results it can be seen that no changes in the blood parameters studied could be found to have resulted from fever.

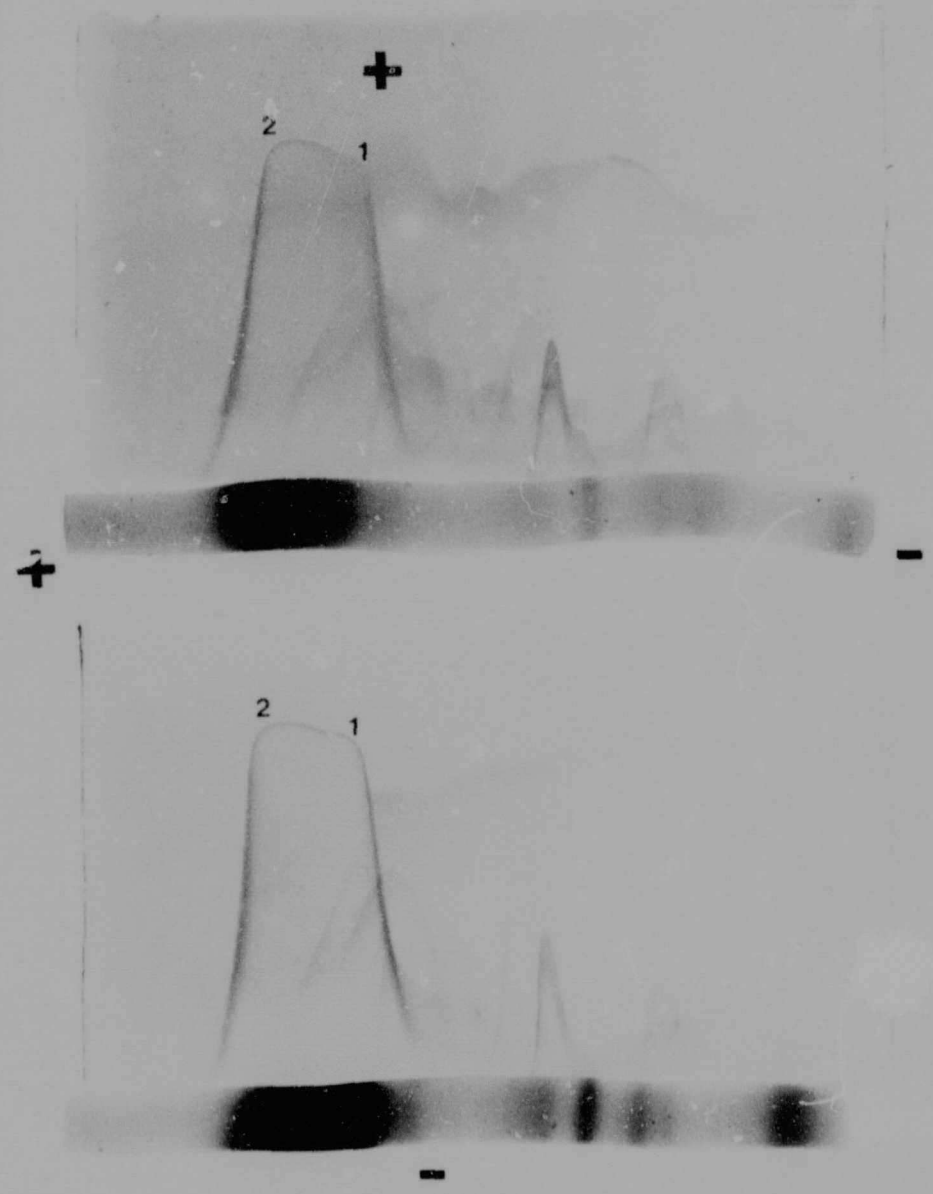


Figure 32

Crossed immunoelectrophoresis of rabbit plasma proteins, separated during SAGE, at normal body temperature before fever (top) and at maximum body temperature during fever (bottom). (1) upper albumin (2) lower albumin.

Table 24

Haematocrit of rabbit blood at normal body temperature before fever and at maximum body temperature during fever.

