

Fig. XII.

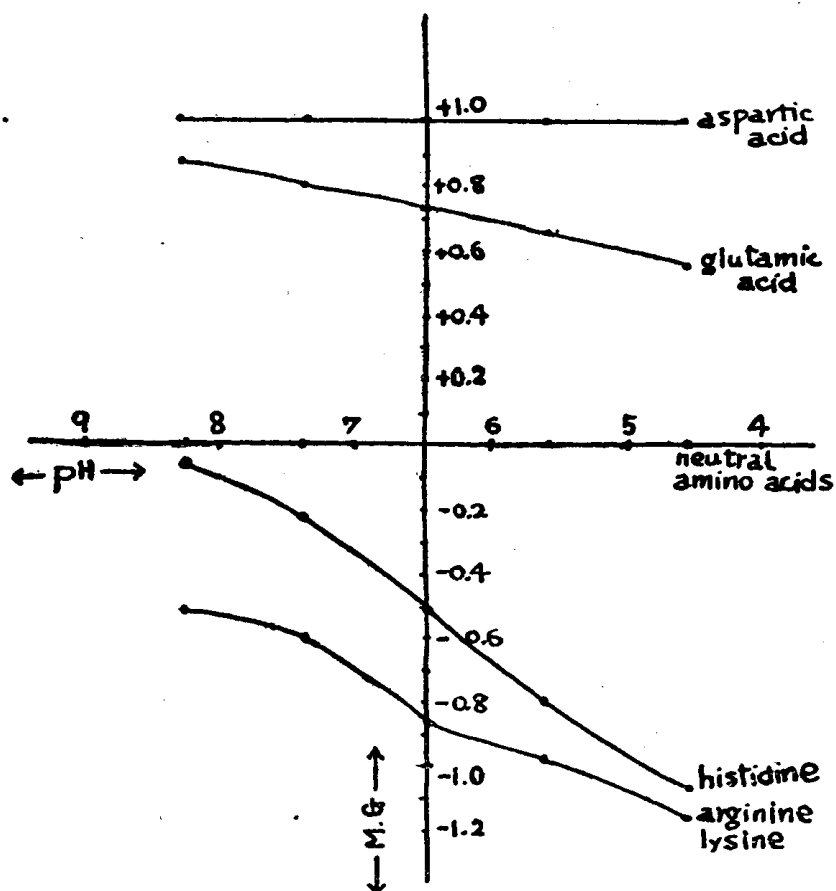


Fig. XII. Plot of M.G of amino acids in paperelectrophoresis against pH of sorrounding buffer solution, at 26.5~27.0° C.

emphasized that amino acids have each static points on filter-paper in paperelectrophoresis and that they have respectively the maximum value of migration distance in each condition.

Literature :

- 1) Foster, A. B., and Stacey, M. ; Appl. Chem. (London), 3, 19, 1953 (C.A., 7857).

Table. IV.

hour amino acid	1	2	3
lysine	-28	-42	-50
histidine	-9	-13	-16
glutamic acid	+37	+54	+63
aspartic acid	+44	+64	+76

Table. IV. The distance of amino acids from glycine, experimented in phosphate buffer of pH 7.38 at 26.5~27.0° C. volt/cm=13.8 (in apparatus C)

Table. V.

amino acid	aspartic	glutamic	lysine	histidin	neutral amino acid
M.G	+1.0	+0.80 (+0.72~ +0.84)	-0.60 (-0.51~ -0.65)	-0.22 (-0.19~ -0.24)	0

Table. V. M.G of amino acids: in phosphate buffer of pH 7.38 at 26.5~27.0° C.

Table. VI.

amino acid pH	aspartic acid	glutamic acid	lysine, arginine	histidine	neutral amino acids
pH 8.30	+1.0	+0.87	-0.52	-0.06	0
pH 7.38	+1.0	+0.80	-0.60	-0.22	0
pH 6.47	+1.0	+0.74	-0.85	-0.50	0
pH 5.59	+1.0	+0.67	-0.99	-0.80	0
pH 4.56	+1.0	+0.54	-1.18	-1.08	0

Table. VI. M.G of amino acids in several pH of phosphate buffer, at 26.5~27.0° C.

From our results we have a doubt about the universal validity of M.G by Foster & Stacey, and believe that it must be more suitable to take as M.G the distance ratio of other amino acids from glycine to that of aspartic acid. In our experiment it was impossible to avoid the influence of Joule's heat and the change of pH on parts of filterpaper. It must be

Table. II.

hour amino acid	1	2	3
lysine	-3.90	-3.80	-3.57
histidine	-1.85	-1.80	-1.60
glutamic acid	+2.70	+2.66	+2.63
aspartic acid	+3.40	+3.26	+3.05

Table. II. Data of M.G of amino acids, experimented in phosphate buffer of pH 7.38 at 26.5~27.0° C. volt/cm=13.8 (in apparatus C)

M.G of amino acids in this definition has the convenience of excluding the influence of time, but cannot exclude other conditions, current, voltage, dropped point and the length of filterpaper. As electroosmosis increases in direct proportion to the current, we don't regard M.G in this definition as the value free from electroosmosis. If we take the following ratio as M.G,

$$\text{M.G} = \frac{\text{migration distance of other amino acids from glycine}}{\text{migration distance of aspartic acid from glycine}}$$

M.G is free from current, voltage, dropped point and the length of filterpaper, but not from pH of surrounding buffer solution. The distance of amino acids from glycine and the value of M.G of amino acids are as following Table.) Table. III, Table. IV, Table. V, Table. VI)

