AGAR ELECTROPHORESIS

Part II. Quantitative Evaluation of Agar Electrophoresis Patterns of Human Serum Proteins by Densitometry and Comparison with Moving Boundary Electrophoresis

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Summary

A comparative study of agar electrophoresis and moving boundary electrophoresis for quantitative evaluation of serum protein patterns has been made. The advantages of agar electrophoresis over paper electrophoresis as a quantitative clinical method for serum protein analysis are discussed.

In earlier publications (Giri, 1956 *a*, *b*, *c*, *d*, *e* and *f*), a technique of zone electrophoresis on agar gel for qualitative analysis of serum proteins and hæmoglobins (Giri and Pillai, 1956 *a* and *b*) was described. The technique has been adapted to two-dimensional procedure for the separation and identification of serum mucoproteins (Giri, 1957). In the first paper of this series, a procedure for the densitometric evaluation of serum protein patterns was briefly outlined (Giri, 1956 *c*).

The methods for quantitative estimation of the different protein components, separated after electrophoresis on agar gel, may be classified as follows:—

1. *Photometric evaluation.*—The protein components after staining can be estimated directly on the dry agar film by photometric evaluation of the stained zones with the help of a suitable densitometer.

2. *Elution analysis of segments.*—For elution analysis the electrophoresis is carried out using cellophane, polythene or 'Mylar' polyester films as described in earlier publications (1956, *e* and *f*). After staining with the dye, the film is cut into segments and the dye is eluted from each segment with N/20 sodium hydroxide. The concentration of the dye eluted is then determined colorimetrically. The amount of dye bound to the protein in each segment can be estimated and an electrophoretic pattern constructed. This method is laborious. It can be adopted in cases, where the substances separated cannot be made visible by any colour reaction or when the colours are transient.

3. *Elution analysis of the zones.*—The stained zones (whole of a fraction) obtained on cellophane or polyester film can be cut out completely and the amount of dye bound by each protein component can be determined by elution with alkali.

The percentage composition of the different protein components of serum can be calculated from the colorimetric readings obtained for each stained zone. As the components separate into compact bands, without leaving any visible traces of proteins due to adsorption between zones, this method gives values which are sufficiently accurate for clinical work. But the chief disadvantage of the method is that the electropherogram will be destroyed and cannot be kept for future reference. The electrophoresis patterns obtained directly on glass plate without the use of cellophane or polyester film can be subjected to elution analysis by eluting the colour of the zones which can be scraped off from the glass with the help of a sharp razor blade into test-tubes and extracting the colour with alkali.

The quicker and simpler procedure is the photometric evaluation by means of a densitometer. The photometric evaluation of serum protein components directly on the medium was introduced by Grassmann and Hannig (1952) for paper electrophoresis.

This paper relates to the investigations on the quantitative photometric evaluation of the protein components after staining with the dye solution by means of a photovolt electronic densitometer, model 525. The results obtained are compared with those obtained by moving boundary electrophoresis. The accuracy and suitability of agar electrophoresis as a clinical laboratory procedure for the quantitative analysis of serum proteins and some of the advantages of this technique over the paper electrophoresis technique are discussed.

Experimental

The serum samples of normal individuals were collected with all the precautions necessary to prevent hæmolysis and stored in a refrigerator at 4° C. The pathological samples were obtained from the patients of local hospitals. Electrophoresis was usually performed on the same day or on the following day. Total N in the serum samples was determined by micro-kjeldahl method.

Apparatus and technique of agar electrophoresis.—The technique employed in the present investigation was essentially the same as described in previous publications (Giri, 1956 *a*, *b*, *c*). Kelab paper electrophoresis equipment was used throughout the investigation. Two samples of serum were examined simultaneously for each run, as the equipment holds two plates. The width of the gel was limited to 2·5 cm. as it is the maximum width that can be used to correspond to the length of the slit of the densitometer.

Electrophoresis was carried out in the medium containing 1 per cent. agar and veronal-acetate buffer of pH 8·6 and ionic strength 0·05. A current of 8–9 mA at 200 volts was employed. Electrophoresis was carried out at room temperature (22–24° C.) and the temperature control was not found to be necessary. The influence of temperature on the quality of the separation has not yet been investigated. But it is generally found that any temperature between 20–25° C. will give satisfactory patterns for quantitative measurement. High temperatures, prevailing

in summer in certain parts of India, should be avoided, as the gel takes a long time to set. In such circumstances suitable arrangements must be made to run electrophoresis at lower temperature.

The staining procedure followed in this investigation was exactly the same as described in the previous publications (Giri, 1956 *b*, *c*, *d*).

Densitometry.—The instrument used, throughout this work, for densitometry of the stained protein zones was the Model 525 Photovolt Electronic Densitometer, with 25×1 mm. slit. No filter was interposed between the slit and the phototube. The procedure adopted for measuring the optical density of the zones was described in the previous publication (Giri, 1956 *c*). The optical densities, obtained against distance of 1 mm. intervals along the plate starting from the forward edge of the albumin zone, were plotted on a graph paper to produce a curve. Each peak corresponded to a protein component and the peaks were drawn as Gaussian curves. After plotting the curves (optical density as a function of distance of migration along the gel), they were extended to the base line by extrapolating and joining through the intersecting point. The area under each peak was measured by means of a p.animeter. The relative amounts of the various components were expressed in terms of the relative area of the components in per cent. No factor for correcting the difference in the dye binding capacity of the proteins was introduced.

The relative areas can also be determined by cutting the areas under the peaks and weighing the pieces of paper on a sensitive ba.ance. By determining the weight of the paper of known area, the area of each peak can be calculated from the weight of the cut-out peaks. This method of excising the peaks was found to be as accurate and reproducible as planimetry, as shown by the results presented in Table I.

Moving boundary electrophoresis.—Moving boundary electrophoresis of the serum samples was carried out in the micro-cell of the Kern Micro-electrophoresis apparatus using Jamin's interferometer instead of Schlieren optics for observation of the boundaries. Serum was diluted 3–4 times with veronal-HCl-buffer of pH 8·6 and 0·1 ionic strength to bring the concentration of the protein into the sensitive range of the instrument. The diluted serum was dialysed with continuous stirring against veronal-HCl-buffer (pH 8·6, ionic strength 0·1) for 4–6 hours. The experimental details and the method for the evaluation of the electrophoretic patterns have been described by Cohly *et al.* (1953). An enlargement of the descending patterns with their projection was drawn on a graph paper and the relative concentrations of the protein fractions calculated in the usual manner.

RESULTS

The relationship between densitometer reading and concentration of protein.—One of the important requisites for the application of planimetric integration s that a linear relationship should exist between the density of the colour as measured by the densitometer and concentration of protein, *i.e.*, Beer's law should be obeyed.

TABLE I

Replicate analysis of the same serum sample (Normal) by area method and weight method

	Albumin	Globulins			
		$Alpha_1$	$Alpha_2$	Beta	Gamma
A. Percentage composition determined by measuring the area with a planimeter					
Expt. No. 1 ..	70·0	3·6	5·6	7·2	13·6
Expt. No. 2 ..	67·1	4·8	6·7	8·1	13·3
Expt. No. 3 ..	71·5	4·1	5·4	6·2	12·8
Expt. No. 4 ..	70·5	3·4	6·0	6·4	13·7
B. Percentage composition determined by weighing the excised peaks					
Expt. No. 1 ..	70·2	3·7	5·6	7·0	13·7
Expt. No. 2 ..	66·2	5·1	7·4	8·1	13·2
Expt. No. 3 ..	72·9	3·9	4·5	6·4	12·3
Expt. No. 4 ..	72·7	4·0	4·7	6·0	12·7

In order to establish a direct relationship between the areas of the peaks and the protein concentration, the following experiment was carried out.

Solutions containing different amounts of human serum albumin (Cutter Laboratories) were prepared by dissolving accurately weighed amount of the protein in water. 10 μl of each protein solution was applied on to the surface of the agar gel and subjected to electrophoresis (200 volts; 8–9 mA, 4 hours run, pH 8·6, ionic strength 0·05). Densitometric evaluation of the protein after staining with the dye was made by plotting graphically the densitometric readings made at 1 mm. intervals along the stained zone to produce Gaussian curves. The area beneath the curves was measured by planimeter. The results are shown graphically in Fig. 1. It illustrates clearly the linear relationship between the area of the peaks and the albumin concentration up to 5 g. per 100 ml.