Rabbit	Hct (%) Normal	Hct (%) Fever
1	44,0	46,0
2	48,0	48,0
3	44,0	44,0
4	40,0	43,0
$\Delta\bar{x}$	1,25	
SD	1,5	
n	4	
P	NS	

Table 25

Albumin/globulin ratios of rabbit plasma at normal body temperature before fever and at maximum body temperature during fever.

Rabbit	A/G Normal	A/G Fever
1	1,63	1,60
2	1,81	1,44
3	2,01	1,89
4	1,79	1,50
$\Delta\bar{x}$	-0,20	
SD	0,16	
n	4	
P	NS	

Table 26

Ratios of rabbit plasma albumin subfractions at normal body temperature before fever and at maximum body temperature during fever. Albumin labeled with ^{14}C-Tryptophan (n=4).

	Ratio
Total albumin normal: total albumin fever	1,29 + 0,11
Upper albumin normal: upper albumin fever	0,97 + 0,07
Lower albumin normal: lower albumin fever	0,54 + 0,21
Upper albumin normal: lower albumin normal	1,18 + 0,09
Upper albumin fever : lower albumin fever	1,00 + 0,13

By labeling the plasma albumin with ^{14}C-tryptophan, a possible increase (0,54± 0,21) in the lower albumin during fever was found(Table 26).This result is, however, seemingly contradicted by the decrease in total albumin during fever (ratio 1,29±0,11) with no significant increase in the upper albumin (ratio 0,97±0,07). These results should thus be viewed with caution.

3.2.2.3 Heat Stress

The effects of heat stress on parameters of blood and interstitial fluid were studied in rats. Heat stress was induced by subjecting the test animals to an environmental temperature of 37°C for a period of three weeks. Blood and interstitial fluid samples were collected before and during the stress period. The experiment was started with 29 test animals of which only four yielded sufficient samples throughout the entire experimental period.

Results of the studies on body fluid parameters during heat stress are reflected in Figures 33 to 37. Body fluid parameters before heat stress were found to be the following: plasma protein concentration: 73,8±14,8 g/l (n=27), interstitial fluid protein concentration: 29,5±8,8 g/l (n=27), plasma A/G ratio: 1,26±0,51 (n=17), haematocrit: 49,6 ±2,9% (n=8). Average body mass before heat stress was found to be 332,7±94,8 g (n=13) with an average rectal temperature of 36,6±1,1°C (n=13).

