and Mrs. Kazuko Tateoka for her assistance in carrying out the experiment.

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Table. IV The MG values of serum proteins in various conditions of paper-electrophoresis in barbital buffer of pH 8.5 (μ=0.045). suspd =suspended, clampd=clamped between glass plates

	albumine	α-globuline		β-globuline		γ-globuline
		α_1	α_2	β_1	β_2	
MG	1.00	0.80	0.57	0.37	0.35	0.25

Table. V The MG of serum proteins in barbital buffer of pH 8.5. (μ=0.045)

Thus the MG values of serum proteins in this definition are the constants regardless of voltage, current, time of electrophoresis and the dropped points of filterpaper.

SUMMARY

The results obtained by a simplified procedure are described in which disturbing factors such as evaporation, heating and buffer concentration gradient were reduced to minimum. The polysaccharide dextran was used as an index of the extent of electroosmotic flow during the course of experiment. A linear relationship was found between the net migration distance of arginine, lysine, histidine, glutamic acid and aspartic acid and the time. The mobilities of amino acids in filterpaper changed their own values as the function of pH in phosphate buffer of constant ionic strength. The MG values of amino acids were found to be the constants regardless of the ionic strength of surrounding buffer. An attempt was made to determine the migration values, the apparent migration distance from original point, the distance of static points from origin and the initial velocity of migrants by the experimental formula obtained in the common apparatus in which the evaporation from paper surface was admitted. In other experiment we defined the distance ratio of serum protein from γ-globuline to that of serum albumine as the MG value, which was the constant regardless of voltage, current, time of electrophoresis and the dropped points of filterpaper.

ACKNOWLEDGEMENT

The authors are indebted to Ass. Prof. Tanaka for some valuable advice and to Prof. Ôbô of the laboratory of biochemistry for supplying dextran for this study. Their thanks are also due to Mr. Yasuo Chûman for the supply of human serum

Table. III The initial velocity of serum proteins, (A) dropped on the point at the distance of 10.0 cm from the center of filterpaper, 4 hours (B) dropped on the center of filterpaper, 3 hours (C) obtained by the method of Tiselius.[4] in barbital buffer of pH 8.5 (μ=0.045)

6) The MG Values of Serum Proteins

As in the case of amino acids[1,2,3] we defined the MG values of serum proteins as follows.

$$MG = \frac{\text{the migration distance of other proteins from } \gamma\text{-globuline}}{\text{the migration distance of albumine from } \gamma\text{-globuline.}}$$

From the purpose of measuring the MG of serum proteins, 1/100 ml of the normal human serum and the serum of a patient with hepatitis were separated in barbital buffer of pH 8.5[5] (μ=0.045) in various conditions of paper-electrophoresis. The results are shown in Table. IV and V.

albumine	α-globuline		β-globuline		γ-globuline	condition
	α_1	α_2	β_1	β_2		
1.00	0.83	0.60	0.41		0	0.46 mA/cm 7.0 V/cm 8 hrs, suspd
1.00	0.82	0.58	0.36		0	〃
1.00	0.77	0.57	0.35		0	〃
1.00	?	0.52	0.32		0	0.38 mA/cm 7.0 V/cm 2 hrs, suspd
1.00	0.82	0.55	0.36		0	0.45 mA/cm 7.0 V/cm 4 hrs, suspd
1.00	0.86	0.62	0.42		0	0.55 mA/cm 7.0 V/cm 6 hrs, suspd
1.00			0.32	0.25	0	0.5 mA/cm 7.0 V/cm 8 hrs, suspd
1.00			0.37	0.30	0	〃
1.00			0.39	0.20	0	〃
1.00	0.77	0.57	0.34		0	0.25 mA/cm 7.0 V/cm clampd
1.00	0.76	0.52	0.35	0.25	0	0.3 mA/cm 7.0 V/cm clampd
1.00	0.75	0.58	0.37		0	0.8 mA/cm 14.0 V/cm 3 hrs, suspd
1.00	0.76	0.60	0.34		0	0.8 mA/cm 14.0 V/cm 2 hrs, suspd
1.00	?	?	0.29		0	0.8 mA/cm 14.0 V/cm 1 hr, suspd

(A)

		MD'				*MD'*s		
		2 hrs	4 hrs	6 hrs	8 hrs	0~2hrs	4~6hrs	6~8hrs
albumine	calcd	−55	−93	−120	−140	−183	−183	−183
	found	−55	−88	−115	−127	−183	−170	−175
α_1-globuline	calcd	(−46)	−78	−101	−116	−153	−153	−153
	found	?	−78	− 98	−110	?	?	?
α_2-globuline	calcd	−35	−60	− 77	− 89	−117	−117	−117
	found	−33	−60	− 75	− 93	−112	−130	−115
β-globuline	calcd	(−30)	−52	− 66	− 76	−100	−100	−100
	found	?	−52	− 60	− 72	?	?	?
γ-globuline	calcd	−18	−31	− 39	− 46	− 60	− 60	− 60
	found	−18	−30	− 38	− 42	− 65	− 60	− 57

(B)

