ELECTROPHORESIS IN MEDICINE*

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Electrophoresis may be defined as the migration of charged particles in electrical fields. In principle, two different methods may be employed in the study of electrokinetic phenomena, namely, (1) by observing with the microscope the motion of inert particles, e.g., quartz, which are coated with the colloid under study, and (2) the measurement by various optical methods of the progress of boundaries formed between colloidal and buffer solutions. In recent years this moving boundary method has largely displaced the microscopic method and the term electrophoresis today is almost synonymous with the former. Studies of the behavior of colloid systems such as proteins date back as far as the first half of the nineteenth century, but the past decade has witnessed an unprecedented development of this field, largely due to the pioneering researches of Arne Tiselius[98] in Upsala and Hugo Theorell in Stockholm. For a full discussion of the history, theory, and practice of electrophoretic analysis in its various forms reference is made to the monograph by Abramson, Moyer, and Gorin[1] and to a symposium published under the auspices of New York Academy of Sciences.[18]

Perhaps the most potent stimulus for a wider application of electrophoretic analysis to biological and medical problems consisted in the availability from commercial sources of apparatus based on the ideas of Tiselius[96] and his coworkers, especially Svensson.[88, 89, 90] In this country one of the most active groups in the field has been that of McInnes and his colleagues, Longsworth[37, 38, 39, 41] and Shedlovsky at the Rockefeller Institute for Medical Research, New York City. One of the first instruments based on the original design of Tiselius was set up in the Department of Physiological Chemistry at Yale University[80] near the end of 1937 and applied to the study of anterior pituitary hormones.[78] This and later instruments, built with the cooperation of the Sloan Physics Laboratory of Yale University, have made it possible to investigate a number of problems of medical interest in close coopera-

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tion with various investigators at the Yale University School of Medicine. This work is now being continued and extended at Polytechnic Institute of Brooklyn in cooperation with the Mt. Sinai Hospital and other metropolitan medical institutions. A similar cooperative effort is being undertaken by the Electrophoresis Laboratory of the College of Physicians and Surgeons, Columbia University, under the direction of Dr. Dan H. Moore. Various other groups associated with a number of other American institutions have entered this field more recently. In England, work centers around the group of MacFarlane* and Kekwick at the Lister Institute of London, while in Scandinavia much clinical work is being carried out in close cooperation between the Medical School and the Institute of Physical Chemistry at Upsala under the leadership of Tiselius.

Commercial instruments, including the optical cells, are now available in the United States as well as in some of the European countries. In view of the ever-increasing interest in electrophoresis as a biochemical and clinical tool, it would appear that the chief impediment in the way of a more rapid expansion of its application is the lack of a simpler, less bulky, and less costly apparatus.

A survey of the literature shows that in the course of the last five years several hundred publications have appeared dealing with electrophoresis in its different aspects, and it is noteworthy that the number of publications increases at a greatly accelerated tempo as time goes on. It has, therefore, been necessary to limit the scope of the present review to a discussion of some of the more significant contributions to problems of predominantly medical interest. It is proposed to discuss electrophoretic studies on normal and pathological blood sera and plasma, reports on the electrochemical properties of isolated serum proteins, and on normal and pathological body fluids. Studies of immunological interest including those dealing with immune bodies and antigens and some recent experiments on formed blood elements will also be considered. This will be concluded by an attempt to correlate changes observed in electrophoretic patterns with the physiological state of the organism. Species differences will be mentioned briefly. Space limitations have made it necessary to exclude from the present discussion experiments on hormones and enzymes except where they are incidental to investigations of

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medical interest. No attempt has been made to treat the subject from a historical point of view or to cover the field completely.

I. *Studies on normal blood serum and plasma*

The first worker to apply the newly perfected electrophoresis technique and optical observation of moving boundaries by Toepler's "schlieren" band method[96] to the analysis of serum was Tiselius himself. He reported[95] in 1937 that horse serum, when examined in his apparatus, showed five boundaries of different electrophoretic mobility. The fastest boundary was identified with that of serum albumin; the three following boundaries were recognized as due to three different globulin components which were designated as alpha, beta, and gamma globulin in decreasing order of mobility. The fifth stationary boundary was attributed to a delta component. Plasma exhibited, in addition to the boundaries just mentioned, a sixth "schlieren" band situated between the gamma and delta boundaries which was shown to be due to fibrinogen.[25, 79] Shortly afterward, the stationary delta boundary observed in the ascending limb of the apparatus and the corresponding epsilon boundary in the descending limb were recognized[100] as boundary anomalies, largely due to the transport of buffer ions by the proteins during electrophoresis, but also partly due to a superimposed protein gradient[38] rather than an additional protein component. A low peak of high mobility (-18×10^{-5} cm^2/sec/V at pH 7.8) observed in normal plasma and serum by Moore and Lynn[51] has been recognized as a "false" moving boundary by Svensson.[91] It is due to the use of a buffer (lithium veronal, lithium chloride) containing two negative ions.

It could be shown[90, 95] that the so-called *pseudoglobulin* and *euglobulin* fractions which may be obtained by salting out and dialysis methods represent mixtures of several globulin components. These two fractions differed chiefly in their solubility behavior rather than in different composition fundamentally (pseudoglobulin was found to contain 85 per cent α globulin and 15 per cent γ globulin, while euglobulin contains more β and γ but less α globulin). In view of these findings the classical terminology was quickly abandoned and the nomenclature of Tiselius based on electrochemical properties of serum proteins has since been generally adopted. These

early observations were subsequently confirmed and considerably extended with the aid of refined optical methods, namely, the optically integrating refractive index techniques of Thovert, Philpot, and Svensson[38, 88] and the mechanically integrating "schlieren" scanning method* of Longsworth.[36, 39, 42]

At present it is only possible to determine the concentrations of the electrophoretically separable proteins by differences in refractive index (correcting for the differences in the refractive index in the various components[42]) and not in terms of protein nitrogen or dry weight. Two methods of distributing the areas among the plasma components are used—drawing an ordinate from the lowest point between two adjacent peaks, following the procedure of Tiselius and Kabat,[100] or resolving the pattern into a series of symmetrical

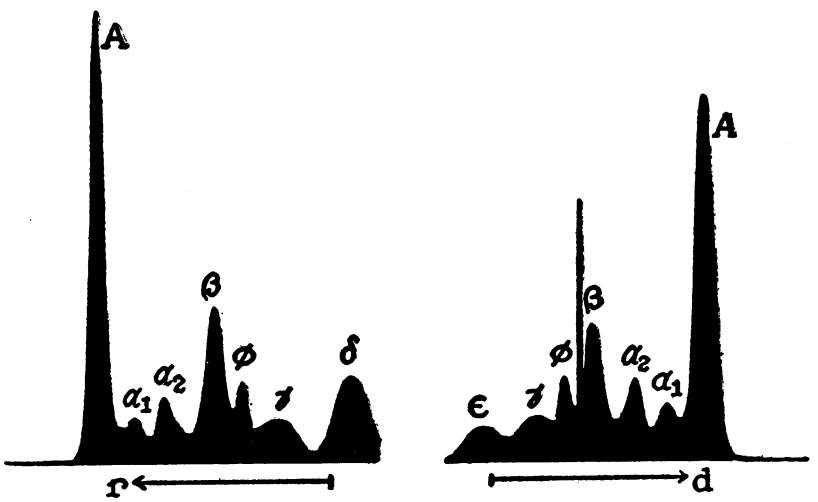

FIG. 1. Electrophoretic pattern of normal human plasma, diluted 1:2 and dissolved in a 0.1 *N* NaV-0.02 *N* HV buffer at pH 8.6. Patterns were obtained after electrolysis for 14,000 sec. at 5.38 volts per centimeter (Longsworth[38]).

curves as described by Pedersen. For a comparison of the two methods illustrated by diagram and table refer to Longsworth.[38]

It could be shown that the number of boundaries observed in the electrophoretic patterns of serum and plasma depends on the type

* Other optical methods such as the light absorption method of Svedberg and the scale refractive index method of Lamm have been employed in electrophoretic investigations.[94, 99]

of buffer employed and on the species under study,* thus the use of a sodium diethylbarbiturate buffer of pH 8.6 and an ionic strength of 0.1 leads to the resolution of human plasma into six well-defined components, namely albumin, α_1, α_2, β, and γ globulin in addition to fibrinogen and the stationary anomalous boundaries[38] as shown in Fig. 1, whereas in phosphate buffer of pH 7.7 and ionic strength 0.2 or lithium veronal buffer of pH 7.9 and ionic strength of 0.05, the α_1 globulin fails to separate from the albumin and the γ globulin remains united with the δ and ϵ boundaries respectively.

The blood sera of different animal species yield markedly different electrophoretic patterns,[14, 49, 90] thus horse serum shows two β-globulin peaks as well as two α components[31, 83, 90] while the blood plasma of dogs gives rise to an even more complex pattern[86, 103]; in addition to albumin, three α-globulin peaks, two β components as well as fibrinogen and γ globulin have been recorded.

As exemplified by the diagram shown in Fig. 1, the albumin peak is by far the tallest and best defined in normal blood sera whereas the globulin peaks are considerably lower and frequently subject to a greater degree of electrophoretic spreading†. The patterns given by the ascending and descending boundaries are not identical. The extent of dissymmetry is a function of the ionic strength of the solvent (cf. ref. 91). In general the rising albumin boundary is better defined than is the falling albumin boundary, and the β peak in the descending limb in the apparatus shows a peculiar anomaly reminiscent of a total reflection phenomenon (see Fig. 1) which has been ascribed to an instability of this component after electrophoretic separation from the accompanying serum proteins (ref. 44, p. 402). Aging of serum for periods as long as 18 months leads to a disappearance of this disturbance in the β-globulin boundary.[17] This phenomenon was correlated by the authors with the simultaneous slight reduction of the β-globulin maximum due to dissociation of lipid from the protein.

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* In the case of horse serum, the use of phosphate buffer yields a higher resolution than does sodium veronal buffer (ref. 38, p. 332).

† A progressive spreading or blurring of the boundary of a colloid migrating in an electrical field is, as a rule, only partly due to diffusion, the remainder is caused by a slight electrochemical inhomogeneity of the material under study. This second component, in contrast to diffusion, is essentially reversible in nature (cf. ref. 1, 75, 77).

The fact that a certain fraction of the serum or plasma migrates at a more or less uniform rate in an electric field should not be accepted as evidence of a chemical homogeneity of this material. Indeed, it has been shown that the various electro-chemically defined components of blood serum may be further fractionated into chemically or biologically different entities, by other methods, e.g., salt and organic solvent precipitations (cf. ref. 10). It is also important to note that the electrophoretic fractions designated throughout this paper as albumin, α, β, and γ globulin do not consist solely of protein but represent native complexes of these protein moieties with low molecular, non-protein substances. Thus, according to Tiselius,[97] the albumin fraction contains bilirubin in addition to carbohydrate, while the β globulin contains an appreciable amount of lipid such as cholesterol. It was subsequently shown[7, 103] that *all* serum protein fractions obtained by electrophoretic separation contain some cholesterol and phosphatids in bound form, although the α and β-globulin fractions are richer in lipids than either albumin or γ globulin, likewise all fractions were found to contain carbohydrate, with the α and β globulins again showing the highest percentage. In spite of this, 50 per cent or more of the total lipids and carbohydrates in serum are contained in the albumin and γ-globulin fractions since α and β globulin constitute only a minor part of the serum proteins.

The β-globulin fraction has recently received careful attention in the Harvard Medical School laboratories with particular reference to its nature as a lipo-protein complex and to the interaction between the two components and with other ions.[54] It now appears that β gobulin or one of its components is closely related to, if not identical with, the "X" protein component observed by Pedersen[57] in his studies of serum proteins in the ultracentrifuge.

The weak bonds between the proteins and these prosthetic groups are broken in the course of chemical isolation procedures; the "purified" serum proteins, e.g., crystalline serum albumin, are actually derivatives of the original complexes as they exist in native blood serum. The majority of antibodies are known to be present in the γ-globulin fraction which is perhaps the most complex and heterogeneous of all.[10]

Quantitative measurements on the relative and absolute concentrations of the various protein components in normal human plasma

and serum have been reported from various laboratories.[15, 29, 44, 51] When comparing these data, it should be borne in mind that some of them were based on only a limited number of observations. Thus, Moore and Lynn tabulate concentration ratios for a total of 25 experiments, 7, 6, and 12 of which were performed in three different laboratories. It is evident that the interpretation of such a small number of observations can have only a limited value even though statistical methods were employed in the evaluation; furthermore some of the data were obtained using buffer systems which do not resolve completely the individual components of human plasma. In the recent studies of Dole and Braun[15] veronal buffer of optimum resolving power was used, and the results are summarized in table 1. The albumin-globulin (A/G) ratio as determined electrophoretically is on the average near 1.5 according to these authors. Higher values for this ratio ranging from 1.94 to 2.25 (cf. ref. 51) are due to lack of resolution of the α globulin and the albumin gradient on account of the buffer used. The agreement with the corresponding value as determined by the sodium sulfate method of Howe, so widely used in clinical laboratories, is not too satisfactory. Thus, Dole and Braun find that the A/G ratio measured electrophoretically is approximately 30 per cent lower than that obtained by chemical fractionation of the same plasma sample. Taylor and Keys[92] find in a comparative study of 8 normal sera that the sodium sulfate technique assigned about 5.2 per cent more nitrogen to the albumin fraction than would be indicated by electrophoretic analysis. That the chemical method rather than the physical method is at fault is indicated by the findings of Pillemer and Hutchinson,[60] who found satisfactory agreement between the A/G ratio obtained by electrophoresis and their low temperature methanol precipitation technique. The values given in table 1 are based on the results obtained with 15 normal plasma samples from young adult males. On the whole no marked differences have been observed in the experience of the present authors between patterns recorded for adults of both sexes or for individuals of widely varying age. Contrary to expectations in a recent study[40] of 10 pairs of maternal and fetal plasmas it was found that the protein components of the maternal samples showed a greater variation from normal than did those of the fetal plasmas, the relative concentration of the β-globulin component being markedly elevated. On the other hand,

TABLE 1 (Dole and Braun[15])

YOUNG ADULT MALES. NORMAL VALUES 15 NORMAL PLASMAS.

Components indicated are albumin, α_1, α_2, and β globulins, respectively, and fibrinogen and γ globulin. A/G denotes albumin-globulin ratio. Concentrations are given as r (ratio of component area to total area exclusive of delta and epsilon peaks) and as grams per 100 cc.

		Albumin		α_1		α_2		β		ϕ		γ		A/G
		r	*gm. per 100 cc.*	*r*	*gm. per 100 cc.*	*r*	*gm. per 100 cc.*	*r*	*gm. per 100 cc.*	*r*	*gm. per 100 cc.*	*r*	*gm. per 100 cc.*	
Concentrations	Mean	0.603	4.04	0.046	0.31	0.072	0.48	0.121	0.81	0.051	0.34	0.110	0.74	1.53
	Standard deviation	0.028	0.27	0.007	0.051	0.013	0.083	0.019	0.126	0.006	0.059	0.025	0.151	0.181
Mobilities (cm^2/volt sec. $\times 10^5$)	Mean	5.94		5.07		4.08		2.83		2.14		1.02		
	Standard deviation	0.267		0.236		0.256		0.241		0.252		0.282		

the γ-globulin concentration of the fetal samples was greater than that of the normal or maternal values, a fact which was correlated by the authors with the well-known natural immunity of newborn infants.

The serum of the newborn calf contains a special globulin component which amounts to 20 per cent of the total serum protein and from 50 to 80 per cent of the globulin fraction.[56, 57] This globulin, which has been designated *fetuin* by Pedersen, has an electrophoretic mobility similar to that of α_2 or β globulin. Fetuin differs from the other globulin components by its solubility in ammonium sulfate solutions and by its sedimentation constant and molecular weight.*

In the serum of calf fetuses the new globulin may amount to as much as one third of the total protein and about 90 per cent of the globulin fraction. A similar substance has been found in the fetus of sheep[56] and in the foal.[56, 57] According to fractionation experiments followed by ultracentrifugal examination this globulin is present only in small amounts in the sera from the human umbilical cord, in agreement with the above-mentioned observations on fetal plasma,[40] and from the fetuses of rabbits.[57] Gross differences in the electrophoretic pattern in the serum of newborn, nursing, and mature calves have previously been reported by Jameson and colleagues.[27] These workers made the interesting observation that serum of newborn calves before the ingestion of colostrum contains no γ globulin and only small amounts of β globulin. During nursing the γ globulin makes its appearance. Moore[49] has observed that the serum of very young rats and kittens contains a minimum amount of γ globulin.

The values obtained for relative concentrations of various protein components as computed from electrophoretic data are affected to some extent by the ionic strength and the protein concentration (cf. ref. 59); thus, in 2 per cent protein solution and with veronal buffer of pH 8.6 the apparent albumin concentration decreases from 57 to 54 per cent while the γ-globulin value rises from 10 to 13 per cent upon increasing the ionic strength from 0.1 to 0.3. Similar variations were observed when the protein concentration was varied

* For information on the behavior of the various serum proteins in the analytical ultracentrifuge reference is made to the recent study by Pedersen.[57]

and the ionic strength of the buffer was maintained at a constant value. The limited number of values reported in the literature for normal plasma and sera and the marked individual differences among them render the demarcation between normal and abnormal or pathological variations in the concentration of individual components somewhat difficult at this time. Future standards will have to be based on a large series of observations made under closely controlled and uniform experimental conditions. For this reason only gross changes in the composition of individual components and the albumin-globulin ratio may be considered significant at this time.

Many of the publications mentioned above also contain data on the electrophoretic mobility of the protein components of serum and plasma (cf. ref. 15 and 44) (see table 1). The chief value of determining the mobility of the various components recorded during electrophoretic separation lies in their use for correlation of the individual maxima with the corresponding components. This however is necessary only in those instances where additional peaks are observed, as in certain pathological sera (see below), or where the entire type of the electrophoretic diagram is changed, as, for example, in nephrosis. As a rule, the mobility of the individual components is affected only to a relatively minor degree by concentration changes.* For this reason the emphasis in the electrophoretic analysis of serum and plasma under a variety of pathophysiological conditions centers at present on the shifts observable in the relative concentrations of the individual components of the protein spectrum.

The effect of various physical factors such as temperature, freezing and thawing, aging, etc., on the electrophoretic pattern of serum and plasma has not yet been studied in a comprehensive manner, although isolated observations have been reported incidental to the investigations of other problems. The changes observed in plasma and serum after storage in the liquid, the frozen, and in the dried

* The values for electrophoretic mobility as computed from observations on the rising and falling boundaries in the apparatus vary somewhat; furthermore the areas under the individual maxima are likewise not identical in all instances. Following Longsworth[38] most workers prefer to use the mobility and concentration values as derived from the descending boundaries although the anomalous shape of the β-globulin peak interferes somewhat with the area measurements by planimetry.

state have been reviewed in detail by Scudder.[67] Storage of pooled plasma from outdated "Bank" blood in the frozen state (−20° C.) and thawing at 37° do not affect the electrophoretic pattern. The same holds true for plasma dried from the frozen state by the lyophile and similar methods. The patterns of plasma samples preserved by drying at 37° in vacuo or by drying in an air-stream in cellophane containers show a decreased albumin component in both instances, a strong turbidity interfering with the optical analysis in the latter. Even in the absence of refrigeration, plasma exhibits a remarkable stability during prolonged storage as judged by electrophoretic examination; however refrigeration appears to increase the stability. Minor changes observed after storage for 53 days concern a decline in the A/G ratio, due mainly to a decrease in the albumin peak. Unrefrigerated and autopsy specimens exhibit more marked changes in their pattern. For further details, reference is made to table 2 in Scudder's paper and to the individual publications quoted by him. Observations similar to those reported on human serum and plasma have been made on aliquots of one batch of horse serum.[81] When horse serum is heated at temperatures ranging from 60 to 70° for periods of from 15 to 30 minutes[65] the serum not only becomes opalescent but the electrophoretic pattern undergoes a series of pronounced changes consisting essentially of a decrease in the albumin peak and a corresponding increase of the α and β globulin gradients which fuse into a single sharply defined peak. Upon continued heating, or increasing the temperature further, the cloudiness and viscosity of the serum increased and gel formation occurred. It has been observed[23] that the heat stability of bovine serum is considerably increased by the addition of relatively large amounts of glucose.

Irradiation with ultraviolet light at from 21 to 25°C. for about 280 hours leads[13] to the appearance of a new peak in the electrophoretic diagram, presumably representing denatured protein at the expense of other components. This material has a mobility like that of β globulin. Similar observations have been made on heat-denatured serum.[13, 65] The effect of the removal of serum lipids by acetone-ether extraction at low temperature on the electrophoretic pattern of blood serum has been studied by Blix.[5] The lipid-free serum fails to show an α-globulin boundary at pH 6.1 or 7.4 in contrast to pH 8.0 where this boundary may be observed although

at lower concentration than in normal serum. Whether this phenomenon is due to the absence of lipids or to an incipient protein denaturation could not be decided. The only other significant change in the pattern of such sera is the absence of the opalescence otherwise noted in the region of the β-globulin boundary.

Studies on pathological sera and body fluids

Two of the earliest studies on this subject were those of Blix[6] and of Hesselvik[24] in Sweden. Blix made the important observation that in *pneumonia* the α-globulin component is increased up to twice the normal concentration, while the other components remain unchanged. This finding has subsequently been confirmed in various other laboratories and extended until it has become accepted generally that the amount of α globulin is usually increased in all conditions accompanied by high body temperatures. Thus, Longsworth *et al.*[44] found for 8 samples of serum obtained from febrile patients, suffering from *rheumatic fever, pneumonia, peritonitis,* etc., an average α-globulin/albumin ratio of 0.30 which is more than twice that found for normal sera. At the same time the A/G ratio was found to be decreased. The same authors made the first electrophoretic survey of normal and pathological sera with the aid of the "schlieren" scanning method which affords diagrams most suitable for the quantitative estimation of relative and absolute concentrations of the various components present in biological fluids. In addition to the febrile conditions already mentioned, they examined sera obtained from 5 cases of *aplastic anemia,* yielding somewhat low values for the A/G ratio due to a relatively high γ-globulin concentration, and one serum from a case of *lymphogranuloma,* showing a low A/G ratio and considerable increases in the relative α, β, and γ-globulin concentration. Their findings in a few cases of *lipoid nephrosis* and *myeloma* will be discussed later. The early work published by these and other authors suffered from the use of buffers which failed to resolve the γ globulin and the anomalous stationary boundaries (δ and ϵ) as well as the albumin and α-globulin maxima. However the patterns reproduced by Longsworth and his colleagues[44] for some of the investigated pathological conditions were sufficiently striking to indicate considerable promise of this new tool in clinical medicine. It was chiefly this paper which initiated

and stimulated a flow of publications on the subject with the result that well over a hundred studies of this type have been published in the last five years.

TABLE 2 (Luetscher[45])

PERCENTAGE COMPOSITION OF PROTEINS OF PLASMA AND EFFUSIONS

	Total protein	*Albumin*	α	β	γ	*Fibrinogen*
NORMAL PLASMA						
Plasma	6.5	62.5	7.0	13.2	11.6	5.7
CIRRHOSIS						
Plasma	5.7	39.3	5.8	14.2	32.0	8.7
Ascites	0.6	41.1	4.5	13.8	34.5	6.1
Plasma	5.3	38.3	5.0	26.2	24.4	6.1
Ascites	0.5	52.0	3.4	20.0	22.5	2.1
Plasma	5.3	35.8	7.0	26.4	27.3	3.6
Ascites	0.3	48.0	6.5	19.5	26.0	—
TERMINAL GLOMERULONEPHRITIS WITH HEART FAILURE						
Plasma	6.0	43.1	3.1	31.2	16.3	6.3
Pleura (left)	0.9	65.6	4.7	12.5	12.5	4.7
Pleura (right)	0.95	67.0	4.5	12.0	12.0	4.5
LOBAR PNEUMONIA						
Plasma	5.6	40.6	10.3	26.8	13.6	8.7
Pleural fluid	4.0	50.5	14.5	19.1	13.8	2.1
Plasma	5.3	33.5	17.0	24.8	17.0	7.7
Pleural fluid	3.4	49.2	14.0	12.2	17.1	7.5
Pericardial	3.3	58.3	12.8	16.5	12.4	
TUBERCULOSIS						
Plasma	6.3	52.2	9.9	17.6	13.1	7.2
Pleural fluid	4.4	61.6	7.0	13.7	14.0	3.7
Plasma	5.7	36.1	9.5	10.9	32.1	11.4
Ascites	2.4	37.5	7.7	8.5	39.3	7.0

Infectious diseases. The observations of Blix[6] and Longsworth et al.[44] on sera obtained from *pneumonia* patients have already been mentioned. Luetscher, in his survey of various pathological states,[46] has studied the plasma as well as the pleural and pericardial effusions in several cases of *lobar pneumonia.* He finds that the plasma has a greatly decreased albumin content with an increased concentration of fibrinogen and usually of β and γ globulin in addition to the doubled α-globulin concentration previously reported by Blix and Longsworth. The protein content of the effusions was found to be as high as 3 or 4 per cent with an albumin concentration similar to that of serum. Globulin and fibrinogen concentrations were low, the latter apparently due to defibrination by the pleura. No evidence was found of a selective secretion of any one protein component into the exudate. The single sample of pericardial fluid examined did not differ significantly electrophoretically from the pleural effusions (see table 2).*

Electrophoretic experiments have recently been reported by Dole[17] and his colleagues of the Rockefeller Institute on 6 patients originally admitted with *scarlet fever,* 3 of whom subsequently developed *rheumatic fever.* Significant changes in electrophoretic pattern of similar nature were observed in the rheumatic as well as in the non-rheumatic scarlet fever patients. The albumin concentration decreased in the early stages of the disease accompanied by increases in the α_1 and α_2 globulin fractions, while the γ-globulin concentration slowly increased in the course of the illness. While there appeared to be a positive correlation between the increase in the latter component and the titer of the antistreptolysin 0, the authors were unable to remove detectable amounts of γ globulin by adsorption on live streptococci of the strain responsible for the scarlet fever of these patients. Excepting for a more delayed development in the abnormal features of the electrophoretic pattern in the case of the rheumatic individuals, no marked differences were found between these sera and those obtained from non-rheumatic scarlet fever patients. Longsworth et al.[44] had previously noted the increase in the α-globulin/albumin and the γ-globulin/albumin ratios

* It should be noted that the experiments forming the substance of this table were carried out with a phosphate buffer of ionic strength of 0.2 and pH 7.7. The mobilities quoted for the individual components by the same author differ therefore somewhat from those contained in table 1.

in 3 cases of rheumatic fever. The latter phenomenon has been confirmed by Luetscher who studied 2 instances of acute rheumatic fever affecting the heart and leading to congestive failure.[45] More recently,[61] Rutstein et al. determined the γ-globulin/albumin and γ-globulin/total protein ratios in 9 samples of sera from 8 patients in acute stages of rheumatic fever. With the possible exception of one, all of the ratios were elevated above the normal value. These ratios persisted on a somewhat elevated level in 3 of these patients during inactive stages of the disease. It is interesting to note that in this series of experiments the α-globulin/albumin ratios were found to be less consistently increased than had previously been reported by other workers in this and other febrile diseases.

In the single instance where the serum of a patient was examined before and after salicylate therapy by Rutstein et al.[61] a drastic decrease took place in the concentration of all globulin components. However, this observation may be fortuitous, since in 2 other cases which had received salicylate therapy, studied by these authors, the γ-globulin concentration was of the same order as in untreated patients.

As in other febrile diseases, the plasma of patients with active tuberculosis shows an increase in the α-globulin fraction.[46] The albumin is reduced to a variable extent while fibrinogen and γ globulin is found to be increased, the latter especially in active cases (see table 2). As may be seen in the data from the same table, the protein content of pleural effusion and of ascitic fluid from 2 cases of *tuberculosis* was found to be relatively high: the distribution of protein over the various components was similar to that in the plasma of the same individual. The most extended electrophoretic studies on crude and on purified tuberculin antigens and of the blood serum of tubercular human patients and rabbits have been carried out by Seibert and her associates at the Phipps Institute of the University of Pennsylvania. Tuberculin preparations were fractionated by electrophoresis into several protein components, polysaccharides, and nucleic acids.[68, 72, 73, 74] The two purified protein components, of different molecular weight, when injected into rabbits elicited the formation of antibodies migrating with the α and γ-globulin components of the serum respectively.[71, 72] The same workers have been able to correlate the clinical progress of the disease with changes in the electrophoretic pattern of the serum of experimental animals

and human patients.[69, 70] With the advance of tuberculosis, a progressive decrease occurred in the absolute and relative albumin concentrations. The first change noticeable in the early stages of the disease is usually a rise in the relative amount of α globulin, as a rule accompanied by the appearance of an abnormal component designated as "X" fraction (not to be confused with Pedersen's "X" protein) having a mobility slightly higher than that of albumin. These changes are interpreted by Seibert and Nelson as probable evidence of sensitization to tuberculin protein. As the disease reaches the terminal stage, the β-globulin concentration increases, whereas a rise in the γ globulin possibly may accompany the development of resistance to the disease.

A careful study of the plasma proteins in *disseminated lupus erythematosus*, a disease of obscure but possibly infectious etiology, has been carried out by Coburn and Moore.[9] On the basis of material covering 30 cases of the disease, the authors conclude that a large increase in γ globulin (hyper gamma-globulinemia) of unknown origin is a constant feature of *disseminated lupus erythematosus*. The γ-globulin fraction as obtained by electrophoretic separation had a sedimentation constant identical with that of normal γ globulin, and contained the relatively highest fraction of an antibody which reacts with certain phosphatids and which is responsible for the false positive Wassermann and Kline tests given by the sera of these patients. It is suggested "that the vascular lesions and disturbances in nutrition observed in this disease may be associated with the presence of a high concentration of circulating γ globulin."

A considerable number of studies has been carried out on the changes in electrophoretic pattern of the sera of experimental animals during immunization and hyperimmunization with bacterial antigens, chiefly for the production of antisera for therapeutic purposes (cf. ref. 1). Inasmuch as studies of this type are somewhat beyond the main scope of this review, only a few of the more significant contributions will be mentioned here. Among the first observations in this field were those of Tiselius[95] on the migration of the antibody in highly potent egg albumin antiserum of rabbits with the γ-globulin fraction and those of Tiselius and Kabat[100] on pneumococcus antisera of various animals. They made the interesting observation that the antibody present in antipneumococcus I horse serum migrates with a mobility intermediate between that of the

β and γ-globulin components, while in the majority of cases the corresponding antibodies in rabbit, pig, and monkey sera form a part of the γ-globulin fraction. Wyckoff and his collaborators (cf. ref. 53) extended these observations considerably. They demonstrated an extraordinary increase in the γ-globulin component in horse serum as a result of hyperimmunization to pneumococcus antigen and the sharp decrease in this component after adsorption with specific polysaccharide. In contrast to the observations with antipneumococcal serum, Wyckoff's group[63, 64] found that antitetanus and various other antibacterial horse sera show, in addition to a considerable increase in the γ-globulin concentration, a new component called by them "T" with an electrophoretic mobility intermediate between β and γ and frequently overlapping with these two globulin fractions. The correlation between the amount of "T" component in hyperimmune sera and their antitoxic activity was poor, suggesting by inference that the latter is associated essentially with the γ globulin rather than with the "T" component. Kekwick and Record[31] attempted to correlate with flocculation time and other immunological characteristics the physical properties of diphtheria antitoxic horse sera, as determined by ultracentrifugal and electrophoretic methods. They demonstrated that the immunization with diphtheria toxoid leads to the formation of two antitoxins which are associated with the β and γ-globulin fractions respectively. This was substantiated by the immunological examination of fractions rich in either β or γ globulin prepared by salt fractionation followed by electrophoretic separation. The changes occurring in the electrophoretic pattern of horse serum as a result of hyperimmunization with diphtheria antigen are illustrated[83] in Fig. 2.

Upon adsorption of such hyperimmune serum on purified diphtheria toxin in vitro, the area under the large maximum in the region of the β-globulin boundary is reduced and its composite nature is clearly indicated by a split in the peak.[83] The reaction between purified diphtheria antitoxic globulin and diphtheria toxin has been studied by Pappenheimer et al.[55] The effect of proteolytic enzymes (pepsin and takadiastase) on the electrophoretic diagram of various antibacterial horse sera has been studied by several investigators.[19, 62] In all instances the patterns of the enzyme-treated sera were considerably simplified.

Relatively few electrophoretic studies have been published on

other infectious diseases, including those caused by protozoa, viruses, or unknown agents. While it has been known for some time that paroxysms in *malaria* are followed by a decrease in the albumin and by a relative increase in the globulin fraction, as determined by the usual methods of clinical chemistry, the electrophoretic changes in the plasma proteins of patients with relapsing malaria have been described only recently by Dole and Emerson.[16] The total serum protein in the plasma of 8 patients with relapsing *P. vivax malariae* was found to be within the normal range; however, there was a shift in the A/G ratio due to a relative increase in the fibrinogen/γ-globulin concentration. In the 2 cases which were studied over a period of time there was a tendency for the electrophoretic pattern of the plasma to return to normal although the infection persisted in a patient suffering from severe *P. falciparum malariae*; the total protein concentration was greatly decreased, mainly due to a lowered albumin content. It was the impression of the authors that the determinations of total protein and of the A/G ratio may serve as indicators of the severity of the disease, but that the changes in the plasma protein pattern possessed no diagnostic significance.

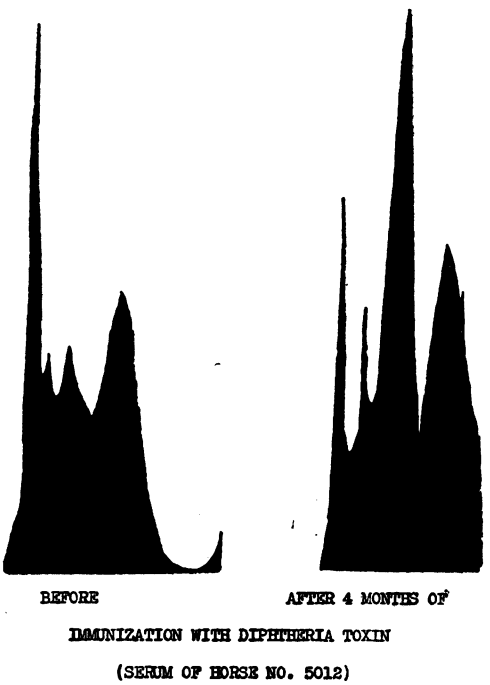

FIG. 2. Horse serum before and after 4 months of immunization with diphtheria toxin (Stern and Fell[88]).

H. B. Bull,[12] in his recent text on *Physical Biochemistry*, states that J. A. Cooper and D. H. Atlas, working in his laboratory, have made the striking observation that the β anomaly which is always observed in fresh normal serum in the descending limb of the Tiselius apparatus was completely absent in the serum of 13 syphilis patients in the primary, secondary, and tertiary stages of the disease.

"So characteristic is the absence of beta anomaly in syphilitic sera that they were able to use this feature as a diagnostic aid for syphilis. As is well known, Wassermann and Kahn tests give false positive reactions which are unrelated to syphilis. In some of these conditions and possibly in all of them, beta anomaly is present."

As yet these findings do not appear to have been published in full, and an independent confirmation would appear to be highly desirable in view of the finding of Dole et al.[17] that aged sera failed to show disturbance in the β globulin. However, Cooper[11] reported electrophoretic experiments on syphilitic sera before and after inactivation by heating to 56°C. for 30 minutes and also after inactivation and flocculation with Kahn antigen. While the heat inactivation alone produced no significant changes in the patterns, the reaction with the antigen removed some of the γ-globulin fraction. Electrophoretic and serological examinations of protein fractions isolated from such sera with the aid of ammonium sulfate precipitation indicated that the β or γ-globulin fractions, or both, are the carriers of the Kahn and Wassermann reagins.

In the virus-induced *lymphogranuloma venereum* an elevation of the globulin fraction of the serum (usually limited to the γ-globulin fraction) appears to be an important feature of the disease.[20] The observations made by Longsworth et al.[44] on a single case of *lymphogranuloma* (Hodgkin's disease) have already been mentioned. One of the present authors,[87] in collaboration with the research group studying this disease of unknown etiology at St. Vincent's Hospital, New York, is conducting a study of the electrophoretic pattern of sera from patients with Hodgkin's disease. Although no definite conclusions may be presented at this time, one has the impression that the changes observed in the electrophoretic diagram of such sera bear a relation to the clinical state of the patients rather than being specific or characteristic for the disease itself.

Non-infectious diseases: An electrophoretic study of 7 cases of *cardiac failure*[46] revealed a lowered plasma albumin and a raised β and γ-globulin concentration, whereas the α-globulin and fibrinogen levels were normal or slightly reduced (see table 2). Effusions (pericardial and ascitic fluids) differ in their protein distribution from the corresponding blood plasma in the sense that the albumin concentration is relatively higher and the β globulin is relatively

lower. The absolute protein concentration in the effusions varied from 1.0 to 3.7 gm./100 cc.

In liver disease only a limited number of cases have been studied with the aid of this new physical chemical tool. The plasma of a patient suffering from *obstructive jaundice* was found to contain a large amount of material moving with the mobility of β globulin, along with an abnormally high content of cholesterol. Extraction of the plasma with ether reduced the β-globulin peak greatly.[44] In a more extended study, Gray and Barron[21] found, however, that *extrahepatic jaundice* caused by gallstones, even though quite severe, does not produce a significant alteration in the electrophoretic pattern of the serum. In spite of the fact that the serum cholesterol was greatly increased in all 5 cases studied, no consistent increases in the β-globulin peak were observed. The authors were inclined to consider the increased β-globulin concentration as a manifestation of a hepatic parenchymatous disorder as evidenced microscopically by focal areas of necrosis and by pyknosis of the liver cell nuclei. Sera of patients suffering from *cirrhosis* of the liver have been examined by several workers. (See Fig. 3 for electrophoretic pattern of a patient with cirrhosis of the liver.[82]) Luetscher[45, 46] finds that the total plasma proteins in the early stages of portal cirrhosis are normal, or occasionally above normal. Electrophoretic analysis discloses, however, that a decrease in the albumin fraction is at least partially compensated by an increase of β and especially of the γ-globulin fraction. These changes are particularly marked when ascites develops (see table 2). For a comparative study of the partition of serum proteins by Howe's sodium sulfate method with the results of the electrophoretic analysis of sera in a number of instances of liver cirrhosis, reference is made to the work of Gutman and his colleagues.[22] More recently, Gray and Barron,[21] after studying 12 patients with cirrhosis of the liver of various types, have stated that the serum protein pattern is more abnormal in this than in any other form of liver disease. The relative serum albumin concentration was decreased on the average 45.7 per cent, varying from 31.6 to 64.5 per cent. The most consistent and characteristic change in the globulin fraction consisted in a mean increase of 100 per cent over the normal in the γ-globulin fraction, whereas the changes in the α and β-globulin components were irregular. As the chief result of their electrophoretic study of the serum proteins in acute parenchy-

matous liver disease, cirrhosis, and cancer of the liver, as well as in extrahepatic jaundice, the authors conclude that the most characteristic change consists in a large increase in the γ globulin and a decrease in the serum albumin concentration, with the extent of abnormality depending on the severity of the disease. It is believed that the serum protein changes occurring in diseases of the liver are caused chiefly by the failure of the liver to synthesize normal serum proteins rather than by the loss of proteins in the ascitic fluid. The mechanism of the flocculation of cephalin-cholesterol emulsions by the γ-globulin fraction isolated from normal and hepatitic serum has recently been studied by Moore et al.[52] with the result that the flocculating power of the γ-globulin fraction may increase in hepatitis whereas the electrophoretically separated albumin fraction, especially from normal sera, may inhibit the phenomenon.

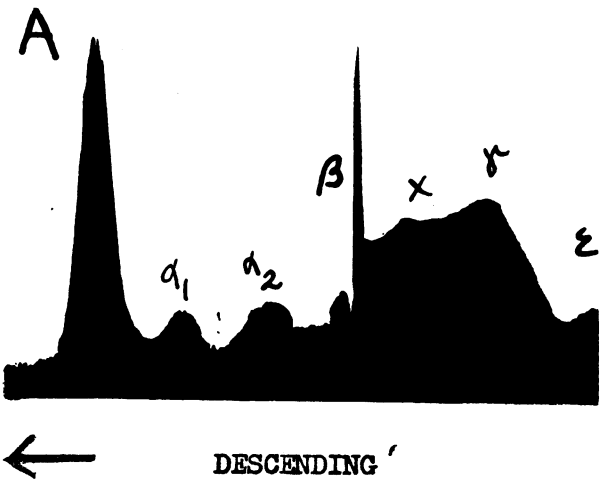

FIG. 3. Electrophoretic pattern of a serum from a patient with cirrhosis of the liver.

One of the most promising problems in the field of electrophoretic analysis of pathological states is that of *renal disease*. The first observation of the highly atypical serum pattern in *nephrosis* may be found in the paper by Longsworth, Shedlovsky, and MacInnes.[44] This diagram showed an extremely low albumin peak and an electrophoretic component migrating with the velocity of α globulin and having a concentration comparable to that of albumin in normal serum. The β-globulin component was also markedly elevated. Shortly afterwards Longsworth and MacInnes[43] published the results of a study of nephrotic sera and urines which, in spite of the small number of cases investigated, were of considerable significance. It was found that the urine of such

patients gave an electrophoretic pattern closely resembling that of normal serum and in striking contrast to the highly abnormal diagram of the serum of the same patients. Electrophoretic patterns of the urine and serum from a case of nephrosis are presented in Fig. 4.

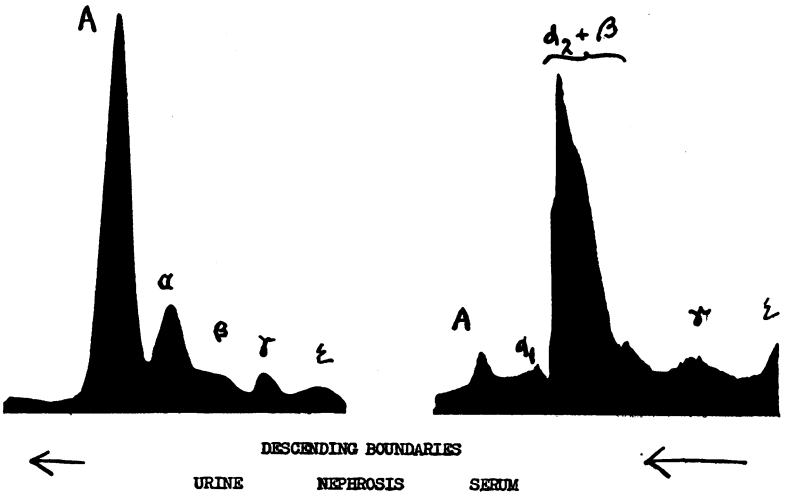

FIG. 4. Electrophoretic patterns of urine and serum from a nephrotic patient.[82]

This observation not only disproves the common notion that the urinary protein in nephrosis essentially represents albumin but it also indicates clearly that the excretion of urinary protein by the kidney is a highly selective process rather than a simple filtration. Ether extraction of a nephrotic serum causes a drastic reduction of the β-component peak which together with chemical lipid determinations strongly suggest that the β peak is due to a lipoprotein complex. Osmotic pressure determinations by Bourdillon and a comparison of the mobilities of the urinary albumin with that of the serum are suggestive of the non-identity of the two proteins although this point remains to be established by future experiments. In the course of this work, Shedlovsky contributed the important observation that nephrotic sera may be clarified by high-speed centrifugation without the loss of protein-bound lipids which are present in true solution. Many of these findings were independently confirmed and extended by Luetscher.[45] Plasma specimens of three

cases of *glomerulonephritis* had an approximately normal protein pattern with minor changes in the albumin and β-globulin components. The urine of these patients showed a lower total protein concentration than was found in nephrosis, but showed similar relative concentrations of the constituents. Electrophoretic and chemical analyses of the proteins present in nephritic urine and of the effect of proteinuria on the human kidney have been described by Blackman and Davis.[3, 4] It was found that the concentration of γ globulin was especially high in the urine of those patients where renal insufficiency was advancing rapidly. In contrast to this finding, the γ-globulin concentration was found to be very low in the urine of a patient with chronic lipoid nephrosis and normal kidney function. The absence of detectable amounts of fibrinogen in the urine of patients with progressing nephrotic nephritis indicates that the hyaline materials which collect in the glomeruli and tubules of the kidney in these patients are, in all probability, formed from globulins rather than from fibrinogen. The phenomena observed in a case of progressive renal insufficiency associated with multiple myeloma[2] are more complex and will be discussed later.

A protein spontaneously precipitated from the cooled blood of a patient having *acro purpura, chronic glomerulonephritis,* and *congestive heart failure* was isolated by Lerner and Greenberg.[32] It resembled γ globulin with respect to its solubility, ultraviolet absorption spectrum, and nitrogen content. The method of isolation, the electrophoretic pattern, and phase rule studies indicated that the purified protein preparation probably was homogeneous.

A limited number of studies has been conducted on the sera of patients with defects in the organs of internal secretion. In cases of *hyperthyroidism* the most notable and consistent deviation from the normal was a low absolute and relative albumin concentration.[33] This was frequently accompanied by an increase in α globulin and occasionally also of fibrinogen. Within several months after thyroidectomy the plasma protein had returned to normal or nearly normal in agreement with the subsidence of the disturbance. Progressive exophthalmus following thyroidectomy or x-ray irradiation of the thyroid was accompanied by strongly abnormal plasma protein diagrams characterized by a low albumin concentration. All adult *hypothyroid* patients showed a decrease in the relative albumin concentration, similar to that observed in *hyperthyroid* cases. How-

ever this time the β-globulin concentration was significantly increased and the α globulin was decreased to a varying extent. Thyroid therapy in these cases caused a fall in the β globulin and a rise in concentration of α globulin.

The observations made on the sera of normal and of *hypophysectomized* rats are somewhat conflicting. Li[35] finds that a component labelled by him as "X," which has a mobility intermediate between albumin and α globulin and which occurs in normal rat as well as in mouse serum,[8] is absent from the serum of hypophysectomized animals. Furthermore, he found an increased total globulin concentration which was responsible for a decrease of the A/G ratio from 3.0 in the normal to 1.33 in the hypophysectomized rat. Moore et al.,[50] on the other hand, state that in the serum of normal rats only β and γ globulin are present (as previously reported by Jameson and Alvarez-Tostado,[26]) but that the α-globulin component appears in rat serum after hypophysectomy, coupled with a moderate increase in the γ-globulin concentration. With regard to the shift in A/G ratio the observations of the two groups of workers are essentially in agreement. It is possible that the discrepancies here mentioned are at least in part due to the different buffers and protein concentrations employed.

Injection of a gonadotropic extract prepared from human pituitary glands into goats[93] produced an increase in the γ-globulin fraction of the serum corresponding to the increase in antihormone activity. Experiments on serum fractions prepared by electrophoresis demonstrated that the entire antihormone activity is associated with the γ-globulin fraction.

The sera of 30 patients with *diabetes mellitus* have been examined in the Tiselius apparatus.[34] In severe states of diabetic acidosis the albumin concentration was decreased while the β globulin was greatly increased, with the result that the total protein value was within the normal range. In cases of mild diabetes the relative protein concentrations were normal, while the total protein level was slightly lowered. Adequate therapy restored to normal the plasma protein concentration. When the diabetes was complicated by diabetic retinitis the albumin concentration in the plasma remained low in spite of the treatment of the underlying condition.

In patients with *Addison's disease* showing definite symptoms of adrenal insufficiency, the total protein was, as a rule, within the

higher normal range.[46] While the relative albumin concentration was significantly decreased throughout, the relative increases in globulin content could not be attributed consistently to any one fraction. Administration of large amounts of crude adrenal extract in contrast to therapy with pure desoxycorticosterone restored the protein distribution to normal or nearly normal values.

Some of the most interesting observations in this field have been made in electrophoretic studies on *multiple myeloma* patients. Electrophoretic patterns of blood serum from 2 multiple myeloma patients[81] are shown in Fig. 5. The report of Longsworth et al.[44] contains the diagram of a plasma cell myeloma, in which the albumin component was decreased about 50 per cent and

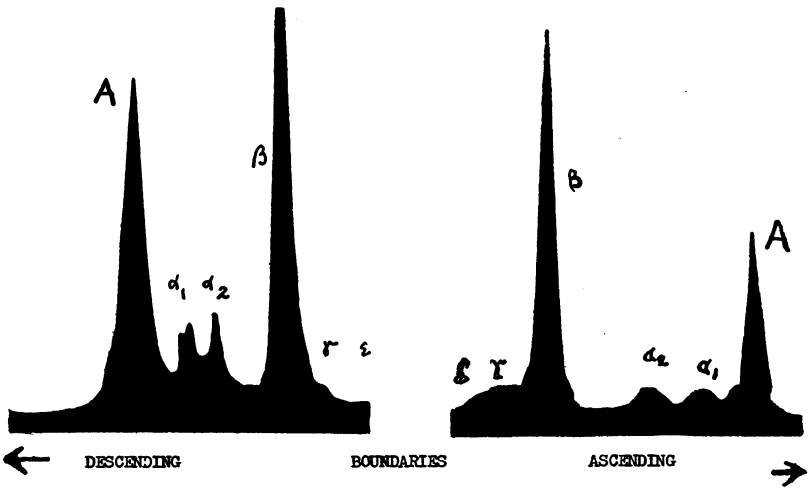

FIG. 5. Electrophoretic patterns of sera from two patients with multiple myeloma.

a large peak in the β-globulin region was the outstanding feature. Of the 3 cases of multiple myeloma studied by these authors another gave a pattern similar to that just described, while the third yielded a normal pattern. Myeloma sera have also been studied independently by several other groups of workers. Kekwick[30] examined 5 human myeloma sera by means of the analytical centrifuge as well as in the Tiselius apparatus. According to this author, the sera of myeloma patients fall into two groups—the first group shows the same number of protein components as normal serum and a large

increase in the γ-globulin fraction; the second group of sera shows up to 5 components in the ultracentrifuge and a greatly increased β-globulin peak in the electrophoresis apparatus. For additional centrifugal studies of myeloma sera the papers of MacFarlane[48] and of Jersild and Pedersen[28] should be consulted. The experiments just cited throw no light on the relationship between serum proteins and Bence-Jones protein: none of the cases studied by Kekwick showed any proteinuria. The most extensive study on myeloma to date has been conducted by Gutman and his colleagues,[22] who examined 7 additional cases of multiple myeloma. Two of these cases showed a very large increase in the γ-globulin peak and an extra component corresponding to that of fibrinogen in plasma; 2 others exhibited a pronounced increase in material of β-globulin mobility but with no "M" boundary; 2 other cases finally yielded patterns simulating those of normal plasma and serum, respectively. These experiments were supplemented by serum protein studies by the Howe method in 38 cases. It is noteworthy that by adding urinary Bence-Jones protein to normal serum the essential pattern observed in myelomatous sera could be reproduced. The electrophoretic examination of the plasma and serum of another patient[75] disclosed a large peak with the mobility of -1.9×10^{-5} units at pH 7.8. This component had the molecular weight of about 160,000 and was found to be identical with neither fibrinogen nor Bence-Jones protein. On dialysis against distilled water a considerable amount of viscous protein precipitated, which these authors believed to be formed by the reaction of two different serum components. The protein excreted in the urine of another patient with multiple myeloma and progressive renal insufficiency[2] was demonstrated by electrophoretic and chemical methods to be Bence-Jones protein. The further claim of these authors that this is identical with the β globulin of normal blood plasma is very doubtful.

The electrophoretic patterns of the sera of 12 cases of *sarcoid* have been studied by Fisher and Davis.[20] In those instances where there were no clinical signs of activity, the diagrams were almost normal showing only a slight decrease of albumin and increase of α globulin. The sera from clinically active cases, on the other hand, showed a marked elevation of the γ-globulin component at the expense of the albumin. The patterns are similar to those observed when antibodies are formed in response to an infectious agent.

Trauma and pathophysiological states: Lymph and serum in experimental *burns* have been examined electrophoretically by Perlman, Glenn, and Kaufman,[58] who compared the patterns of normal lymph and serum in calves with those obtained after the animal was subjected to burns. Normal lymph contained the same components as serum but lymph from the burned tissue revealed an additional boundary, migrating with half the speed of γ globulin, which they designate as "s" component. The serum of the burned animals showed a slight decrease in the A/G ratio with an increase in the α-globulin fraction.

The electrophoretic pattern of plasma protein regeneration has been investigated in dogs by Zeldis and Alling.[101] Acute depletion is accomplished by massive bleeding with the simultaneous return of washed red cells—*plasmapheresis*. During the first 24 hours following depletion of appreciable quantities of all electrophoretic components, all plasma proteins enter the circulating blood stream even when food is not given. In such fasting periods, albumin and globulin appear in approximately normal proportions. Both α and β globulins continue relatively elevated during the early portion of the recovery period, suggesting a rapid formation from tissue reserves as well as a rapid synthesis from dietary protein. Initial albumin levels are regained more slowly than are those of total globulin, so relative proportions of the electrophoretic pattern may be disturbed for as long as from 2 to 3 weeks after the total protein level has returned to normal. Similar observations have been made by R. H. Silber and one of the present authors.[86]

Depletion of plasma proteins in dogs due to many weeks of low protein feeding has been investigated in the same laboratory[102] by electrophoretic as well as by chemical analyses. Long-continued restriction of dietary protein results in striking decreases in plasma albumin, although the depletion of electrophoretic albumin is considerably greater than that of the chemical albumin fraction. All of the electrophoretic globulin areas are increased during depletion, especially the α globulin. This increase is not detectable by chemical protein analysis, but is found to be associated with the elevated plasma lipid levels which occur in these depleted dogs. Normal chemical albumin concentrations are usually restored within one week when large amounts of protein are fed, while electrophoretically determined albumin requires 3 weeks or longer to reach the normal amount. Total circulating globulin concentrations are only slightly

decreased or may actually increase, particularly β globulins. Besides the type of protein fed, the restoration of total plasma protein may be influenced by the degree of depletion of tissue protein reserves, the quantity of protein fed, or the specific amino acid requirement, or possibly the vitamin supplement.

During the last year, the present authors had the opportunity to investigate 2 cases of *hypoproteinemia* in children, characterized not only by a low protein content but also by an *almost complete absence*

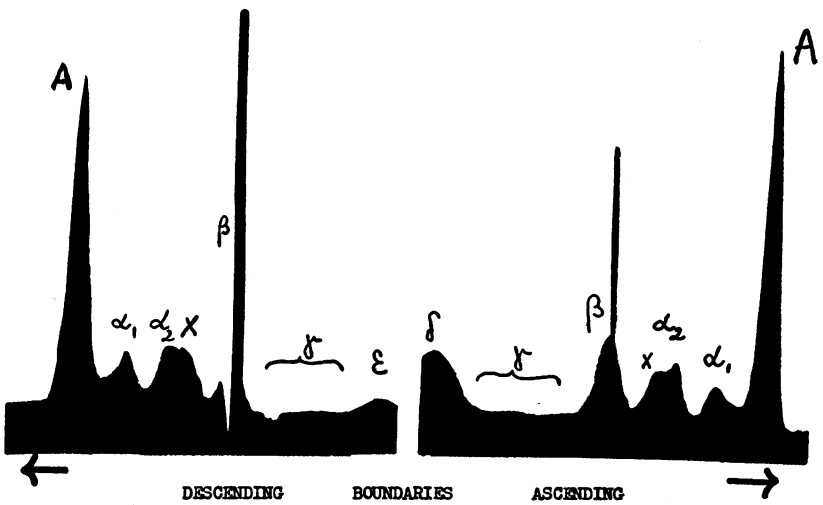

FIG. 6. Electrophoretic pattern of 3-year-old boy (F. P.) showing absence of γ-globulin.

of γ globulin. The progress of the 12-year-old girl has been followed since birth[66] and all attempts to raise the total protein have been only temporarily successful. Contrary to expectations she has been singularly free from infections. The electrophoretic pattern of a similar case in a 3-year-old boy (F. P.) demonstrates this lack of γ globulin (see Fig. 6).[81]

Since the sedimentation rate is increased in many pathological conditions, especially in acute infections, Shedlovsky and Scudder[77] have attempted to correlate it with the electrophoretic patterns of 21 normal or pathological human bloods. No significant deviation from normal was found in chemically induced shock in *demented patients.* The α-globulin level and the sedimentation rate are sig-

nificantly increased in febrile infections as well as in cases of extensive tissue destruction, as is evidenced by the patterns for coronary thrombosis, burns, and fractures. The correlation between sedimentation rates and α-globulin levels is as good as that with fibrinogen. The addition of purified fibrinogen to normal blood failed to increase the rate of sedimentation of erythrocytes to the same extent as does a comparable amount present in pathological blood.

Preliminary electrophoretic experiments[85] on hemolysates of red blood cells from several species — man, dog, chicken — have disclosed patterns considerably less complex than those for the sera of the same species. The major component is represented by hemoglobin but, in addition, all hemolysates showed the presence of a small amount of an *opalescent, colorless* fraction of an anodic mobility of about 6×10^{-5}cm^2 per volt per second at pH 8.6, as compared to about 3×10^{-5} for hemoglobin. The mobility of the opalescent material is of the same order as that of intact erythrocytes, suggesting that it is derived from the membrane or stroma of the cells. This fraction, called *a-component,* has recently been separated into a clear, low-molecular and an opalescent, macromolecular protein fraction by high-speed centrifugation. The relationship of these proteins to choline esterase is under investigation in collaboration with Dr. R. Brauer of Harvard Medical School.

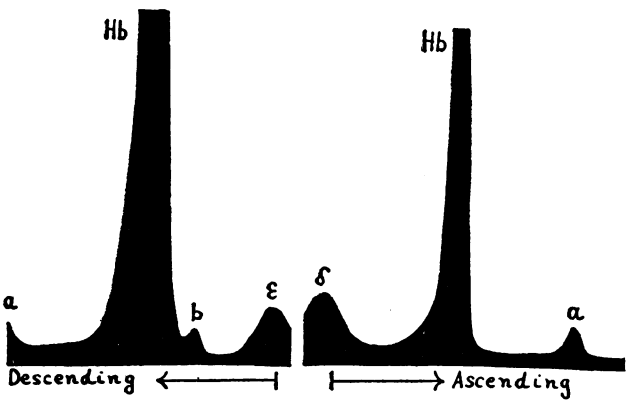

FIG. 7. Electrophoretic patterns obtained with human red blood cell hemolysates. The diagrams of the descending and ascending boundaries were obtained in different experiments with different samples of human erythrocytes (Stern, Reiner, and Silber[85]).

Conclusion

From the foregoing discussion it is evident that the diagnostic value of the Tiselius technique in the field of clinical medicine does not lie in the absolute specificity of electrophoretic patterns for

specified diseases. The protein spectrum in plasma and serum is the resultant of a host of factors concerned with the formation, the interaction, and the destruction of the individual components. The blood, while often considered a tissue in itself, is perhaps more dependent on the physiological state of the organism as a whole than is any other tissue. It is, therefore, only natural to find that there exists a close correspondence between the blood, the protein system, and the physiological state of the individual as a whole, rather than to a given pathological condition.

This explains why in all diseases accompanied by fever and tissue destruction the concentrations of the α globulin components are significantly increased and why in all instances, with few exceptions, where an antigen-antibody system is involved specifically, the γ-globulin concentration is found to be markedly elevated. There is a strong indication that future work in this field might most profitably be focused on long-range studies in which an attempt is made to correlate the clinical course of various diseases with shifts in the electrophoretic patterns of the blood and plasma of selected patients, with due consideration to the effect of therapeutic measures on these variables. In any event, there can be no doubt that the electrophoretic analysis of serum protein affords a more detailed and reliable picture of this labile biological system than does any other chemical or physical chemical method used thus far, including even the ultracentrifugal method of analysis. Not only does this new tool yield accurate values for the albumin/globulin ratio but it yields quantitative information on a whole series of well-defined blood proteins which cannot be differentiated by other methods and which, no doubt, are of primary importance in the physiology of the organism.

NOTE: As this paper goes to press, Dr. Eric L. Alling of the University of Rochester School of Medicine is reported to have stated, at the Centennial Celebration of the University of Buffalo, held in September 1946, that of 125 sera of cancer patients examined in the electrophoresis apparatus only one showed a normal pattern. A critical evaluation of Dr. Alling's findings will have to await a detailed publication of his experiments.

REFERENCES

1 Abramson, H. A., L. S. Moyer, and M. H. Gorin: *Electrophoresis of Proteins.* Rheinhold Publishing Company, New York, 1942.

2 Blackman, S. S., W. H. Barker, M. V. Buell, and B. D. Davis: J. Clin. Invest., 1944, *23*, 163.

3 Blackman, S. S., and B. D. Davis: J. Clin. Invest., 1943, *22*, 545.
4 Blackman, S. S., and B. D. Davis: South. Med. J., 1943, *36*, 247.
5 Blix, G.: J. Biol. Chem., 1941, *137*, 495.
6 Blix, G.: Ztschr. f. d. ges. exper. Med., 1939, *105*, 595.
7 Blix, G., A. Tiselius, and H. Svensson: J. Biol. Chem., 1941, *137*, 485.
8 Bourdillon, J., and E. H. Lennette: J. Exper. Med., 1940, *72*, 11.
9 Coburn, A. F., and D. H. Moore: Bull. Johns Hopkins Hosp., 1943, *73*, 196.
10 Cohn, E. J.: Chem. Rev., 1941, *28*, 395.
11 Cooper, J. A.: Proc. Soc. Exper. Biol. & Med., 1944, *57*, 248.
12 Cooper, J. A., and D. H. Atlas [cited by H. B. Bull in *Physical Biochemistry*, John Wiley and Sons, New York, 1943, p. 177].
13 Davis, B. D., A. Hollaender, and J. B. Greenstein: J. Biol. Chem., 1942, *146*, 663.
14 Deutsch, H. F., and M. B. Goodloe: J. Biol. Chem., 1945, *161*, 1.
15 Dole, V. P., and E. Braun: J. Clin. Invest., 1944, *23*, 708.
16 Dole, V. P., and K. E. Emerson, Jr.: J. Clin. Invest., 1945, *24*, 644.
17 Dole, V. P., R. F. Watson, and S. Rothbard: J. Clin. Invest., 1945, *24*, 648.
18 *Electrophoresis*: Ann. N. Y. Acad. Sci., 1939, *39*, 105.
19 Fell, N., K. G. Stern, and R. D. Coghill: J. Immunol., 1940, *39*, 223.
20 Fisher, M. A., and B. D. Davis: Bull. Johns Hopkins Hosp., 1942, *71*, 364.
21 Gray, S. J., and E. S. Barron-Guzman: J. Clin. Invest., 1943, *22*, 191.
22 Gutman, A. B., D. H. Moore, E. G. Gutman, V. McClellan, and E. A. Kabat: J. Clin. Invest., 1941, *20*, 765.
23 Hardt, C. R., I. F. Huddleson, and C. D. Ball: Science, 1943, *98*, 309.
24 Hesselvik, L.: Acta med. Scandinav., 1939, *101*, 461.
25 Horsfall, F. L.: Ann. N. Y. Acad. Sci., 1939, *39*, 203.
26 Jameson, E., and C. Alvarez-Tostado: Proc. Soc. Exper. Biol. & Med., 1939, *40*, 476.
27 Jameson, E., C. Alvarez-Tostado, and H. H. Sortor: Proc. Soc. Exper. Biol. & Med., 1942, *51*, 163.
28 Jersild, M., and K. O. Pedersen: Acta path. et microbiol. Scandinav., 1938, *15*, 426.
29 Kekwick, R. A.: Biochem. J., 1939, *33*, 1122.
30 Kekwick, R. A.: Biochem. J., 1940, *34*, 1248.
31 Kekwick, R. A., and B. R. Record: Brit. J. Exper. Path., 1940, *22*, 29.
32 Lerner, A. B., and R. G. Greenberg: J. Biol. Chem., 1946, *162*, 429.
33 Lewis, L. A., E. P. McCullagh, and J. Clark: Am. J. Med. Sci., 1944, *208*, 727.
34 Lewis, L. A., R. W. Schneider, E. P. McCullagh, and J. Clark: J. Clin. Endocrinol., 1944, *4*, 535.
35 Li, C. H.: J. Am. Chem. Soc., 1944, *66*, 1795.
36 Longsworth, L. G.: J. Am. Chem. Soc., 1939, *61*, 529.
37 Longsworth, L. G.: Ann. N. Y. Acad. Sci., 1939, *39*, 187.
38 Longsworth, L. G.: Chem. Rev., 1942, *30*, 323.
39 Longsworth, L. G.: Indust. and Engin. Chem. (Anal. Ed.), 1946, *18*, 219.

40 Longsworth, L. G., R. M. Curtis, and R. H. Pembroke Jr.: J. Clin. Invest., 1945, *24*, 46.
41 Longsworth, L. G., and D. A. MacInnes: Chem. Rev., 1939, *24*, 271.
42 Longsworth, L. G., and D. A. MacInnes: J. Am. Chem. Soc., 1940, *62*, 705.
43 Longsworth, L. G., and D. A. MacInnes: J. Exper. Med., 1940, *71*, 77.
44 Longsworth, L. G., T. Shedlovsky, and D. A. MacInnes: J. Exper. Med., 1939, *70*, 399.
45 Luetscher, J. A. Jr.: J. Clin. Invest., 1940, *19*, 313.
46 Luetscher, J. A. Jr.: J. Clin. Invest., 1941, *20*, 99.
47 McCullagh, E. P., L. A. