

CHANGES IN THE PLASMA PROTEIN PATTERN (TISELIUS  
ELECTROPHORETIC TECHNIC) OF PATIENTS WITH  
HYPERTENSION AND DOGS WITH EXPERIMENTAL  
RENAL HYPERTENSION

BY LENA A. LEWIS, Ph.D., AND IRVINE H. PAGE, M.D.

(From the Research Division of the Cleveland Clinic Foundation, Cleveland)

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The modifications which occur in the plasma proteins in association with changes in the arterial blood pressure have been studied only incompletely. Variations have been reported in the plasma fibrinogen level with increases and decreases in the pressure. Goreckzy and Berencsi (1) found an increased plasma fibrinogen in 15 cases of hypertension while Gayet-Hallion, Cuny, and Quivy (2) observed lack of correlation between the level of the arterial pressure and fibrinogen concentration. Chemical methods for estimation of the fibrinogen were used. It was felt that additional information could be obtained by studying the plasma protein pattern by the electrophoresis technic and determining the relative concentration of the albumin, of  $\alpha$ -,  $\beta$ -,  $\gamma$ -globulin, and fibrinogen. The plasma of patients with essential or malignant hypertension was studied and of dogs before, during, and after the development of hypertension following wrapping of the kidneys in silk.

*Methods and Materials*

Total plasma protein was determined by the Pregl modification of the micro-Kjeldahl method (3). Potassium oxalate was the anticoagulant used. The fractionation of the proteins was done by Longsworth's (4) modification of the Tiselius electrophoresis technic. Phosphate buffer, pH 7.8 was employed, ionic strength 0.16.

Electrophoresis studies were made of the plasma of 10 patients with essential hypertension who had no known complications likely to affect the plasma protein distribution. Studies were also made of the plasma of 16 patients with malignant hypertension, classified into 3 groups according to severity of the disease; there were 4 patients in group 1, the mildest, 8 in group 2, and 4 in group 3, the severest. The plasma proteins of 6 dogs were studied before and following the development of hypertension induced by wrapping both kidneys in silk according to the method of Page (5). Three dogs were studied to determine the effect of wrapping of another organ than the kidney, namely, the spleen.

RESULTS

The plasma protein pattern of the 10 patients with uncomplicated essential hypertension showed as a whole only slight deviations from the normal range (Table I). Three of the 10 showed  $\alpha$ -globulin below normal both in terms of grams per 100 ml. and in percentage of total protein. Three showed slightly low  $\gamma$ -globulin in terms of grams per 100 ml., but in terms of percentage of the

total were within the normal range. The normal values obtained in this laboratory have been published in detail previously. The same conditions as employed in these studies were used (7).

In contrast with the minimal changes found in the plasma protein pattern in essential hypertension, alterations were great (Table II) in the protein pattern of patients with relatively severe malignant hypertension (groups 2, 3) (Fig. 1). The patients in group 1 showed less extensive alterations.

TABLE I  
Plasma Protein Pattern of Patients with Essential Hypertension

No.	Blood pressure day of protein study	Total protein	Albumin		$\alpha$ -globulin		$\beta$ -globulin		$\gamma$ -globulin		$\phi$	Mobilities, $\mu \times 10^6$ cm. <sup>2</sup> sec. <sup>-1</sup> volt <sup>-1</sup>							
														A	$\alpha$	$\beta$	$\phi$	$\gamma$	Dilution of plasma
			gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent		gm./100 ml.	per cent	mm. Hg	gm./100 ml.	per cent	gm./100 ml.	per cent	
1	180/110	5.89	3.65	62.0	0.39	6.7	0.91	15.3	0.55	9.4	0.39	6.6	6.9	4.7	3.7	2.5	1.0	1:3	
2	240/130	6.37	4.02	63.0	0.41	6.5	0.95	14.8	0.63	10.0	0.36	5.7	6.8	4.8	3.6	2.9	1.2	1:3	
3	226/136	5.84	3.78	64.5	0.33	5.7	0.94	16.2	0.55	9.5	0.24	4.1						1:3	
4	190/140	6.84	4.28	62.5	0.46	6.7	0.97	14.3	0.71	10.4	0.42	6.1	6.9	4.8	3.4	2.2	1.0	1:4	
5	160/100	6.47	3.67	56.7	0.45	7.0	1.10	17.1	0.80	12.3	0.45	6.9	7.0	4.8	3.4	2.0	1.1	1:4	
6		6.42	4.20	65.5	0.41	6.3	0.77	12.0	0.72	11.2	0.32	5.0	6.8	4.7	3.7	2.2	1.0	1:4	
7		6.27	4.16	66.4	0.32	5.0	0.80	12.8	0.70	11.2	0.29	4.6	6.8	4.9	3.7	2.7	1.2	1:3	
8	200/115	6.17	3.73	60.6	0.38	6.1	0.88	14.2	0.65	10.5	0.53	8.6	7.1	5.4	3.9	2.9	1.4	1:3	
9		6.64	4.15	62.5	0.49	7.4	0.62	9.3	0.87	13.1	0.51	7.7	6.9	4.8	3.5	2.7	1.1	1:4	
10		6.42	3.90	60.7	0.46	7.1	0.97	15.1	0.71	11.1	0.38	6.0	7.2	5.0	3.8	2.6	1.0	1:4	
Normal.....		6.51	4.09	62.7	0.47	7.2	0.81	13.1	0.77	11.7	0.33	5.4							
(Average of 25, range).		5.94-7.82	3.72-5.11	60.1-67.2	0.39-0.66	6.0-8.7	0.65-1.07	11.0-15.9	0.60-0.91	8.6-14.8	0.16-0.48	2.8-7.2							