		MD'				*MD'*s	
		1.5 hrs	3 hrs	4.5 hrs	6 hrs	0~1.5 hrs	4.5~6 hrs
albumin	calcd	−22	−39	−51	−61	−93	−93
	found	−22	−37	−50	−60	−93	−88
α_1-globuline	calcd	(−16)	−27	−37	−44	−66	−66
	found	?	−27	−34	−48	?	?
α_2-globuline	calcd	−10	−17	−23	−28	−42	−42
	found	− 9	−17	−20	−25	−45	−45
β-globuline	calcd	− 3	− 5	− 7	− 8	−12	−12
	found	?	− 5	− 6	− 6	?	?
γ-globuline	calcd	+ 7	+12	+16	+19	+29	+29
	found	+ 7	+11	+14	+14	+25	+25

Table. II The MD' and MD'S of serum proteins, (mm) (A) dropped on the point at the distance of 10.0 cm from the center in the cathodic side of filterpaper, $a'/a=0.70$, (B) dropped at the center of filterpapar, $a'/a=0.76$, in barbital buffer of pH 8.5 ($\mu=0.045$)

sec^{-1}) by the following formula.

$$V_0 = \frac{(MD'_1 - MD'_{dex})\log(a'/a)}{a'/a-1} E \cdot t \ cm^2 \cdot volt^{-1} \cdot sec^{-1}, \cdots (10)$$

where V_0 the initial velocity of serum protein, MD_1' the apparent migration distance at the time of t_1 (three or four hours), MD'_{dex} the apparent migration distance of dextran at the same time, a'/a the decrement ratio of mobility of serum protein at the interval of t, E the voltage (V/cm) and t the time of electrophoresis (sec). The V_0 of serum proteins obtained by the formula (10) agreed closely with those obtained by the method of Tiselius.[4] (Table III)

	albumine	α_1-globuline	α_2-globuline	β-globuline	γ-globuline
(A) $V_0\times10^5$	8.8	7.5	5.2	4.1	1.3
(B) $V_0\times10^5$	8.9	7.3	5.7	3.7	1.1
(C) $U\times10^5$	8.9	7.7	5.8	3.8	1.5

the method of dropping amino acids on the several points of filterpaper.

The difference between the values of MD'_s, (A) and (B) is perhaps due to the increase of osmotic flow of buffer into filterpaper during the course of experiment. Several experiments were carried out with the following results: the experimental values of MD' of four hours showed 85% (76~109%) of those by calculation, MD'_s (B) 75% (66~86%) and other values, MD' in three hours MD'_s (A) and V_0 agreed closely. The above formulas are applicable to the migration of amino acids on the leveled filterpaper, but not to the filterpaper folded double.[10]

5) The Determination of Migration Distance of Serum Proteins

1/100 ml of normal human serum was dropped on the center of filterpaper or on the point at the distance of 10.0 cm from the center in the cathodic side and the migration distance at a definite interval and the distance of the static points of serum proteins from their original points were measured. As in the case of amino acids the MD' and MD'_s were calculated by the following formulas,

$$MD' = MD'_1 \frac{(a'/a)^n - 1}{a'/a - 1} \quad \cdots\cdots (6)'$$

$$MD'_s = \frac{-MD'_1}{a'/a - 1} \quad \cdots\cdots (7)'$$

in which MD' is the migration distance of serum proteins from the original point at the time of t after the beginning of electrophoresis, MD'_1 the migration distance at the time of t_1, MD'_s the distance of the static points of serum proteins from the original point, n the ratio t/t_1 and a'/a the decrement ratio of mobilities at the interval of t_1. In this experiment, a'/a was determined from the experimental values, MD'_1 and MD'_s at the time of t by the following formula.

$$\frac{a'}{a} = 1 - \frac{MD_1}{MD'_s} \quad \cdots\cdots (9)$$

Table. II shows the MD' and MD'_s of serum proteins.

It will be seen from Table. II that the experimental values agree closely with those by calculation. In these experiments we also measured the net migration distance of serum proteins and calculated the initial velocity of migration ($cm^2 \cdot volt^{-1} \cdot$

$$V'_0 = \frac{MD'_1 \cdot \log(a'/a)}{a'/a - 1} \quad \cdots\cdots(5)$$

Substituting in (4), we have

$$MD' = MD'_1 \frac{(a'/a)^n - 1}{a'/a - 1} \quad \cdots\cdots(6)$$

When t becomes ∞, the migration distance is expressed by

$$MD'_s = \frac{-MD'_1}{a'/a - 1} \quad \cdots\cdots(7)$$

in which MD'_s shows the distance of the static points of amino acids from original point. The net initial velocity of amino acids V_0 is obtained from (5),

$$V_0 = \frac{(MD'_1 \pm MD'_{dex}) \cdot \log(a'/a)}{a'/a - 1}, \quad \cdots\cdots(8)$$

where MD'_{dex} is the distance of dextran from original point at the time of t_1. The V_0 theoretically accords with the net migration distance MD in the formula (1) at the time of t_1.