Table. III.

amino acid		histidine		lysine		glutamic acid		aspartic acid	
apparatus	mA/cm time	low	high	low	high	low	high	low	high
apparatus C	1hr	-6	-7	-16	-17	+20	+21	+28	+30
	2hrs	-9	-9	-27	-25	+33	+35	+47	+46
	3hrs	-11	-12	-30	-31	+44	+45	+59	+61
	4hrs	-13	-13	-34	-35	+49	+50	+65	+69
apparatus A	1hr	-10	-11	-28	-29	+33	+34	+44	+45
	2hrs	-14	-15	-42	-40	+50	+52	+65	+67
apparatus B	1hr	-11	-11	-29	-27	+33	+34	+45	+47
	2hrs	-15	-14	-43	-41	+53	+52	+66	+68
	3hrs	-17	-16	-54	-48	+66	+68	+82	+83

Table. III. The distance of amino acids from glycine, experimented in phosphate buffer of pH 7.38 at 26.5~27.0° C. volt/cm=6.9

cine and in buffer of various higher pH, even in barbiturate, and no regular relation was found between pH of surrounding buffer solution and migration distance. The fact—that although the current had no relation to the mobility of electrophoresis, the increase of current shifted amino acids to anodic side and their mobility gradually decreased—was perhaps due to the difference of electroosmosis and the osmotic flow of buffer into filterpaper. As the current is proportional to the degree of saturation of buffer into filterpaper, the expression that migration distance increases in inverse proportion to the current may be unsuitable. If we wish to have the same migration value, volt/cm, mA/cm, dropped point and the length of filterpaper must all be identical.

When the definition of M.G by Foster & Stacey is applied to amino acid, we get the following ratio:

$$\text{M.G} = \frac{\text{migration distance of other amino acids}}{\text{migration distance of glycine}}$$

M.G of several amino acids in this definition when dropped in the center of filterpaper is shown in Table. I and Table. II.

Table. I.

M.G			M.G average current = 0.6 mA/cm		M.G average current = 0.8 mA/cm		M.G average current = 0.5 mA/cm	M.G average current = 0.7 mA/cm
amino acid			appara- tus A	appara- tus B	appara- tus A	appara- tus B	appara- tus C	appara- tus C
lysine	hour	1	−3.44	−3.72	−3.70	−4.25	−3.00	−3.50
		2	−3.55	−3.58	−3.56	−3.99	−2.98	−3.40
		3	—	−3.50	—	−3.99	−2.95	−3.18
		4	—	—	—	—	−2.90	−3.00
histidine	hour	1	−2.00	−2.00	−2.11	−2.15	−1.75	−2.00
		2	−1.83	−1.88	−1.81	−2.10	−1.69	−1.90
		3	—	−1.81	—	−2.00	−1.68	−1.81
		4	—	—	—	—	−1.66	−1.67
glutamic acid	hour	1	+1.81	+2.00	+2.55	+3.12	+1.50	+2.50
		2	+1.72	+1.88	+2.26	+3.00	+1.54	+2.50
		3	—	+1.82	—	+2.90	+1.69	+2.38
		4	—	—	—	—	+1.72	+2.26
aspartic acid	hour	1	+2.52	+2.98	+3.55	+4.88	+2.50	+4.16
		2	+2.40	+2.51	+3.26	+4.10	+2.96	+3.90
		3	—	+2.68	—	+3.94	+2.61	+3.70
		4	—	—	—	—	+2.50	+3.60

Table. I. Data of M.G of amino acids, experimented in phosphate buffer of pH 7.38 at 26.5~27.0° C. volt/cm

Fig. XI.