It is necessary for quantitative estimation of the stained protein zones by densitometric method to have a simple relationship ideally linear, between the density of colour as measured by densitometer and concentration. The failure to observe this relationship by some workers on paper electrophoresis is due partly to the following causes:

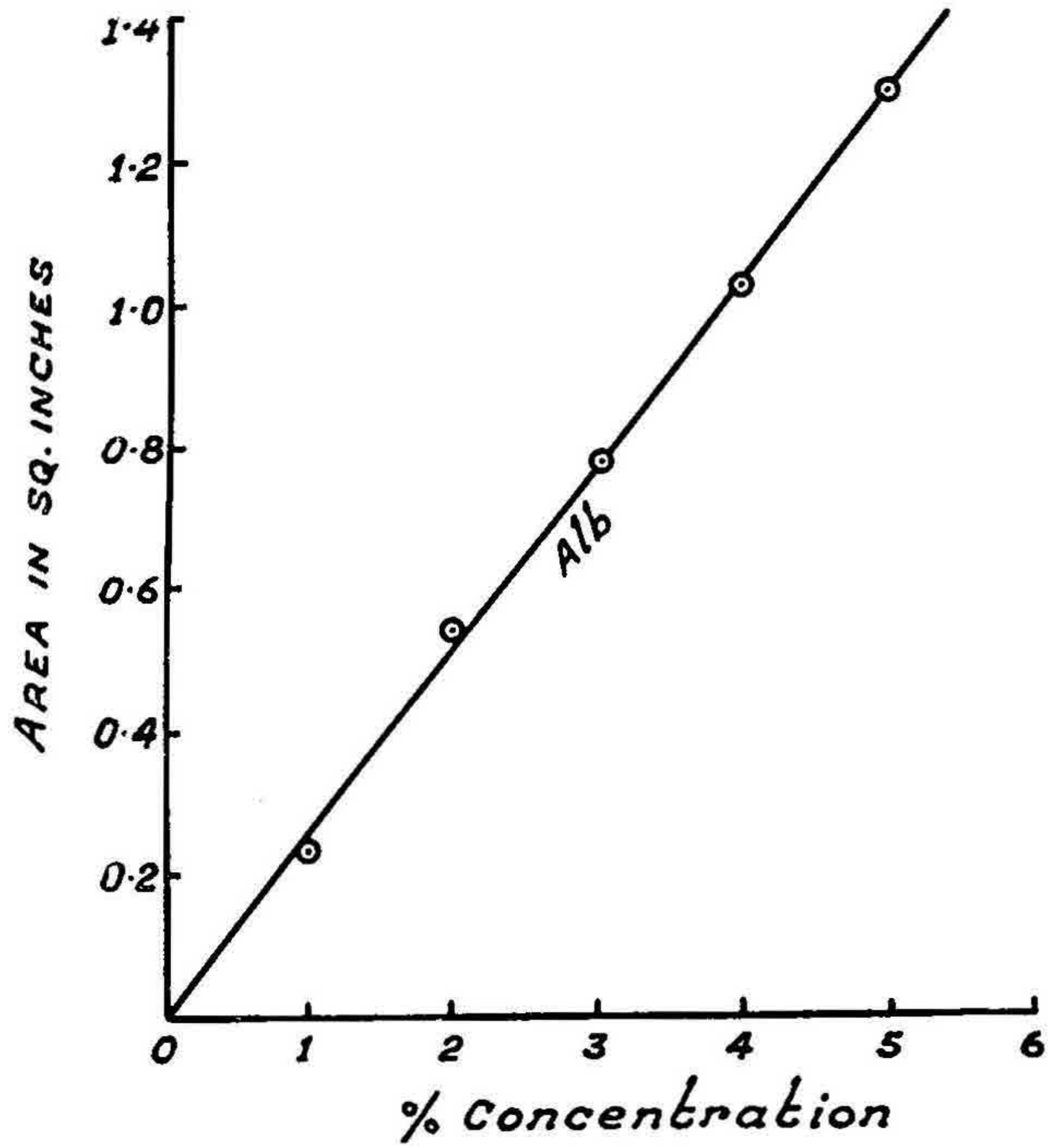

FIG. 1. Relationship between the area of the optical density peak and concentration of serum albumin.

1. The paper and the stained bands are not transparent;
2. The colour is not uniformly distributed throughout the zone; and
3. Light is transmitted unevenly through the stained paper fibres.

Agar film as a photometric medium.—For densitometric measurements to be reproducible one of the important requisites is that the colour must be uniformly distributed in the medium. This requirement has been satisfied in the case of agar gel. In paper electrophoresis, however, it is not possible to eliminate this defect which is inherent in the paper. Furthermore, the uniformity of colour depends on several factors such as the type of paper used and the method of drying the paper before staining. Filter-paper is a highly inhomogeneous medium. It consists of fibres of varying size and degree of compactness and each fibre contains number of fibrils of varying degree of orientation. In such a medium the buffer solution is held within interstices of the matrix and the proteins migrate through these interstices during electrophoresis. On drying the paper after staining, the protein collects

around the individual fibres. The colour of the protein stain is, therefore, not distributed uniformly through the paper, some areas being stained leaving the adjoining areas without stain. This is clearly shown in the photomicrograph published by Jencks *et al.* (1955).

It is, therefore, obvious that accurate measurement of colour by densitometry in such a medium is very difficult. Furthermore, the paper itself scatters light. Although attempts have been made by several workers to minimise the influence of these inherent defects of paper by carrying out the measurement of colour of the stained protein bands by reflectance densitometry and by treating the paper with immersion fluids to make it optically homogeneous, it is not possible to eliminate completely these defects which are inherent in the paper. The quantitative results obtained by some authors which are comparable to moving boundary method are due to certain compensating errors. The departure from Beer's law observed by some workers is primarily due to the non-uniformity of the distribution of the coloured material in the paper.

The important requisites for obeying Beer's law are that the medium should be translucent, homogeneous and free from light leak due to scatter. These conditions are satisfied better in agar electrophoresis than in paper electrophoresis. Figure 2 (Plate IX) shows the difference between the stained zones of albumin after electrophoresis of serum in agar gel and paper. The photomicrographs clearly indicate the more homogeneous nature of the stained albumin zone on agar film compared to the one obtained on paper. The albumin zone in paper electrophoresis shows considerable inhomogeneity.

Quantitative evaluation of the protein components in serum.—In Tables II, III and IV, the results obtained on the quantitative evaluation of the protein components in normal and pathological samples of sera by agar electrophoresis and moving boundary electrophoresis are presented.

Discussion

The comparative study of the protein components of serum obtained from 10 normal healthy individuals by agar electrophoresis and moving boundary electrophoresis (Table II) leads to the following observations.

Albumin.—The values for albumin obtained by agar electrophoresis (A.E.) method are consistently higher than the corresponding values obtained by moving boundary electrophoresis (M.E.), the average value being 55·5 for M.E. and 62·3 for A.E. If the combined values of albumin and α_1 are considered a fair agreement is obtained and the average values would be 61·9 for M.E. and 64·9 for A.E. The higher values obtained for albumin in A.E. may be attributed to the higher dye binding capacity of albumin compared to globulins. Cooper *et al.* (1954) have reported lower values for albumin in paper electrophoresis (P.E.) compared to the values obtained by M.E.

TABLE II

Electrophoretic analysis of normal serum proteins by agar and moving boundary electrophoreses at pH 8·6 and ionic strength 0·1

Sl. No.	Albumin		$Alpha_1$ globulin		$Alpha_2$ globulin		Beta globulin		Gamma globulin		Albumin/globulin		N×6·25
	M.E.	A.E.	M.E.	A.E.	M.E.	A.E.	M.E.	A.E.	M.E.	A.E.	M.E.	A.E.	
1	54·7	56·0	6·2	5·0	10·3	10·0	12·9	12·3	15·9	16·7	1·21	1·27	7·81
2	57·8	70·6	6·1	1·6	6·6	7·2	13·2	8·1	16·3	12·5	1·37	2·40	7·95
3	57·2	63·2	8·3	2·9	12·9	8·2	6·2	8·8	15·4	16·8	1·34	1·72	7·87
4	53·3	59·5	8·2	3·4	10·3	9·8	11·8	7·4	16·4	20·0	1·14	1·47	7·95
5	56·4	63·5	6·1	..	7·7	8·0	13·4	9·2	16·4	19·3	1·30	1·74	8·23
6	57·5	62·2	5·0	3·2	7·5	8·1	13·0	9·9	17·0	16·6	1·35	1·65	7·37
7	52·5	61·4	5·0	1·9	7·5	6·0	18·5	9·8	16·7	20·8	1·12	1·60	8·04
8	53·9	60·0	7·7	4·2	9·7	9·0	11·8	11·3	16·9	15·5	1·17	1·50	8·16
9	55·5	60·1	6·0	1·8	7·0	5·7	12·0	12·1	19·5	20·2	1·25	1·51	8·16
10	56·0	66·6	6·0	2·5	6·5	5·7	13·5	7·8	18·0	17·5	1·27	1·99	7·16
Aerage values													
											Albumin +$Alpha_1$ globulin		
											M.E.	A.E.	
	55·5	62·3	6·5	2·6	8·6	7·8	12·6	9·7	16·9	17·6	1·25	1·68	61·9	64·9
Standard mean deviation														
	1·72	3·57	1·13	1·07	1·93	1·41	2·81	1·66	1·23	2·42	0·09	0·31	2·05	3·11

M.E. = Moving boundary electrophoresis. A.E. = Agar electrophoresis.

TABLE III

Electrophoretic analysis of pathological serum samples by agar and moving boundary electrophoreses at pH 8·6 and ionic strength 0·1

Sl. No.	Serum	Albumin		$Alpha_1$ globulin		$Alpha_2$ globulin		Beta globulin		Gamma globulin		Albumin/globulin		N × 6·25
		M.E.	A.E.	M.E.	A.E.	M.E.	A.E.	M.E.	A.E.	M.E.	A.E.	M.E.	A.E.	
1	Cirrhosis (Infantile) ..	34·4	30·9	7·7	5·0	11·8	16·5	16·9	16·2	29·2	31·6	0·52	0·45	8·67
2	Cirrhosis (portal) ..	28·0	36·5	11·0	..*	11·0	6·2	16·0	15·6	34·0	41·6	0·39	0·57	5·60
3	Nephritis	19·5	21·6	6·5	3·6	7·5	4·6	14·0	13·5	53·0	57·0	0·24	0·28	5·22
4	Cancer (stomach) ..	28·3	40·0	10·1	5·2	23·1	20·0	5·7	8·4	31·8	25·9	0·39	0·67	7·25
5	Jaundice	45·5	53·8	5·5	..*	12·5	9·4	14·5	12·6	23·0	24·1	0·83	1·16	8·12
6	Pulmonary T.B. ..	27·8	35·0	8·7	9·4	13·3	15·3	22·4	10·8	27·8	29·5	0·38	0·54	8·67
7	,, ..	36·5	39·9	11·5	3·3	15·0	15·0	16·0	15·9	21·0	25·8	0·57	0·66	7·54
8	,, ..	14·0	24·3	14·0	7·1	16·5	15·5	12·0	15·5	43·5	37·6	0·16	0·32	6·94
9	,, ..	36·3	48·6	13·3	2·8	5·1	9·1	22·9	12·5	22·3	21·2	0·57	0·95	..
10	,, ..	41·0	51·5	8·9	3·5	8·3	9·3	19·7	14·4	22·3	21·2	0·69	1·10	7·49

* In cases where the $Alpha_1$ values are not given, the value under albumin relates to the combined values of albumin and $Alpha_1$ globulin.

M.E. = Moving boundary electrophoresis. A.E. = Agar electrophoresis.

TABLE IV

Analysis of pathological serum samples by agar electrophoresis at pH 8·6 and ionic strength 0·1

Sl. No.	Serum	Albumin	Alpha$_1$* globulin	Alpha$_3$ globulin	Beta globulin	Gamma globulin	Albumin/ globulin
1	Cirrhosis (Infantile) ..	44·7	..	16·0	14·9	24·5	0·81
2	,, ,, ..	61·6	..	11·6	12·1	14·0	1·60
3	Cirrhosis (Portal) ..	42·4	..	9·1	12·1	36·4	0·74
4	Nephritis	58·4	..	12·3	15·0	14·6	1·46
5	,, ...	55·0	..	13·5	14·0	16·5	0·82
6	,, (acute) ..	23·1	4·6	11·5	17·7	43·1	0·30
7	Albuminataria ..	42·6	5·7	11·4	20·9	19·0	0·74
8	Kwasiorkar	53·4	5·3	9·4	7·6	24·0	1·15
9	Pulmonary T.B. ..	44·0	7·1	14·7	7·3	26·7	0·79
10	,, ..	30·6	5·5	14·1	13·7	36·1	0·44
11	,, ..	50·4	5·9	16·5	13·2	13·8	1·02

* In cases where Alpha$_1$ values are not given, the value under albumin relates to the combined values of albumin and Alpha$_1$ globulin.

α_1, α_2 and β-globulins.—The values of α_1, α_2 and β components obtained by A.E. are low in comparison with those obtained by M.E. The mean value of α_1-globulin component by M.E. method is 6·5 which is much higher than the value (2·6) obtained by A.E. method. The values for α_2-globulin component are 7·8 (A.E.) and 8·6 (M.E.). Similar observations were made by Cooper *et al.* (1954) who found that slightly higher values were obtained for α_2-globulin by M.E. method compared to the values obtained by P.E. method. In most of the samples of serum investigated the β-globulin component was resolved into two components in A.E. method and the comparison was made between the combined values of β_1 and β_2 components and the β-component by M.E. method. In general, the values for the β-globulin component was found to be lower than that of M.E. method, the mean values of the β-globulin component being 9·7 (A.E.) and 12·6 (M.E.).

γ-Globulin.—A good agreement in the values for γ-globulin obtained by A.E. and M.E. methods has been observed. Cooper *et al.* (1954) have reported higher values for γ-globulin by paper electrophoresis (P.E.) method compared to M.E.

method. The average values for γ-globulin are 16·9 (M.E.) and 17·6 (A.E.), the percentage variation being 4·1.

The pathological samples of sera (Table III) show similar discrepancies between the values obtained by A.E. and M.E.

The results obtained with the pathological serum samples (Table III) show the characteristic changes in protein con.ponents in the various types of diseases investigated.

The discrepancies observed between the values for α_1, α_2 and β globulins obtained by A.E. and M.E. methods are to be expected. In free (boundary) electrophoresis the registration is based on the refraction in the concentration gradients of the boundaries. Substances other than proteins, such as lipids and carbohydrates have an index of refraction different from that of other components. These non-protein constituents contribute to the formation of boundaries. It is known that α_1, α_2 of human serum contain carbohydrates in a varying degree and the β-globulin contains lipids. The presence of these constituents in the protein components is responsible for the increase in concentration observed in M.E. method. On the other hand, in agar electrophoresis only the proteins combine with the stain and hence the values for the α_1, α_2 and β-globulins are usually low. Furthermore, the dye binding capacity of the protein components differ from each other.

Albumin-globulin ratio (*A/G*).—Albumin-globulin ratio (A/G) has been widely used by clinicians as an index of the health of the patient and for prognosis of diseases. In many types of diseases the ratio is found to change from normal range to sub-normal value. The albumin concentration in serum decreases under conditions of impaired synthesis of the protein, or increased breakdown by the liver or by the elimination of this fraction *via* the kidney. The increase in globulins occurs in diseases in which abnormal proteins accumulate as in myelomatosis or as a result of reticuloendothelia response.

The defects inherent in paper electrophoresis, such as failure to obey Beer's law and trailing of the albumin band leads to under estimation of the A/G ratio. The values obtained for the ratio by A.E. method are shown in Tables II and III. The average value for the A/G ratio is found to be 1·68 for normal serum which is higher compared to the value 1·25 obtained by M.E. method. The A/G (A.E.) ratio is found to vary from 1·2–2·4 and only in one case the higher value of 2·4 was observed. The pathological samples of sera investigated gave values less than 1·0, except in one case of tuberculosis and jaundice in which slightly higher value of 1·1 was obtained. Based on the results obtained in the present investigation, the provisional threshold value for normality can be conveniently fixed at 1·0, for agar electrophoresis.

It would be of interest to compare the value for A/G ratio obtained by A.E. method with those obtained by other methods (Table V).

The values show that by salt fractionation method higher value for the ratio is obtained. This is due to the contamination of globulins in the albumin fracti on

TABLE V

Albumin-globulin ratio obtained by different methods of fractionation and electrophoresis

	Methods	Albumin-globulin Ratio A/G	Reference
1.	Salt fractionation	2·0 (Normal)	Gutman (1948)
2.	Methanol fractionation ..	1·3–1·6 (Normal)	Pillimer and Hutchinson (1945)
3.	Moving boundary electrophoresis (M.E.)	1·5 (Normal)	Dole (1944)
4.	Paper electrophoresis (P.E.) ..	1·0 (Normal) Threshold value for normality	Abdal-Wahab *et al.* (1956)
5.	Paper electrophoresis (P.E.) ..	Less than 1·0 (Pathological samples)	Rees *et al.* (1954)
6.	Agar electrophoresis (A.E.) ..	1·25 (Normal) 1·00 (Threshold value for normal) Less than 1·0 (For pathological samples)	Giri *et al.*

The values obtained by paper electrophoresis method for normal sera corresponds approximately with the values obtained by the agar electrophoresis method. The value 1·5 obtained by moving boundary method by Dole is slightly higher than the value (1·25) obtained in the present investigation.

Dye binding capacity of serum proteins.—The dye binding capacity of human serum albumin and human γ-globulin was determined by using different concentration of the proteins. The proteins, after subjecting them to agar electrophoresis individually, were stained with Amidoschwarz dye solution and the intensity of the stained bands was measured densitometrically. The albumin binds relatively more dye than the γ-globulin and a factor of 1·8–2·0 was obtained for the ratio of the dye bound by the same amount of albumin and γ-globulin. The values obtained for γ-globulin were not corrected by multiplying the values by this factor. The satisfactory correlation between the values obtained by A.E. and M.E. methods for γ-globulin without introducing any correction factor leads to the suggestion that there is a high probability that the dye binding

capacity of the protein components present in native serum may be different from that of the isolated proteins, which may be altered perceptibly by techniques of isolation, fractionation or storage. Changes in dye binding capacity of proteins may be caused by secondary changes produced during purification process or storage.

It has been observed before by several workers that the dye binding capacity of the protein components of serum differ widely with each component, the binding capacity of albumin being always higher than that of globulins. Discrepancies have been observed by several workers on paper electrophoresis, between the results obtained by paper electrophoretic methods and the boundary electrophoresis procedure. These discrepancies have been attributed largely to the difference in the dye binding capacity of the individual protein fractions. This led to the introduction of ' correction factors ' by which the binding value of each component is to be multiplied to bring it close to the values obtained by moving boundary method and other units of measurement (Cremer and Tiselius, 1950; Kunkel and Tiselius, 1951; Grassmann and Hannig, 1952; Pezold and Peiser, 1953; Sommerfelt, 1952; Conn and Klatskin, 1954; Hardwicke, 1954; Latner *et al.*, 1954; Mackay *et al.*, 1954). Factors ranging from 0·9–8·1 and varying with each globulin of normal and pathological sera have been reported by Koiw *et al.* (1952). As the main protein fractions of serum are heterogeneous and of variable composition containing carbohydrates and lipids in varying proportions, it is very difficult to obtain a standard for comparison. As a result of the heterogeneous nature of the components, the specific refractive increments, the dye binding capacity and the nitrogen content of the fractions differ from protein to protein. It has been observed by Jencks *et al.* (1955) that the bromophenol blue binding capacity, as compared to biuret value, nitrogen content, or refractive index increment, is not the same for different proteins, more than two-fold variation being observed among different proteins. In view of the above, the conversion of the values obtained by zone electrophoresis to these obtained by moving boundary electrophoresis is rendered difficult, and the observed discrepancies or agreement between the results obtained by both these techniques are not to be taken into serious consideration. It would, therefore, appear that the introduction of arbitrary correction factors, which vary considerably from protein to protein, for the differences observed between the values obtained by the two methods, is not necessary. In agreement with the view expressed by Jencks *et al.* (1955), it is suggested that normal values for each type of zone electrophoresis of serum with a standard staining procedure should be established and the values assessed on their own merit without comparing them with the values obtained by other " classical " methods. The choice of an analytical method depends on the balance between the simplicity, reproducibility and complexity of the method. Considering the several advantages of agar-electrophoresis technique compared to paper electrophoresis and moving boundary methods, the technique affords a convenient, simple and accurate method for routine quantitative analysis of serum proteins.

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