No single fraction was consistently altered, but each of the 4 in group 1 showed values outside the normal range in more than one fraction.

The changes in group 2 were much larger than in the first group. In many instances none of the protein fractions was present in normal concentration. Two of the four studies in group 3 were particularly interesting as the electrophoresis pattern showed separation of  $\alpha_1$ -globulin from the albumin. This has never been observed in normal human plasma when phosphate buffer pH 7.8 is employed, as used in these studies.

The plasma albumin was decreased in 12 patients and fibrinogen was elevated beyond the normal range in 14 of the 16 patients with malignant hypertension.

Ten of 12 patients with grade 2 and 3 malignant hypertension showed in-

creased  $\beta$ -globulin. The  $\alpha_2$ -globulin was greatly elevated in some instances while in others in the same group abnormally low values were observed.

The changes observed in the plasma protein patterns of the dogs during the

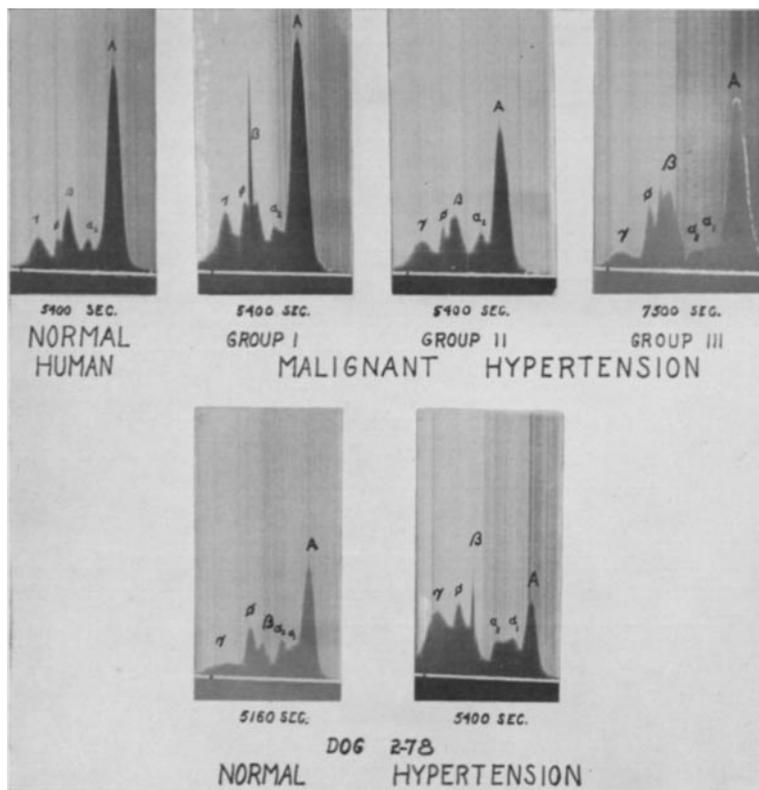


FIG. 1. Electrophoretic patterns of normal human plasma and of plasma of patients with malignant hypertension, and of normal and hypertensive dog plasma. Phosphate buffer pH 7.8, ionic strength 0.16, 0.0044 mho at 2°C. current 22 ma., time indicated under each picture.

development of hypertension are summarized in Tables III and IV. Increase in  $\gamma$ -globulin was a consistent finding. In 3 of the 6 dogs, elevation in  $\beta$ -globulin and fibrinogen level was also observed. No consistent changes in the  $\alpha_1$ - or  $\alpha_2$ -globulin occurred.

The plasma protein pattern in 2 of the 3 dogs studied whose spleens were wrapped with silk showed no significant alterations from the normal. The third dog showed some increase in  $\gamma$ -globulin and fibrinogen. This may have been due to an extensive infection of the lower jaw which developed during the period of study.

TABLE II  
Plasma Protein Pattern of Patients with Malignant Hypertension

No.	Group	Total protein		Albumin		$\alpha_2$ -globulin		$\beta$ -globulin		$\gamma$ -globulin		$\phi$		Mobilities, $\mu \times 10^8 \text{ cm.}^2 \text{ sec.}^{-1} \text{ volt}^{-1}$						
		gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	A	$\alpha_1$	$\alpha_2$	$\beta$	$\phi$	$\gamma$	Dilution of plasma
1	1	5.64	3.63	64.3	0.25	4.4	0.70	12.5	0.56	10.0	8.8	7.3			5.1	3.8	2.3	1.3	1.3	1:3
2	1	5.36	3.32	61.9	0.27	5.0	0.80	14.9	0.70	13.1	5.1	6.9			4.6	3.3	2.3	0.9	1:3	1:3
3	1	7.59	4.23	55.7	0.59	7.8	1.31	17.3	0.85	11.2	8.0	6.8			4.9	3.5	2.3	0.9	1:4	1:4
4	1	5.89	2.92	49.5	0.32	5.5	0.80	13.5	1.19	20.2	11.3	7.0			5.3	4.1	3.0	1.2	1:3	1:3
5	2	7.00	3.58	51.2	0.93	13.3	1.08	15.4	0.77	11.0	9.1	6.9			5.5	3.9	2.6	1.5	1:4	1:4
6	2	7.90	4.29	54.4	0.35	4.4	1.58	20.0	1.13	14.3	6.9	7.1			5.1	3.6	2.7	1.2	1:4	1:4
7	2	6.85	2.92	42.6	0.71	10.3	1.27	18.5	1.03	15.1	0.92	6.4			5.1	3.6	2.0	0.9	1:4	1:4
8	2	6.60	3.26	49.4	0.70	10.6	1.20	18.2	0.94	14.2	7.6	6.8			5.1	3.9	2.7	1.2	1:4	1:4
9	2	6.88	3.69	53.7	0.53	7.7	1.46	21.9	0.65	9.4	8.0	6.6			4.9	3.4	2.4	1.0	1:3	1:3
10	2	8.14	4.41	54.2	0.89	10.9	0.89	10.9	0.97	11.9	12.1	7.1			5.1	3.5	2.8	1.2	1:3	1:3
11	2	6.50	2.05	31.4	1.44	22.2	1.07	16.5	1.07	16.5	13.4	6.7			4.9	3.3	2.7	1.1	1:4	1:4
12	2	7.06	3.44	48.7	0.80	11.3	1.32	18.8	0.82	11.6	9.6	7.0			4.7	3.3	2.3	1.4	1:4	1:4
13	3	6.39	3.40	53.2	(0.24 gm.) $\alpha_1$ (3.7 per cent)	10.4	1.08	16.9	0.92	14.5	9.2	7.4	6.0		5.2	4.3	3.0	1.7	1:3	1:3
14	3	5.92	2.75	46.4	(0.29 gm.) $\alpha_2$ (4.5 per cent)	8.1	1.55	26.2	0.41	6.9	11.2	7.1	5.6		4.8	3.7	2.4	1.0	1:3	1:3
15	3	6.95	3.64	52.4	(0.33 gm.) $\alpha_1$ (5.5 per cent)	10.4	1.20	17.3	0.88	12.6	9.6	7.3			5.6	3.8	3.0	1.1	1:3	1:3
16	3	7.65	3.89	50.9	8.1	10.4	1.51	19.7	0.93	12.2	6.8	6.8			4.7	3.6	2.7	1.2	1:4	1:4