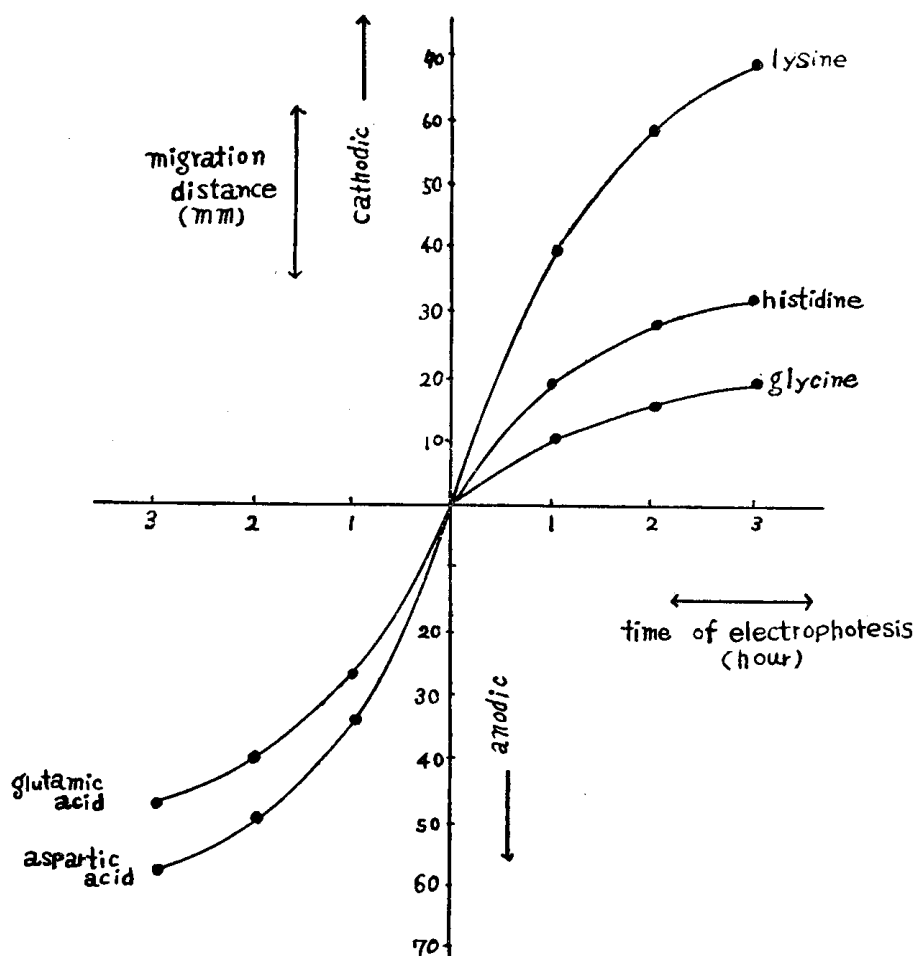


Fig. XI. Migration distance of amino acids, time study. Amino acids migrated in phosphate buffer of pH 7.38 at 26.5~27.0° C. volt/cm=13.8, average current=1.29 mA/cm (in apparatus C)

Thus migration distance of amino acids from each other became about twice when voltage was heightened double, and the static points of amino acids gathered towards the center of filterpaper with higher voltage. The angle between the migration distance lines of amino acids relative to their dropped points and the abscissa increased with voltage.

Discussion of Results

Many factors as demonstrated above have effects on the migration distance of amino acids in paperelectrophoresis. The reason why glycine flowed to cathodic side in phosphate buffer of pH 7.38, is perhaps due to the effect of electro-endosmotic flow and disturbance of isoelectric point by phosphate ion. In our experiment glycine flowed to cathodic side in phosphate buffer of pH 6.1, that is, equal to the isoelectric point of gly-

Fig. X.

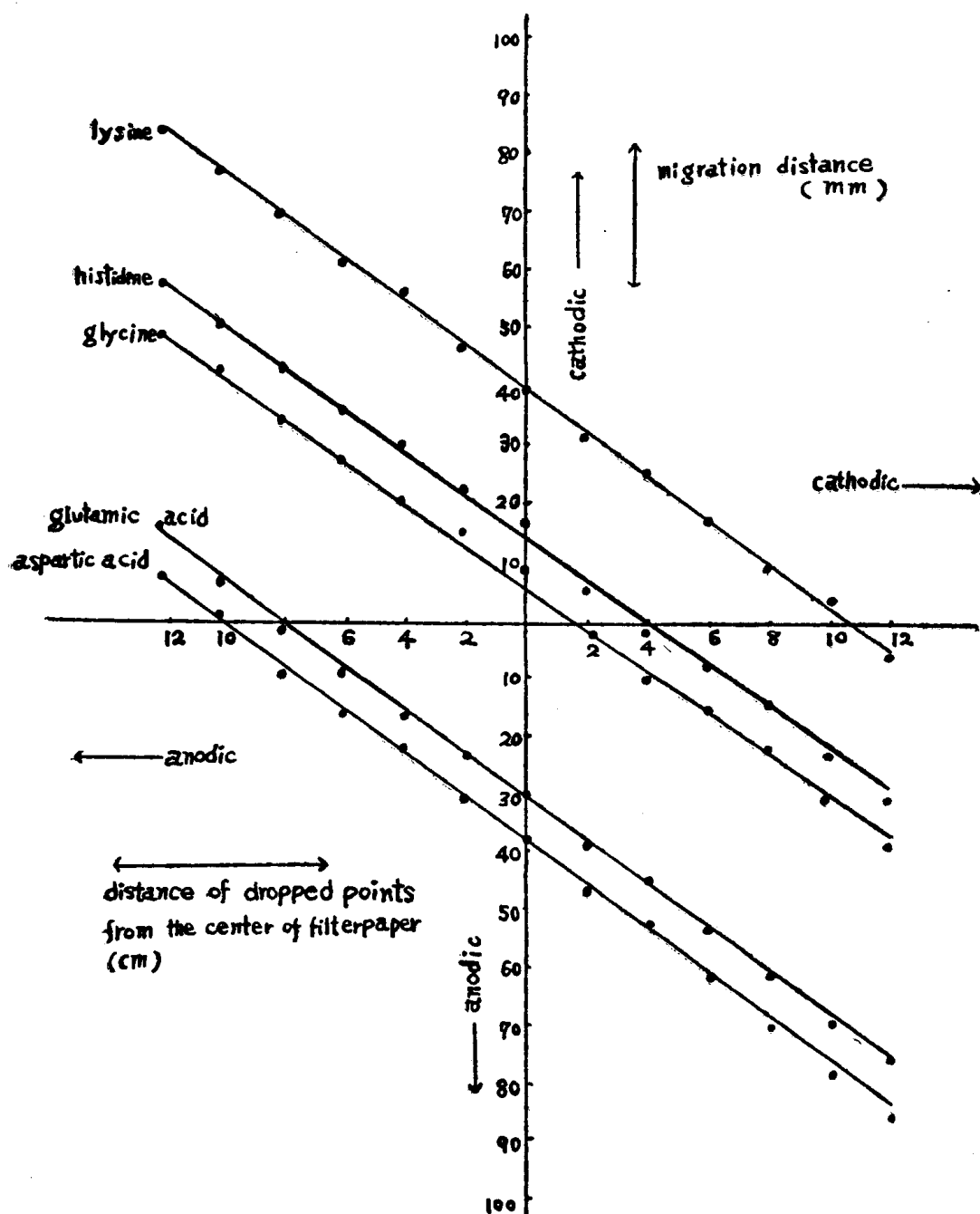


Fig. X. Plot of migration distance of amino acids against the distance of dropped points from the center of filterpaper in apparatus C. Amino acids migrated in phosphate buffer of pH 7.38 at 26.5~27.0° C. volt/cm=13.8, average current=1.16 mA/cm

The migration distance of amino acids in each time experimented in apparatus C with higher voltage is shown in Fig. XI.

Fig. IX.

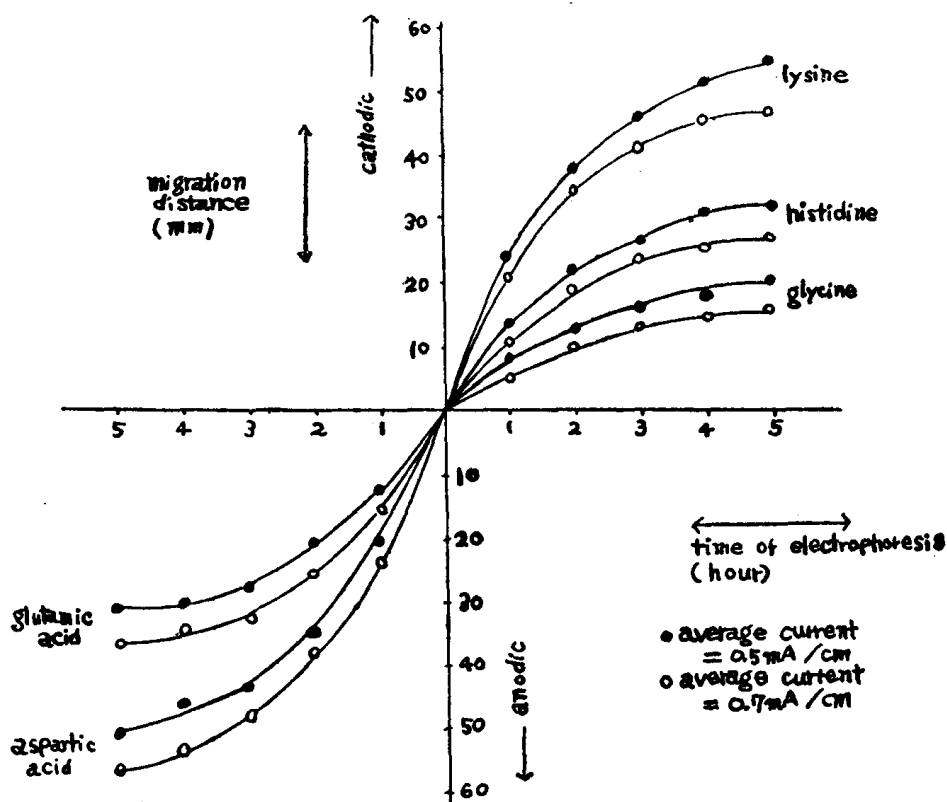


Fig. IX. Migration distance of amino acids, time study. Amino acids migrated in phosphate buffer of pH 7.38 at 26.5~27.0° C. (in apparatus C) volt/cm=6.9

These results showed that in spite of other same conditions migration distance was not identical when the length of filter-paper was different.

§ Effect of Voltage on the Migration Distance

The above-mentioned amino acid solutions were each dropped on the points at the distance of 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 cm on both sides from the center of filterpaper in apparatus C and the current of higher voltage (13.8 volt/cm) was turned on. The results are shown in Fig. X.

Fig. VIII.

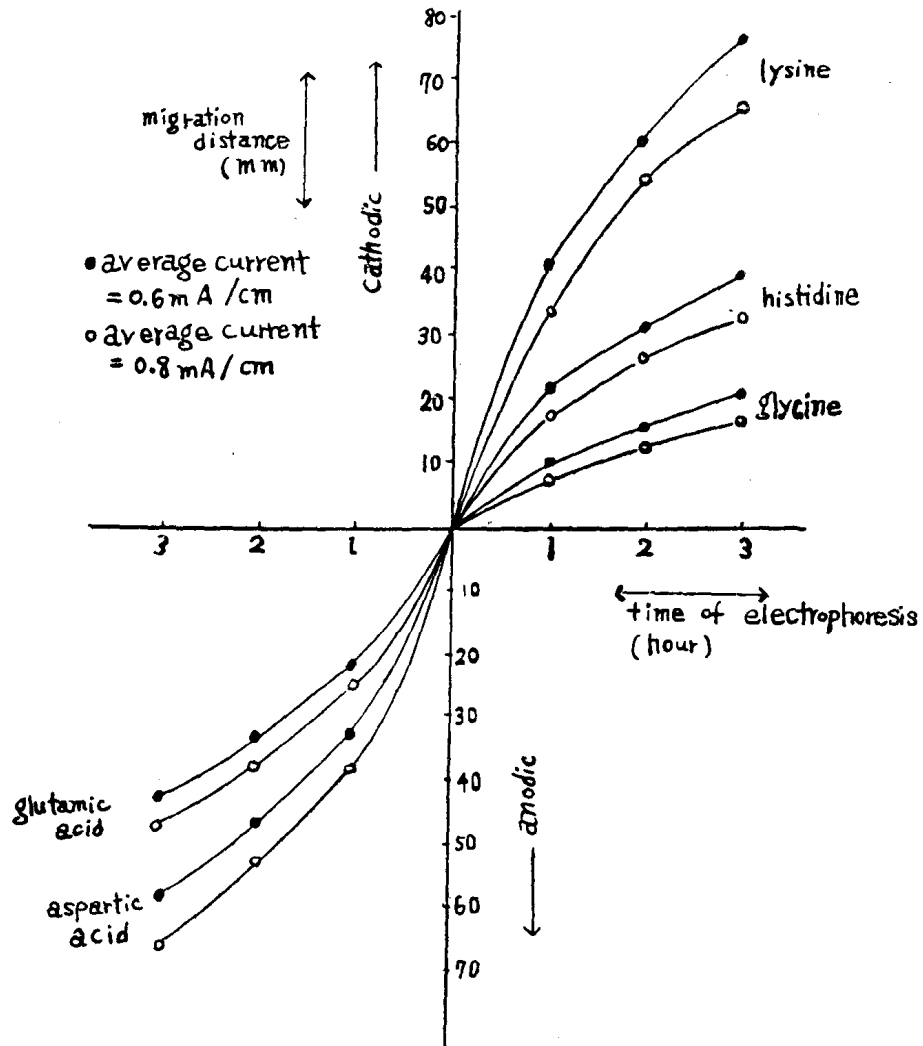


Fig. VIII. Migration distance of amino acids, time study. Amino acids migrated in phosphate buffer of pH 7.38 at 26.5~27.0° C. (in apparatus B) volt/cm=6.9

The migration distance of neutral amino acids, valine, serine, leucine, tyrosine, tryptophane, proline, oxyproline and methionine was equal to that of glycine and the distance of lysine was equal to that of arginine. The plot of migration distance of other amino acids against their dropped points ran parallel with that of glycine. Therefore after electrophoresis the amino acids kept the same relative distance from each other without reference to the dropped points and the mobility of amino acids gradually decreased with equal ratio. As in the case of glycine increase of current shifted the static points of amino acids to anodic side. The migration distance of amino acids in each time in apparatus A almost accorded with that in apparatus B, but not with that in apparatus C. (Fig. VII, Fig. VIII, Fig. IX)

Fig. VII.

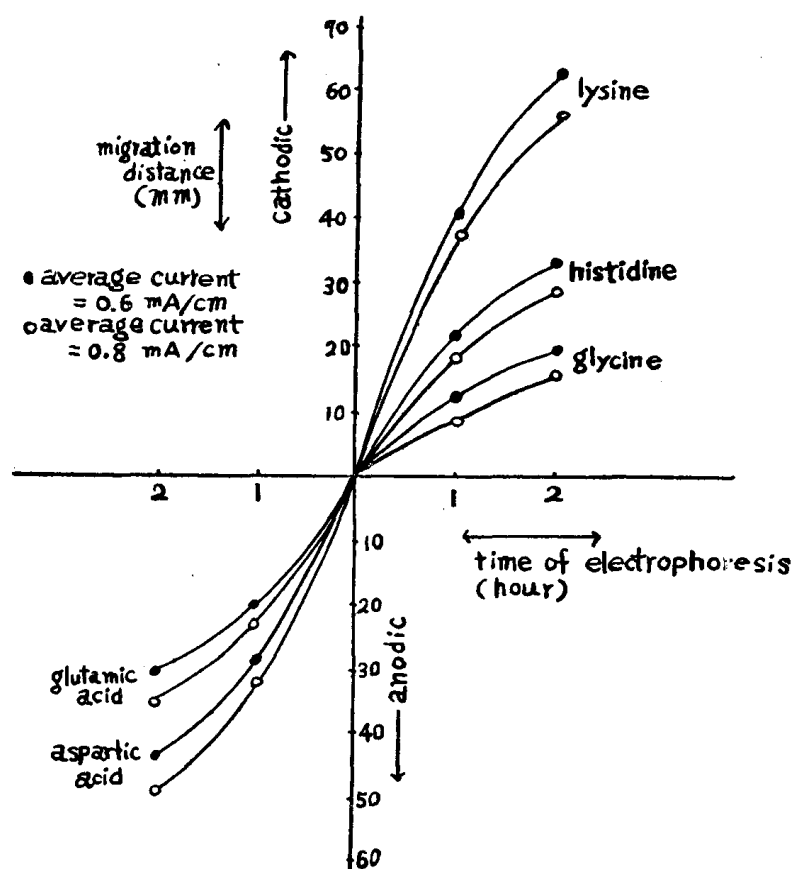


Fig. VII. Migration distance of amino acids, time study. Amino acids migrated in phosphate buffer of pH 7.38 at 26.5~27.0° C. (in apparatus A) volt/cm=6.9

Fig. V.

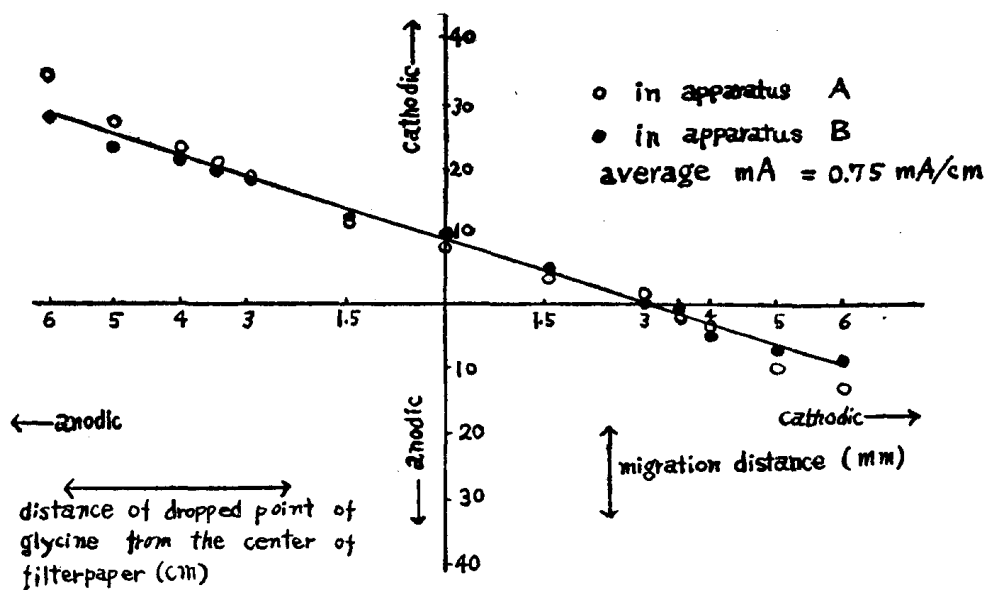


Fig. V. Plot of migration distance of glycine against the distance of dropped points from the center of filterpaper, experimented in apparatus A and B. Glycine migrated in phosphate buffer of pH 7.38 at 26.5 ~27.0° C. volt/cm=6.9

Fig. VI.

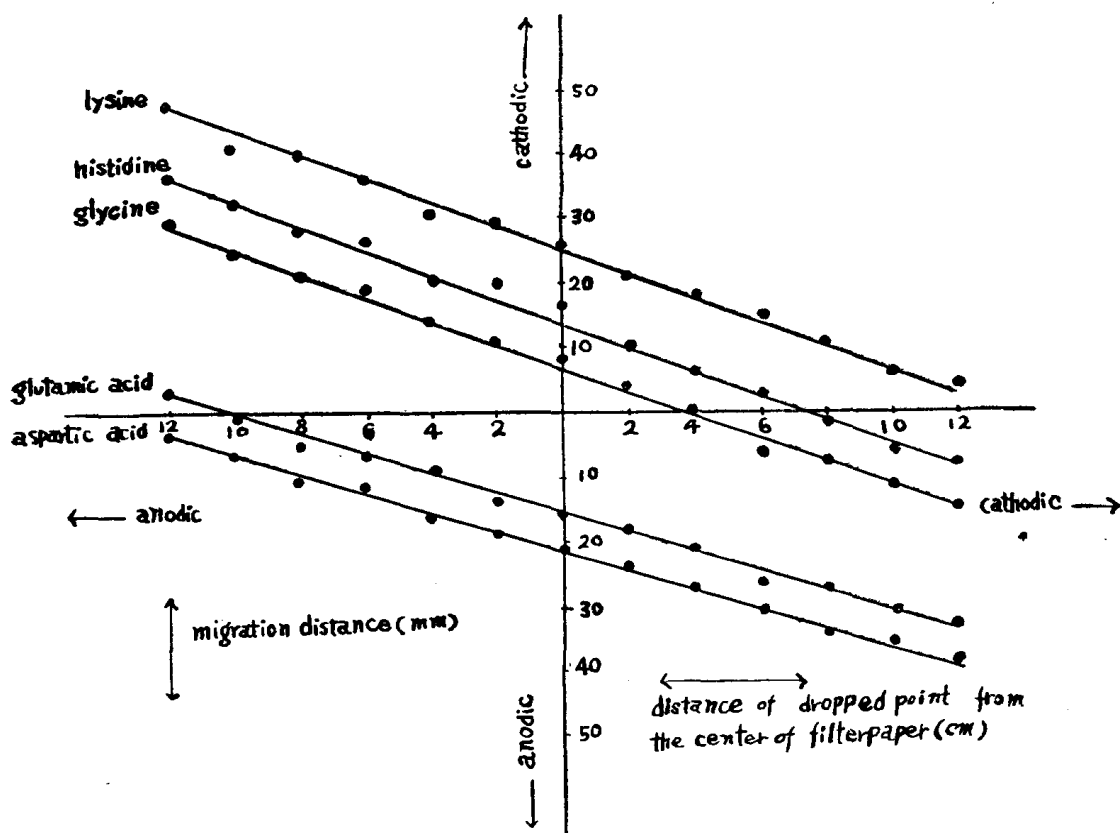


Fig. VI. Plot of migration distance of amino acids against the distance of dropped points from the center of filterpaper in apparatus C. Amino acids migrated in phosphate buffer of pH 7.38 at 26.5~27.0° C. volt/cm=6.9, average current=0.4 mA/cm

five times every one hour after the beginning of electrophoresis. The results are shown in Fig. III.

When glycine was dropped on the points, at the distance of 1.5, 3.0, 3.5, 4.0, 5.0 and 6.0 cm on both sides from the center of filterpaper, glycine distanced on 3.5cm cathodic side (average current = 0.61 mA/cm) stayed on the original point, and glycine distanced farther on the cathodic side migrated to anodic side, glycine farther on the anodic side migrating to cathodic side. In other word glycine dropped on the filterpaper gathered towards its own "static point" in each condition. Increase of current shifted the static point of glycine to anodic side. (Fig. IV)

Fig. IV.

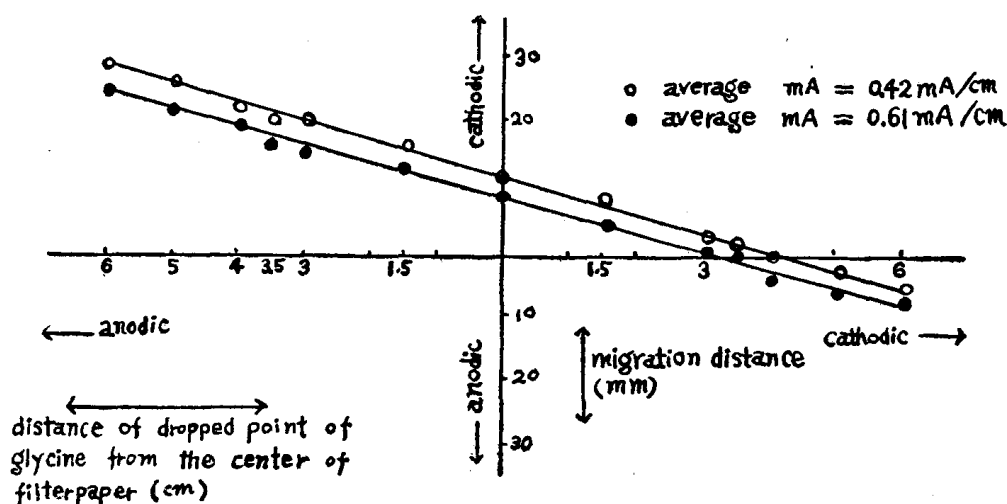


Fig. IV. Plot of migration distance of glycine against the distance of dropped points from the center of filterpaper, experimented in apparatus C. Glycine migrated in phosphate buffer of pH 7.38 at 26.5~27.0° C. volt/cm = 6.9

When glycine was dropped on the above-mentioned points on filterpaper in apparatus A and B, which had filterpaper each of 30 cm in length of different level parts, the migration distance in both apparatus almost accorded with each other in the same current, but not with that in apparatus C. (Fig. V)

§ Migration Distance of Other Amino-acids

The amino acid solutions, arginine, lysine, histidine, valine, glycine, serine, leucine, tyrosine, tryptophane, proline, oxyproline, methionine, aspartic acid and glutamic acid were each dropped on the points at the distance of 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 cm on both sides from the center of filterpaper in apparatus C. The average current was kept in the same value to one tenth mA. The results are presented in Fig. VI.

Fig. II.

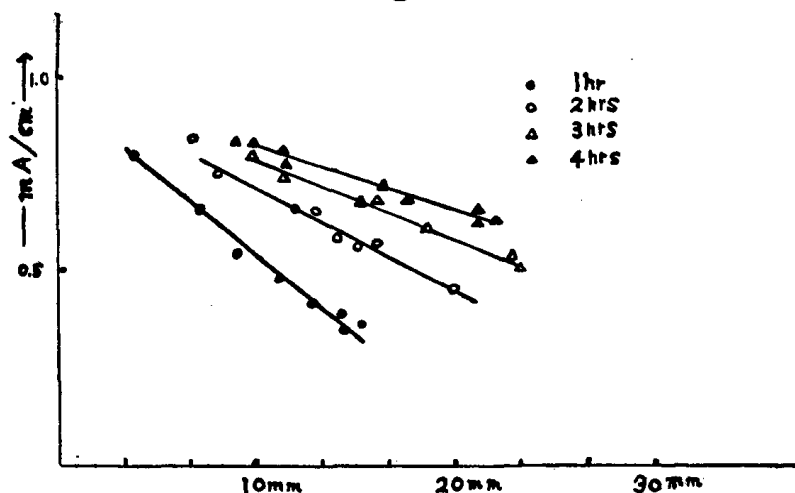


Fig. II. Plot of migration distance of glycine against the current of electrophoresis in various time. Glycine migrated in phosphate buffer of pH 7.83 (ionic strength=1.29) at 26.5~27.0°C. (in apparatus. A) volt/cm=6.9

Though glycine (isoelectric point=pH 6.1) was in phosphate buffer of pH 7.38, it flowed to cathodic side. The migration distance increased in inverse proportion to the average current and its mobility gradually decreased with the lapse of time. As it was impossible to measure the migration distance of each time, five filterpapers (Tōyō, No. 50, 2×50 cm) were put in apparatus C and migration distance was measured

Fig. III.

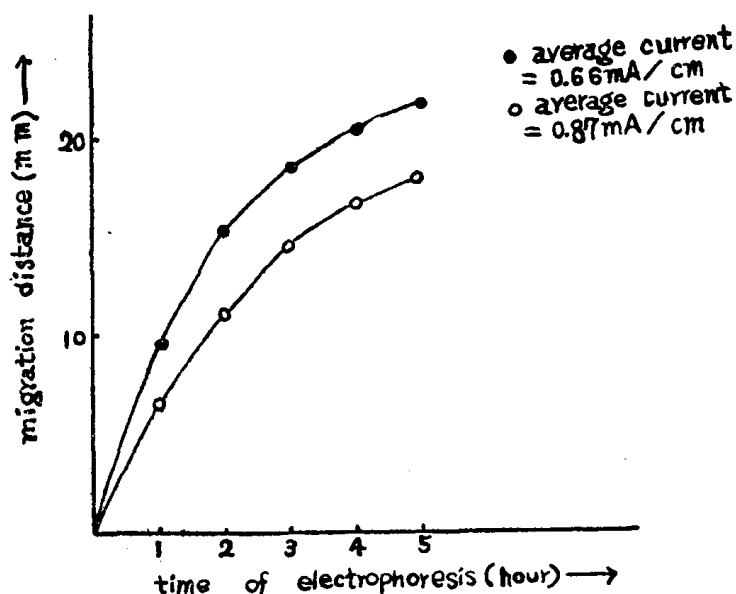


Fig. III. Migration distance of glycine, time study. Glycine migrated in phosphate buffer of pH 7.38 at 26.5~27.0°C. volt/cm=6.9 (in apparatus C)

mic acid were each dissolved in 5 ml of water, and 1/1000 ml of the solution was used for paperelectrophoresis.

§ Migration Distance of Glycine

Filterpaper (Tōyō, No. 50, 2×30 cm) treated with *n*-HCl and *n*-NaOH were set between Petri-glasses, 12.5 cm in diameter, both ends of which were put in phosphate buffer solution of pH 7.38. (ionic strength=1.29) The above apparatus was placed in thermostat and kept at 26.5~27.0° C and in saturated vapour pressure. (Apparatus. A)

In more than 30 minutes after buffer soaked through the filterpaper, the current was turned on for about 20 minutes, 1/1000 ml of glycine solution was dropped on the central original point of filterpaper and electrophoresis was began. The current was measured at the interval of 5 minutes and the arithmetical mean was shown as the average current. Glycine was coloured with 0.25 % ninhydrin butanol solution. The results are shown in Fig. II.

Fig. I.

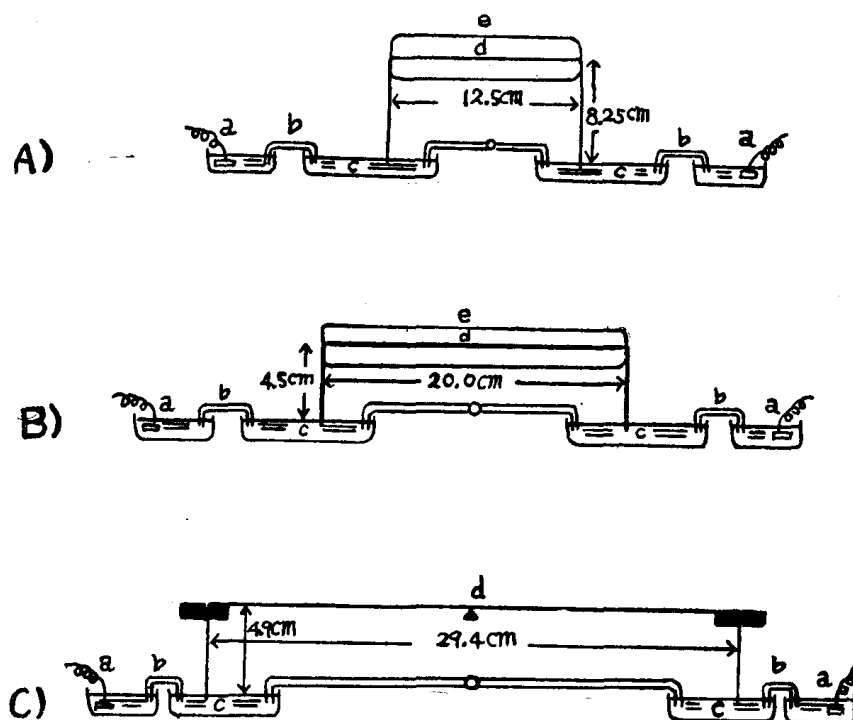


Fig. I.

A) Apparatus. A B) Apparatus. B C) Apparatus. C
a: electrode b: agar bridge c: buffer solution d: filterpaper
e: Petri-glasses or enamelled plate

The Separation of Amino-acids by Paperelectrophoresis: The Migration Distance of Several Amino-acids (3rd Report)

By Kiyoshi Sakamoto
Kazuko Tateoka

In a recent paper in this series we reported on the separation of several amino acid mixtures and protein hydrolysates in various pH with 100~400 V D.C. without describing migration distance from the original point. Although the migration distance in paperelectrophoresis is to be theoretically determined by the conditions of volt/cm, mA/cm, buffer solution used and the time of electrophoresis, we found it difficult to have definite migration distance even in the identical condition owing to the influence of electro-endosmotic flow, evaporation by Joule's heat, the chemical and electrochemical properties of electrolytes and the lack of uniformity in filterpaper etc. Few studies seem to have been reported on the migration distance under various conditions of paperelectrophoresis. Foster and Stacey¹⁾ (1953) defined M.G of monosaccharides and their derivatives as the following ratio,

$$\text{M.G} = \frac{\text{M.G of monosaccharides (derivatives)}}{\text{M.G of } D\text{-glucose}}$$

excluding the influence of electroosmosis.

In the case of amino acids, paperelectrophoresis is more complicated because of their dipolar ion, on which the valence of buffer solution, the hydrogen ion concentration and the ionic strength have an important effect. This time we experimented about the migration distance of several amino acids and obtained almost systematic M.G.

Experimental

§ Amino-acid Solutions

50 mg of *l*-arginine-HCl, *l*-lysine-2HCl, *l*-histidine-HCl, *d*, *l*-valine, glycine, alanine, serine, leucine, tyrosine, *d*, *l*-tryptophane, proline, oxyproline, methionine, aspartic acid and gluta-