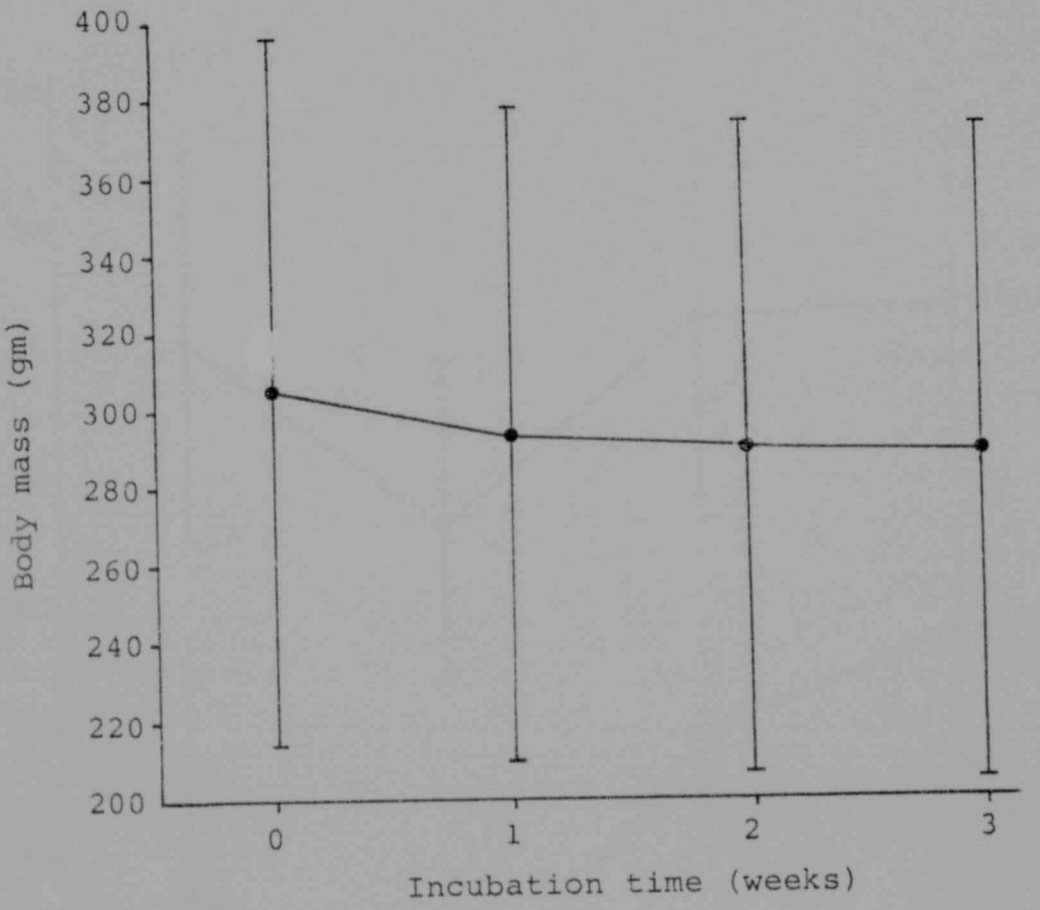


Figure 33

Average body mass of rats before and during a three-week period of heat stress at 37°C. Means ± SD(n=4).

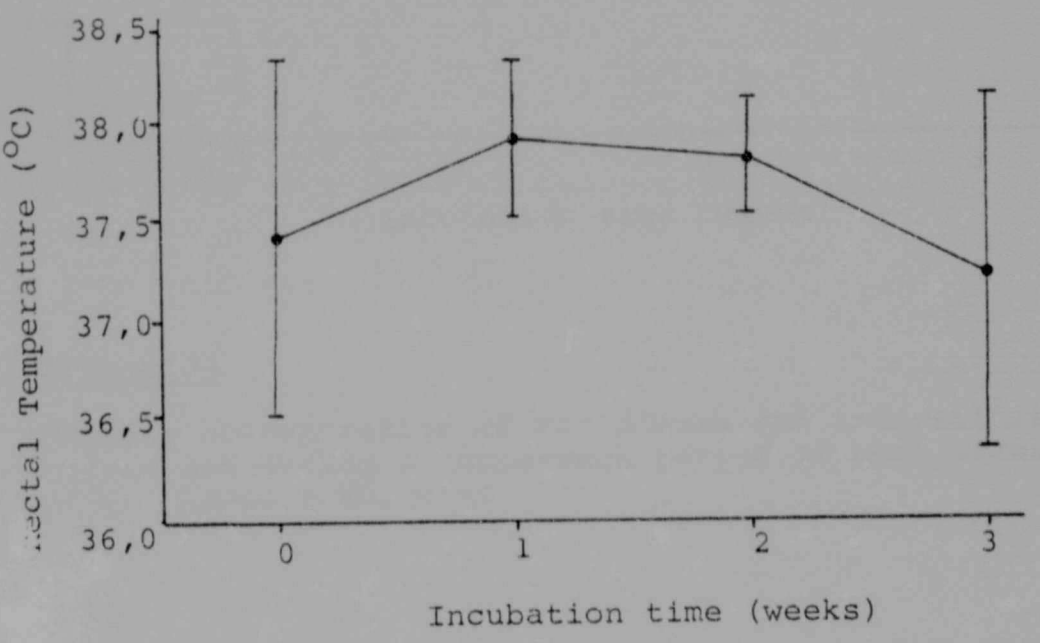


Figure 34

Average rectal temperature of rats before and during a three-week period of heat stress at 37°C. Means ±SD,(n=4).