TABLE III  
Plasma Protein Pattern of Dogs before and during Hypertension

Dog No.	Blood pressure mm. Hg	Total protein		Albumin		α <sub>1</sub> -globulin		α <sub>2</sub> -globulin		β-globulin		γ-globulin		φ		Mobilities, μ × 10 <sup>4</sup> cm. <sup>2</sup> sec. <sup>-1</sup> volt <sup>-1</sup>				
		gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	A	α <sub>1</sub>	α <sub>2</sub>	β	γ
3-41	Normal	5.47	2.11	38.5	0.74	13.5	0.22	4.1	0.82	15.0	0.70	12.9	0.88	16.0	7.6	35.2	3.6	2.7	1.1	1:3
	176	7.34	2.33	31.8	0.68	9.2	0.76	10.3	1.28	17.5	1.32	17.9	0.97	13.3	7.9	35.6	3.8	2.8	1.1	1:4
	198	7.12	2.14	30.1	0.68	9.5	0.58	8.1	1.11	15.6	1.34	18.8	1.27	17.9	7.8	35.3	3.8	2.7	1.0	1:4
2-78	Normal	5.03	2.08	41.2	0.47	9.4	0.63	12.6	0.54	10.7	0.54	10.7	0.77	15.4	7.6	45.4	3.8	2.8	0.9	1:3
	210	7.28	1.73	23.7	0.73	10.0	0.29	4.0	0.98	13.5	2.18	29.9	1.38	18.9	7.9	55.4	3.8	2.7	0.9	1:3
2-54	136(normal)	5.45	2.32	42.4	0.46	8.4	0.55	10.0	0.80	14.8	0.52	9.6	0.80	14.8	7.6	45.7	3.9	2.9	1.0	1:3
	160	5.86	1.76	30.1	0.52	8.8	1.01	17.2	1.01	17.2	0.74	12.6	0.82	14.1	7.6	45.6	3.9	2.8	1.1	1:3
	190	6.36	2.22	35.0	0.65	10.2	0.76	11.9	0.80	12.6	1.03	16.2	0.90	14.1	7.6	35.6	3.8	2.8	1.0	1:3
3-58	140(normal)	4.81	2.13	44.3	0.44	9.1	0.45	9.3	0.54	11.3	0.42	8.8	0.83	17.2	7.6	45.4	3.9	2.9	0.9	1:3
	174	5.53	1.78	32.2	0.64	11.5	0.74	13.3	0.77	13.8	1.07	19.3	0.55	9.9	7.6	35.6	4.1	3.0	0.9	1:3
3-05	136(normal)	5.89	2.65	45.0	0.66	11.3	0.39	6.6	0.86	14.6	0.55	9.3	0.78	13.2	7.6	35.7	4.1	2.5	1.0	1:3
	220	7.15	1.27	17.8	0.49	6.8	0.71	9.9	1.44	20.2	1.61	22.5	1.63	22.8	7.6	45.4	3.9	2.4	1.0	1:3
4-03	140(normal)	5.76	2.36	41.0	0.55	9.6	0.67	11.6	0.96	16.6	0.55	9.6	0.67	11.6	7.6	45.3	3.8	3.0	1.1	1:3
	170	5.72	1.93	33.6	0.69	10.9	0.91	16.1	0.77	13.4	0.84	14.7	0.58	10.2	7.6	25.3	4.0	2.9	1.0	1:3
	230	6.51	2.18	33.5	0.52	8.0	0.63	9.7	0.84	12.9	1.41	21.7	0.92	14.2	7.6	25.4	3.8	2.9	1.1	1:3

TABLE IV  
*Blood Pressure and Plasma Protein Studies on Dog 3-41 before, during, and following the Development of Hypertension*

Date	Arterial blood pressure mm. Hg	Total protein		Albumin		$\alpha_1$ -globulin		$\alpha_2$ -globulin		$\beta$ -globulin		$\gamma$ -globulin		$\phi$		Mobilities, $\mu \times 10^4 \text{ cm}^2 \text{ sec}^{-1} \text{ volt}^{-1}$				
		gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	gm./100 ml.	per cent	A	$\alpha_1$	$\alpha_2$	$\beta$	$\gamma$
5-28-46	Normal,*	5.47	2.11	38.5	0.74	13.5	0.22	4.1	0.82	15.0	0.70	12.9	0.88	16.0	7.6	6.3	5.2	3.6	2.7	1.1
6-6-46	pre-op																			
6-7-46	170																			
6-14-46	167																			
6-14-46	176																			
6-19-46	170																			
6-25-46	176	7.34	2.33	31.8	0.68	9.2	0.76	10.3	1.28	17.5	1.32	17.9	0.97	13.3	7.9	6.3	5.6	3.8	2.8	1.1
7-5-46	196																			
7-11-46	188																			
7-12-46	198	7.12	2.14	30.1	0.68	9.5	0.58	8.1	1.11	15.6	1.34	18.8	1.27	17.9	7.8	6.3	5.5	3.8	2.7	1.0
7-16-46	214																			
7-19-46	220																			
7-24-46	208	5.65	1.74	30.8	0.37	6.6	0.60	11.7	0.84	14.9	1.06	18.7	0.98	17.3	7.7	6.5	5.3	3.7	2.6	1.1
7-25-46	204																			
7-25-46	202																			

\*Normal—before operation (wrapping of kidneys with silk).

## DISCUSSION

The finding of no significant change from normal in the plasma fibrinogen in essential hypertension would indicate that no direct relationship existed between blood pressure level and fibrinogen concentration. The fact that the  $\alpha_2$ -globulin level in the patients with essential hypertension was in the lower normal range or actually decreased is interesting, because of the finding of Plentl, Page, and Davis (6) that renin substrate has the same electrophoretic mobility as  $\alpha_2$ -globulin.

Other diseases in which a low plasma  $\alpha_2$ -globulin has been observed are untreated myxedema (7) and hypoadrenal activity of pituitary origin (8). In both of these a low arterial blood pressure is present.

The finding of a greatly elevated  $\beta$ -globulin in many of the patients with malignant hypertension suggests that a modified  $\beta$ -globulin level may be an important change coexistent with vascular disease. Among the conditions in which an elevated  $\beta$ -globulin level is found are: the nephrotic phase of Bright's disease, terminal glomerular nephritis (9), diabetes (10) when uncontrolled, and in controlled patients who have diabetic retinitis and frequently also, increased capillary fragility.

The increased  $\beta$ -globulin was observed consistently in diabetic patients with progressing diabetic retinitis. In some of these cases, marked improvement in visual acuity and in the eyegrounds occurred following ingestion of a high protein diet and satisfactory diabetic control for long periods. Simultaneously, the plasma albumin level approached normal levels and in some cases the  $\beta$ -globulin decreased to normal. In the patients who failed to show improvement in the eye grounds as a result of therapy, no shift toward normal levels was observed in the plasma protein pattern.

The partial separation of  $\alpha_1$ -globulin from albumin during electrophoresis of human plasma, when phosphate buffer pH 7.8 is employed, is observed very infrequently. It has never been encountered in normal plasma. In the last 750 electrophoresis studies carried out on human plasma at the Cleveland Clinic, 14 showed this phenomenon. In the group of patients were 2 with malignant hypertension, 3 with glomerulonephritis, 3 with cirrhosis of the liver, 2 with avitaminosis, 3 with lupus erythematosus, and 1 with congestive heart failure. In all these the plasma protein pattern was markedly abnormal.

The fact that little change was observed in the plasma protein pattern of the dogs whose spleens were wrapped with silk indicates that production of scar tissue induced by foreign irritants in the abdomen is not in itself cause for the increased  $\gamma$ -globulin observed in the hypertensive animals. There was no direct correlation in all cases between the blood pressure and the  $\gamma$ -globulin level in the animals with hypertension, however. In one example (dog 3-41) the  $\gamma$ -globulin level was markedly increased when the blood pressure was only

moderately elevated and failed to rise much higher when the pressure rose from 176 mm. Hg to 198 mm. Hg. It is interesting that the one dog showing marked increase in  $\beta$ -globulin, both in grams per 100 ml. and in percentage of total protein, was the animal exhibiting the malignant syndrome as evidenced by papilledema and detachment of the retinas. Further investigation of this phase of the problem is in progress.

#### SUMMARY

The plasma protein pattern of patients with uncomplicated essential hypertension showed only slight variations from the normal while that of patients with severely malignant hypertension showed marked shifts. The fibrinogen and  $\beta$ -globulins were usually elevated beyond the normal range and the albumin decreased. In less severely malignant hypertension, the changes were less marked.

In dogs with experimental renal hypertension, the  $\gamma$ -globulin level was greatly elevated, and in one animal exhibiting the malignant syndrome  $\beta$ -globulin and fibrinogen were also increased.

Elevation of  $\beta$ -globulin seems in some manner associated with the occurrence of severe vascular disease.

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