In the experiment, a'/a is determined by measuring the distance of amino acids from original point two times at the interval of t_1. In general the value falls in the range from 0.7~0.8, when t is one hour. Table. I shows both these values by the experimental determination and the calculation from above-mentioned formulas (6), (7) and (8). In this table the experimental values of V_0 (mm/hour) were obtained by the method of formula (1) taking one hour as t_1, and those of MD'_s (A) and (B) were respectively measured on the filterpaper at one and six hours after the beginning of electrophoresis by

amino acid	method	MD' 1 hr	MD' 2 hrs	MD' 3 hrs	MD' 4 hrs	MD'S (A)	MD'S (B)	V_0	a'/a
glycine	calcd	10	17	23	27	38		− 2	0.74
	found	10	18	24	28	35	30	− 2	0.80
aspartic acid	calcd	−25	−44	−57	−67	−96		−44	0.74
	found	−25	−43	−51	−59	−81	−69	−41	0.72
glutamic acid	calcd	−18	−31	−41	−48	−70		−32	0.74
	found	−18	−30	−38	−44	−59	−50	−29	0.66
histidine	calcd	14	24	32	37	54		5	0.74
	found	14	24	30	34	−51	40	6	0.71
arginine lysine	calcd	33	57	75	84	126		27	0.74
	found	33	60	68	70	112	89	31	0.81

Table. I The apparent migration distance, the distance of static points from origin and the initial velocity of amino acids by the experiment and calculation. (mm) The experimental values were obtained in phosphate buffer of pH 7.38, 7.0 V/cm, 0.7 mA/cm.

Fig VII

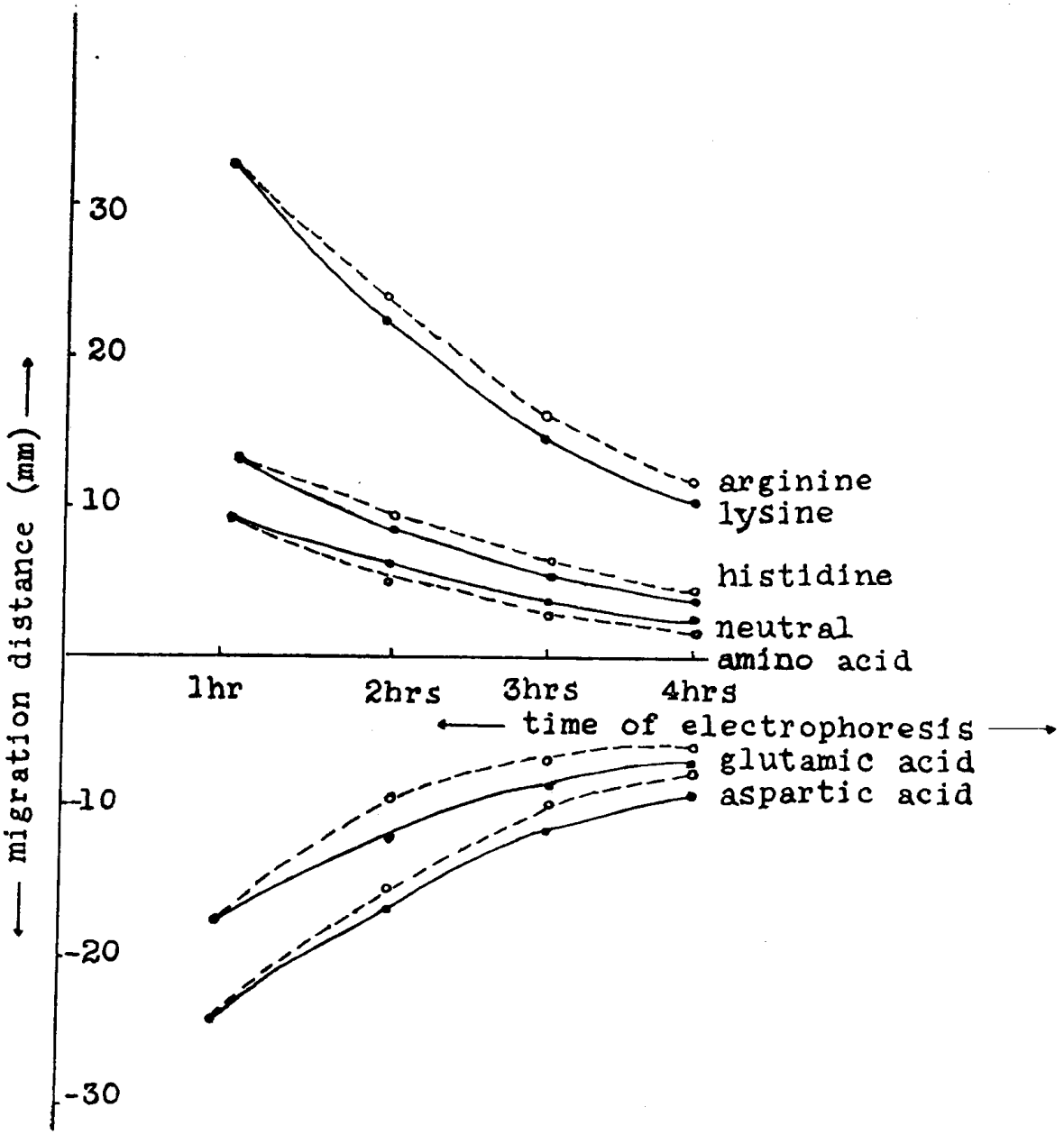

Fig. VII The curves of the mobilities of amino acids as the function of time, in phosphate buffer of pH 7.38, 6.9 V/cm, 0.7 mA/cm, dotted lines show the logarithmic decrement.

so that we have

$$MD' = \frac{V'_0}{\log(a'/a)}\{(a'/a)^n - 1\}. \quad \cdots\cdots(4)$$

The apparent migration distance at the time of t after the beginning of electrophoresis, MD'_1 is expressed by

$$MD'_1 = \frac{V'_0}{\log(a'/a)}(a'/a - 1),$$

so that V'_0 is

Fig VI

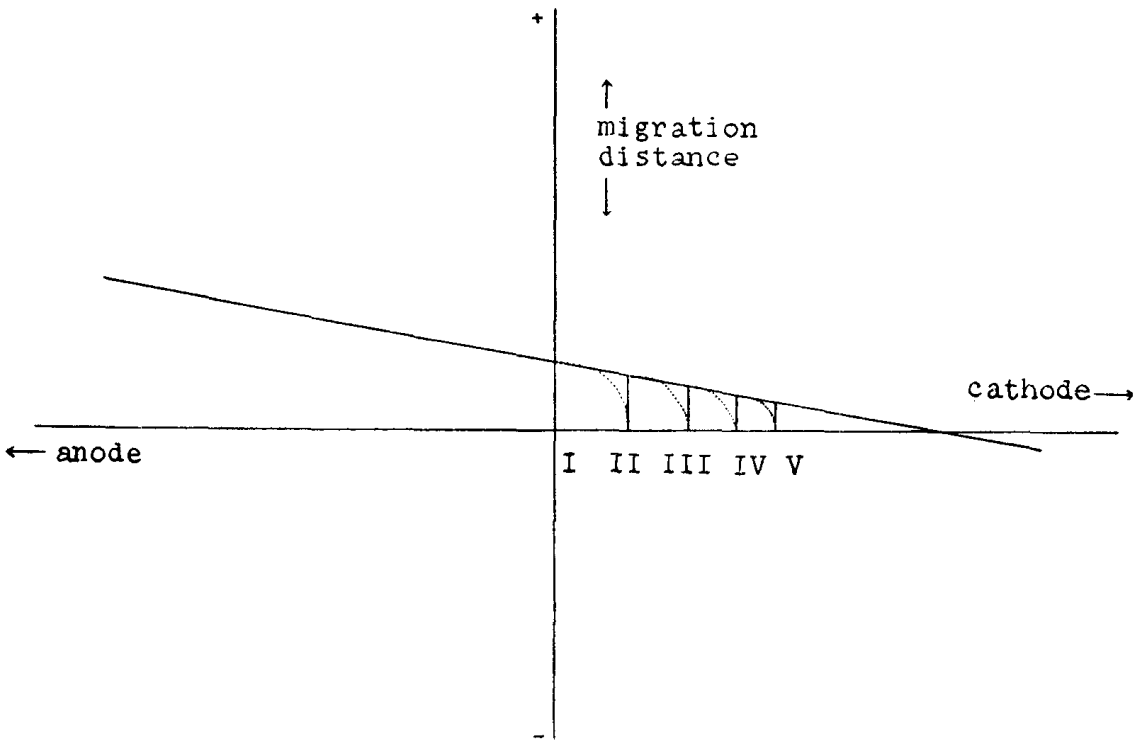

Fig. VI The decrement of mobility of migrant at a definite interval. The migration distance, I, II, III, IV, and V show the logarithmic decrement.

paper was permitted were shown in Fig. VII. It will be seen from Fig.VII that the curves obtained by the experiment differ only slightly from those of logarthimic decrement.

If this assumption is valid, then the apparent migration distance MD' at the time of t is approximately to be calculated by the following formula,

$$\int_0^t V'_0 \left(\frac{a'}{a}\right)^t dt = MD', \quad \cdots\cdots(2)$$

where a'/a the ratio of the decrement of mobilities of amino acids at the intervals of t_1 and V'_0 is the apparent initial velocity of migration. Therefore MD' is expressed by

$$MD' = V'_0 \frac{(a'/a)^n}{\log(a'/a)} + C, \quad \cdots\cdots(3)$$

where n is t/t_1. When t is zero, we have

$$C = -V'_0 \frac{1}{\log(a'/a)}.$$

Substituting in (3), MD' is

$$MD' = V'_0 \frac{(a'/a)^n}{\log(a'/a)} - V'_0 \frac{1}{\log(a'/a)},$$

in which D_{am} the distance that amino acid travels from origin, D_{dex} the distance that dextran travels in the same or opposite direction and t is the time of electrophoresis.

Previously[1,2] we reported that the mobilities of amino acids gradually decreased with the lapse of time by the effect of osmotic flow of buffer into filterpaper. Afterwards experiments were made to determine the decrement of the mobilities of amino acids versus time. Fig. V illustrates the relationship between the movements of amino acids and the dropped points of them, which appeared to be a convenient method for determining the static points of migrants. As it is seen in Fig. V that the static points of them showed slight displacement during three hours' course of electrophoresis, the possibility that the decrease of mobilities at a definite interval may be represented by a logarithmic decrement must be admitted. Fig. VI shows the logarithmic decrement of mobilities at a definite interval.

Some examples of the migration distance of amino acids in the common apparatus in which the evaporation from filter-

Fig V

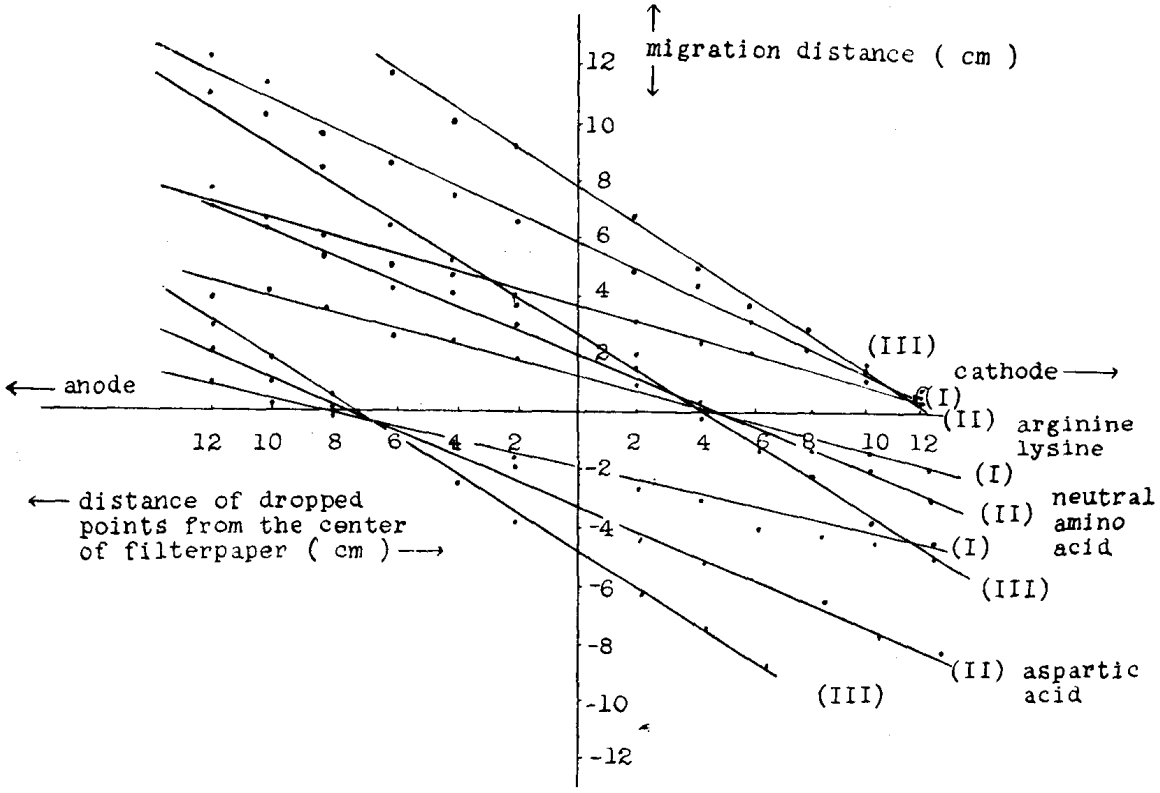

Fig. V Plot of migration distance against the dropped points, (I) 1 hr, (II) 2 hrs, (III) 3 hrs, in phosphate buffer of pH 7.38, 7.1 V/cm, 0.7 mA/cm.

Fig IV

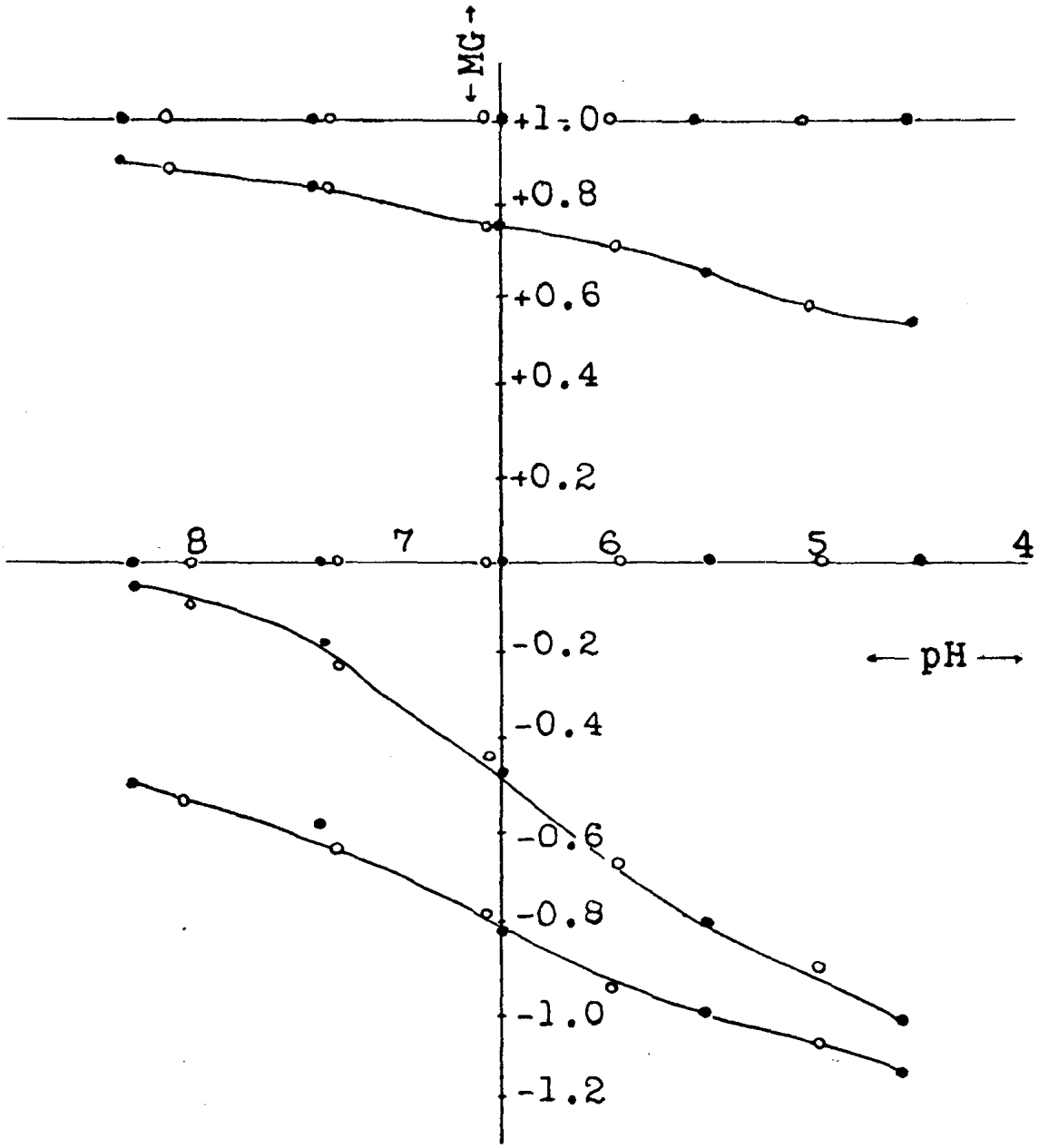

Fig. IV The MG values of amino acids plotted against pH, in phosphate buffer, ○ : $\mu=0.17$, ● : $\mu=0.06\sim0.20$

4) The Determination of Migration Distance of Amino Acids

In paper-electrophoresis, as demonstrated above, a linear relationship is obtained between the net migration distance of amino acids and the time, excluding the effect of electroosmotic flow and evaporation of buffer from paper surface. So the migration distance MD at the time of t is given by the following formula,

$$MD=(D_{am}\pm D_{dex})t \cdots\cdots (1)$$

Fig Ⅲ

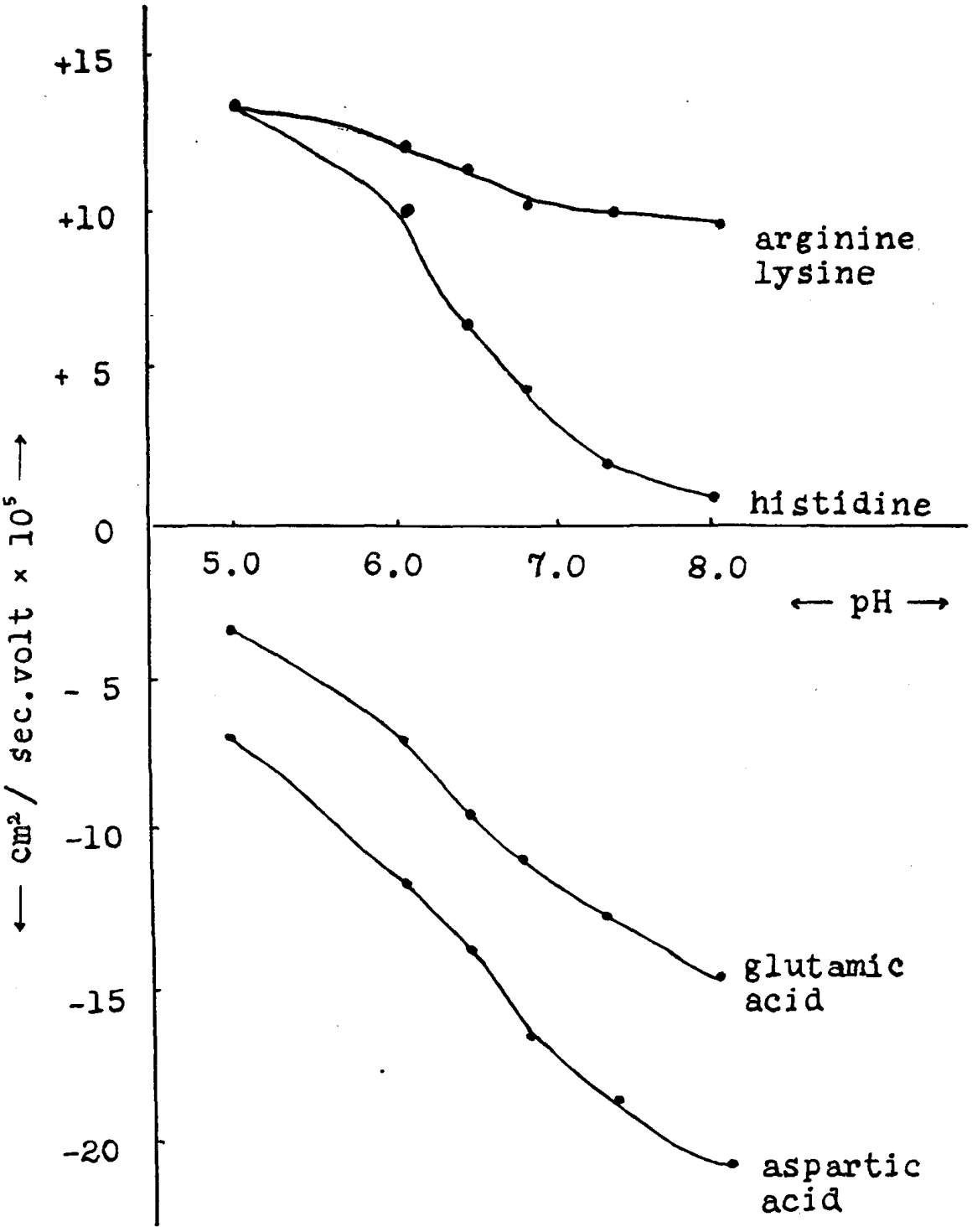

Fig. III The movements of amino acids as the function of pH in phosphate buffer of constant ionic strength, (μ=0.17) 6.9 V/cm, 0.7 mA/cm.

Fig II

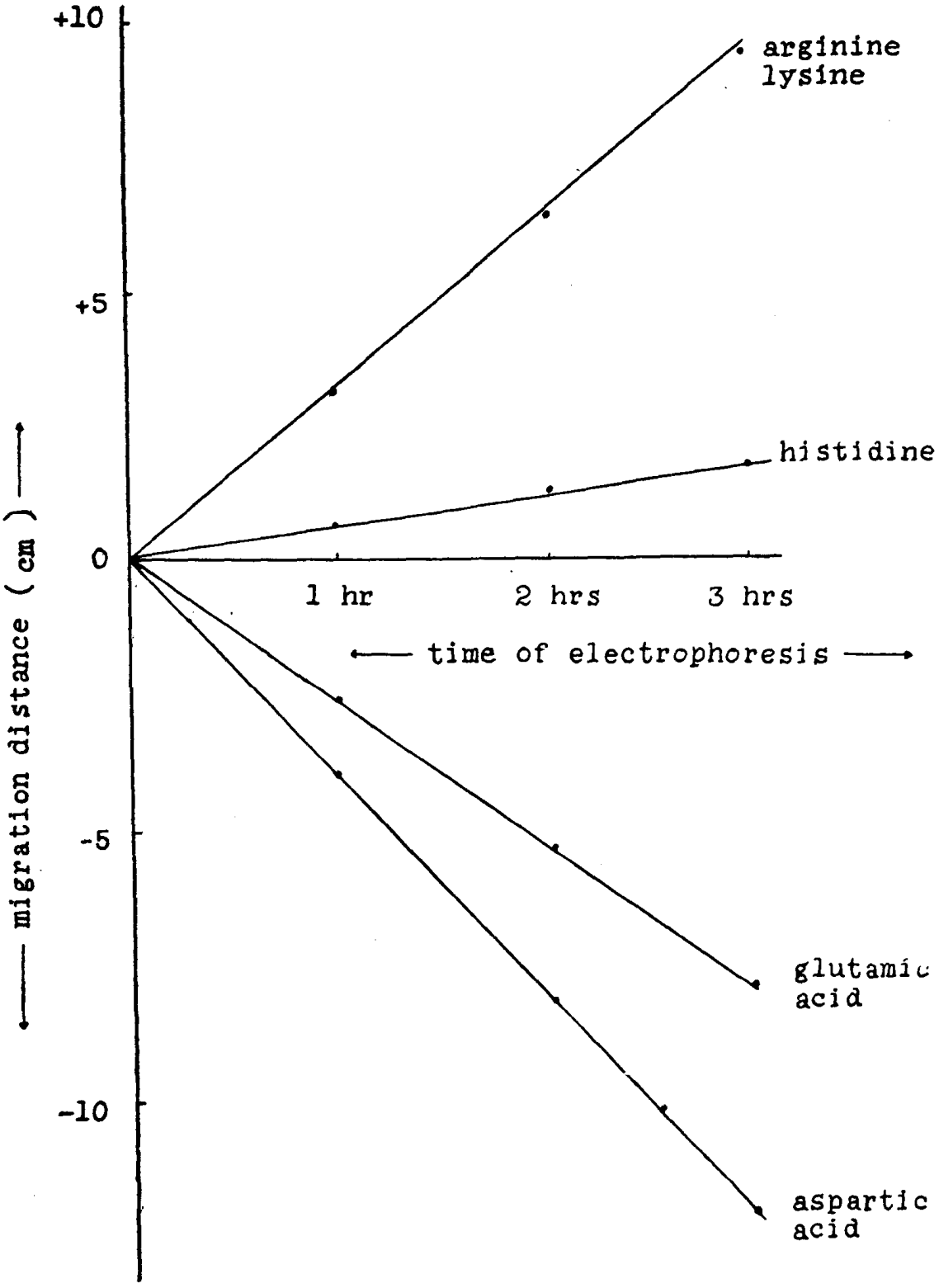

Fig. II The movements of amino acids as the function of time in phosphate buffer of pH 7.38, 6.9 V/cm, 0.6 mA/cm

2 per cent of acetic acid. Dextran was localized[4] as a white spot when the dried paper was placed in deeply clored ethyl alcohol solution of bromphenol blue.

EXPERIMENTAL RESULTS

1) Movements of Amino Acids as the Function of Time

Previously[1,2,3] we reported that the mobilities of amino acids gradually decreased with the lapse of time under the effect of evaporation from paper strips. In this experiment the evaporation from paper surface was prevented by clamping strips between glass plates and a linear relationship was found between the movements of arginine, lysine, histidine, glutamic acid and aspartic acid and the time of electrophoresis, employing the distance of amino acids from dextran as the net migration distance. In Fig. II is shown a plot of movement of amino acids versus time. The pH, potential and ionic strength were held constant during the course of experiment.

2) Movements of Amino-acids as the Function of pH

The mobilities of amino acids, aspartic acid, glutamic acid, histidine, lysine and arginine were studied over a wide pH range of the phosphate buffer solution of constant ionic strength. (μ=0.17) In electrophoresis voltage was kept at constant value of 6.9 V/cm and average current was always kept at 0.7 mA/cm. The results are shown in Fig. III.

Thus the mobilities of amino acids change their own values as the function of pH in phosphate buffer of constant ionic strength like the results obtained by Schlieren[6,7] method and paper-electrophoresis.[8,9] The reason why histidine migrated to the cathodic side in higher pH than the isoelectric point may be due to the faultiness in measuring the electroosmosis owing to the viscosity of dextran solution and the disturbance of the isoelectric points by phosphate ion.

3) The Effect of Ionic Strength on the MG Values of Amino Acids

The MG values of amino acids obtained in phosphate buffer of constant ionic strength are plotted in Fig. IV. The result shows that the pH-MG curves of amino acids in phosphate buffer of constant ionic strength, 0.17, accord with those of different ionic strength, 0.06~0.20. Thus the MG values of amino acids were the constants regardless of ionic strength of surrounding buffer.

glutamic acid-HCl, L-aspartic acid, DL-valine, glycine, DL-alanine, DL-serine, L-leucine, L-tyrosine, DL-tryptophane, L-proline, L-hydroxyproline, DL-methionine and 200 mg of dextran were each dissolved in 5 ml of water (or acidified water with hydrochrolic acid) and 1/1000 ml of the solution was used for electrophoresis. In other experiment 1/100 ml of normal human serum and the serum of a patient with hepatitis was used for electrophoresis.

2) **Buffer Solutions**

Buffer solutions of pH 8.05, 7.38, 6.84, 6.50, 6 07 and 5.02 of constant ionic strength (μ=0.17) were prepared from M/6~M/17 KH_2PO_4~and Na_2HPO_4. Barbital buffer of pH 8.5[5] (μ=0.045) was used for the separation of serum proteins.

3) **Apparatus and Method**

Filterpaper (Tôyô, No. 50, 2×30 cm) treated with n-HCl and n-NaOH were suspended horizontally or clamped between glass plates, 27.6 cm in length, both ends of which were put in phosphate buffer. The above apparatus was placed in thermostat and kept at 26.5~27.0°C under saturated vapour pressure.

Fig. I

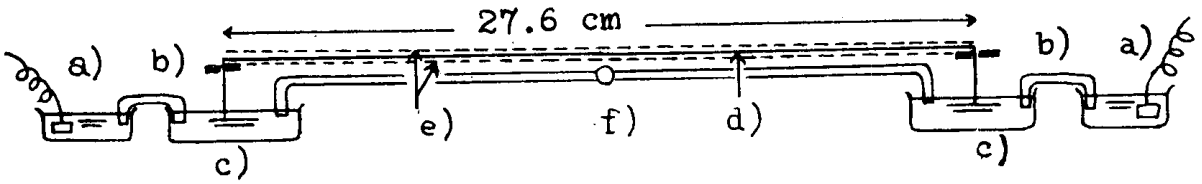

Fig. I Apparatus
a) electrode b) agar-bridge c) buffer solution d) filterpaper
e) glass plates f) glass tube

In more than thirty minutes after buffer soaked through the filterpaper, the current was turned on for forty minutes, the sample solutions were dropped on the original points and electrophoresis was began. The current was measured at the intervals of five minutes and the arithmatical mean was assumed as the average current. The displacement of the migrating compound was detected by spraying the paper, previously dried in a stream of warm air, with suitable spot test agent to produce colored bands. In the experiments involving amino acids, a solution of 0.25 per cent ninhydrin in butanol was used to spray the paper. Serum proteins were colored with an aqueous solution containing 1 per cent bromphenol blue and

On the Migration Distance of Amino Acids and Serum Proteins in Paper-electrophoresis

Part. IV*

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(Recieved July 15, 1956)

Peviously[1,2,3] we reported on the migration distance and newly defined MG values of amino acids in paper-electrophoresis and pointed out that the MG of amino acids in our definition——the distance ratio of amino acids from glycine to that of aspartic acid——changed their own values as the function of pH of surrounding buffer solution. These results suggested that in paper-electrophoresis the mobilities of amino acids were the function of pH of buffer. Kunkel and Tiselius[4] decided the mobilities of serum proteins by measuring the distance of migrants from dextran as the net migration distance, clamping paper strips between glass plates to exclude the effect of electro-osmotic flow and evaporation of buffer from paper surface. In the present paper a description is given of measuring the net migration distance by the above method, in which disturbing factors such as evaporation, heating and buffer concentration gradient were reduced to minimum, and of applying the MG values to serum proteins. An attempt was made to determine the apparent migration distance from original point, the distance of static point of migrant from origin and the initial velocity of migration by the experimental formulas obtained in the common apparatus, in which the evaporation from filterpaper was admitted.

MATERIALS AND METHOD

1) Sample Solutions

50 mg of L-arginine-HCl, L-lysine-HCl, L-histidine-HCl, L-

* Part of this work has been presented at the anual meeting of the agricultural chemical society of Japan, at Tokyo university, march, 1956.