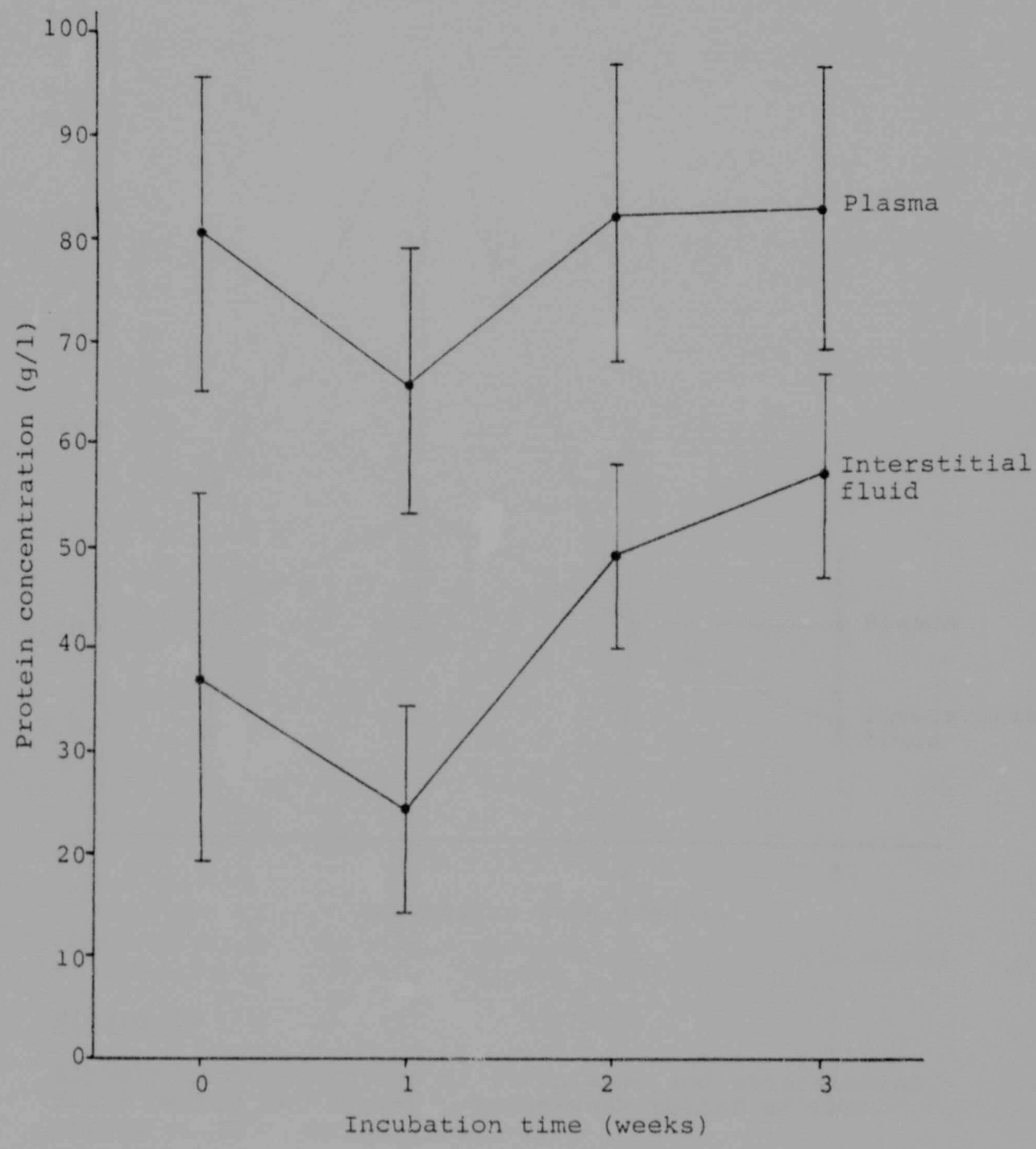


Figure 35

Protein concentration of rat plasma and interstitial fluid before and during a three-week period of heat stress at 37°C. Means ± SD, (n=4).

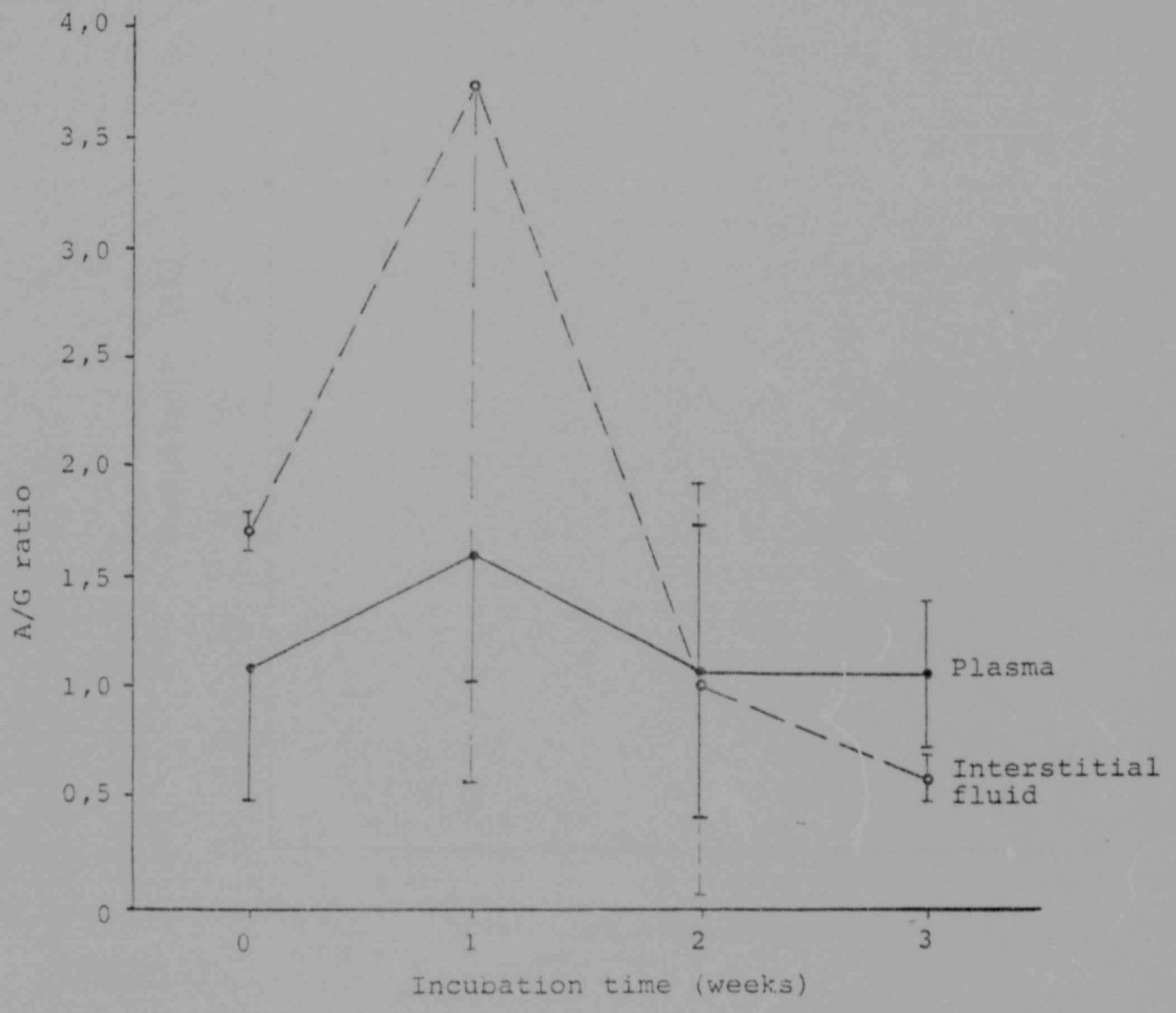


Figure 36

Albumin/globulin ratios of rat plasma and interstitial fluid before and during a three-week period of heat stress at 37°C. Means ±SD, (n=4).

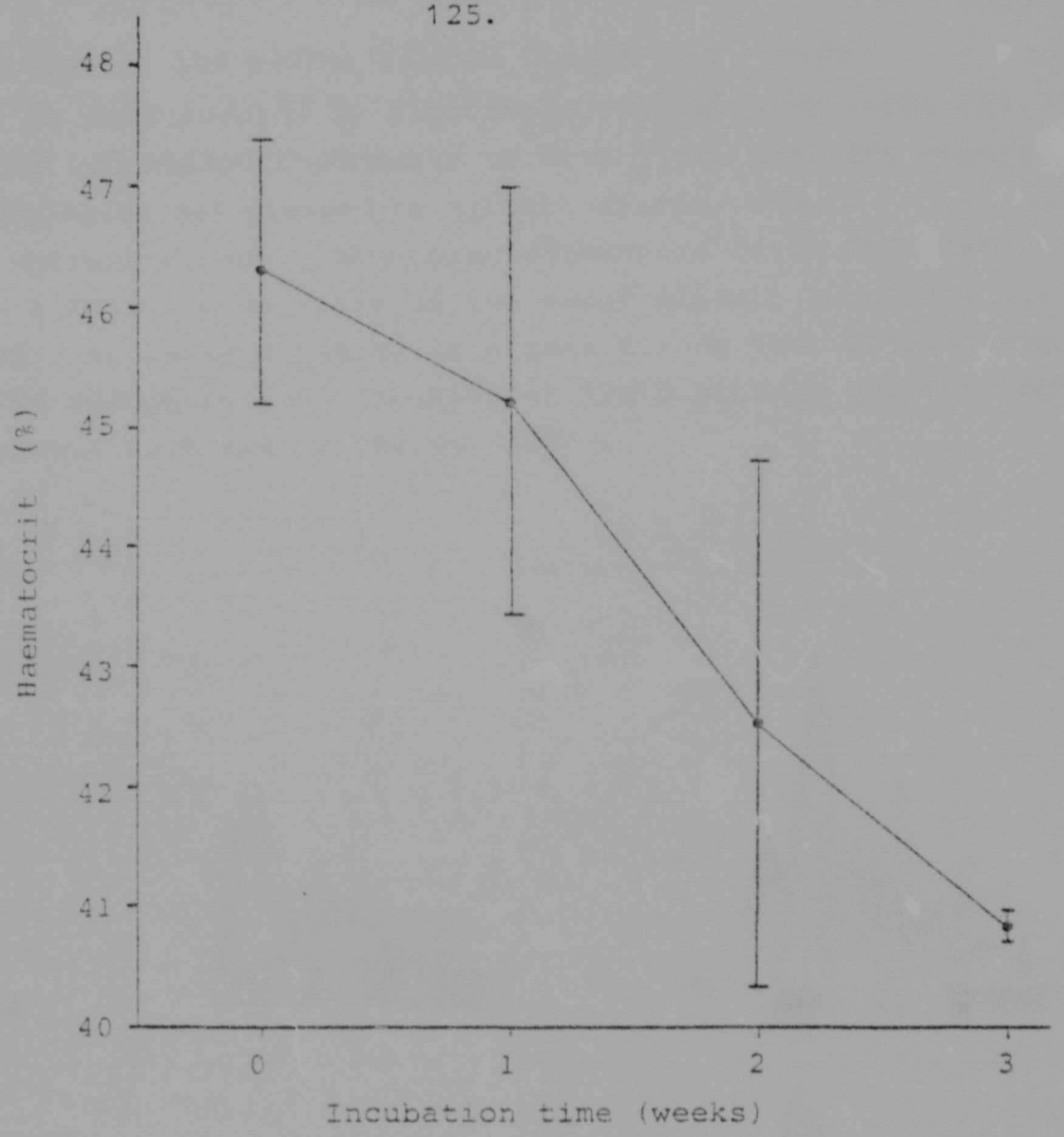


Figure 37

Haematocrit of rats before and during a three-week period of heat stress at 37°C. Means ± SD, (n=4).

From the results in Figs. 33 and 34 it can be seen that no significant change in either body mass or body temperature occurred during the period of heat stress. Neither could any significant changes in plasma protein concentrations be demonstrated during heat stress although a significant increase ($P < 0,05$) in interstitial fluid protein concentration occurred (Fig. 35). Interstitial fluid A/G ratio was found to have significantly decreased, $P < 0,001$ (Fig. 36). Significant changes in plasma A/G ratio could, however, not be established. During the period of heat stress a significant decrease ($p < 0,001$) in haematocrit occurred (Fig. 37), indicating possible haemodilution due to excessive water intake.

No changes in the plasma albumin during heat stress could be demonstrated by electrophoresis on acrylamide gel. Crossed immunoelectrophoresis of body fluid proteins separated on acrylamide gel yielded no albumin change (Fig. 38), whereas crossed immunoelectrophoresis after SAGE has shown a relative decrease of the upper albumin component (or increase in lower albumin) in plasma during heat stress (Fig. 39). No change in the interstitial fluid albumin could be demonstrated with the latter procedure.

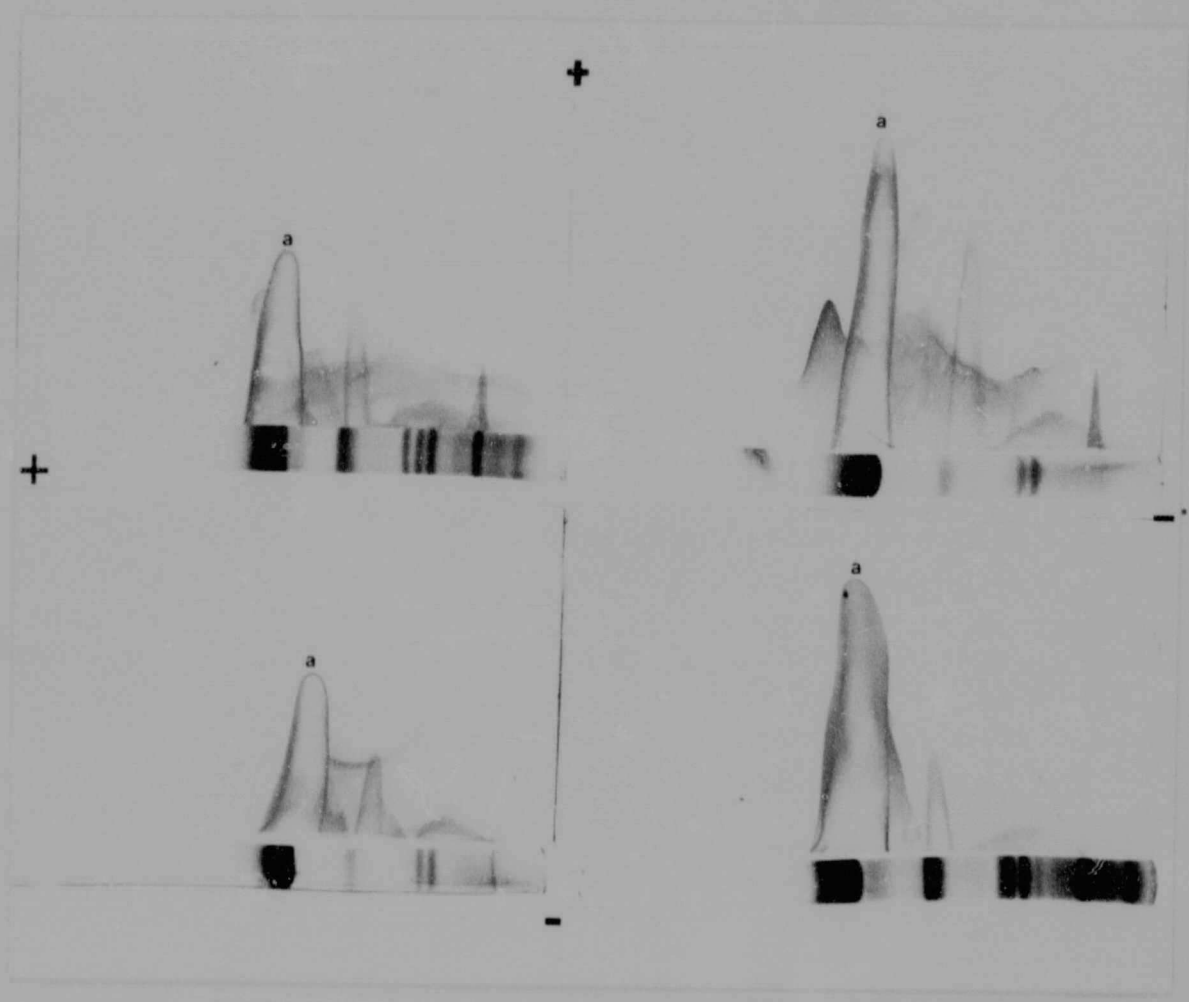


Figure 38

Crossed immunoelectrophoresis of rat plasma (top) and interstitial fluid (bottom) proteins separated on acrylamide gel, before (left) and after (right) a three-week period of heat stress at 37°C. (a) albumin.

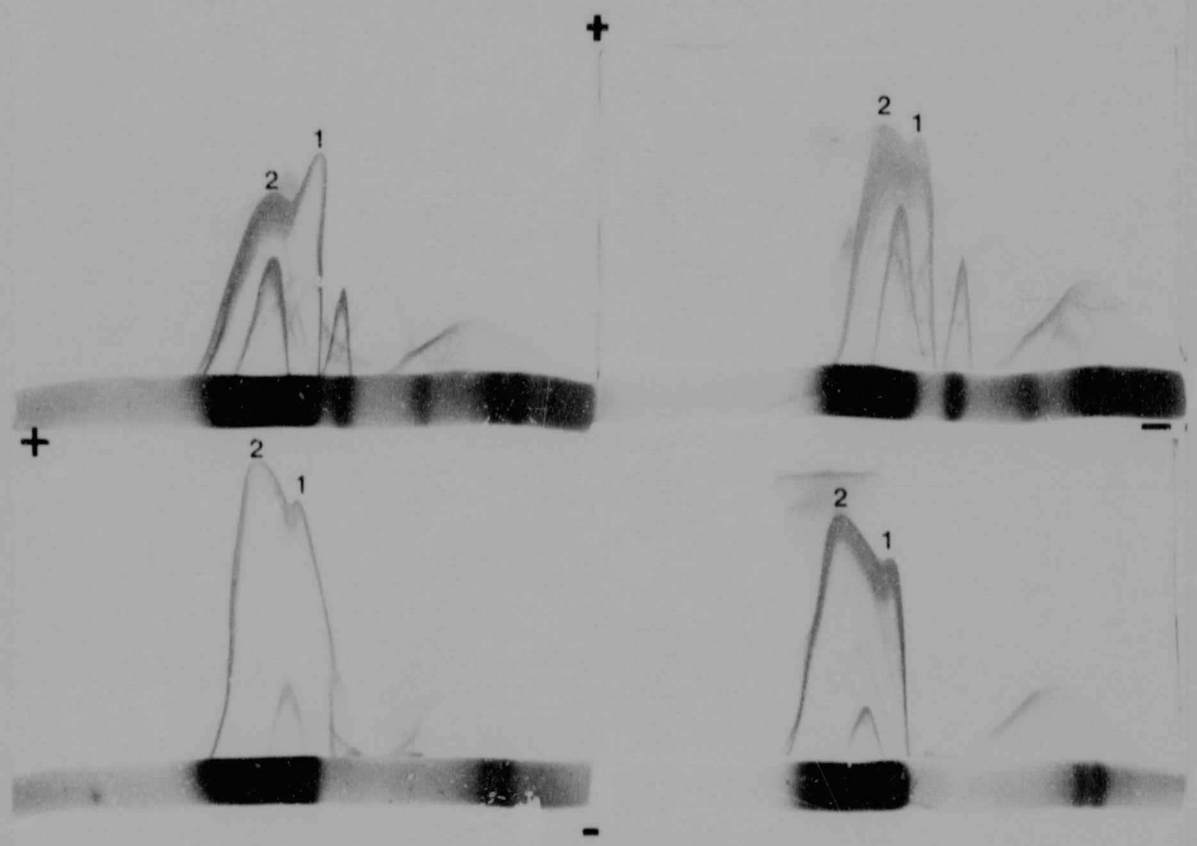


Figure 39

Crossed immunoelectrophoresis of rat plasma (top) and interstitial fluid (bottom) proteins separated on sephadex-acrylamide gel, before (left) and after (right) a three-week period of heat stress at 37°C. (1) upper albumin (2) lower albumin.

Author Coetzee N
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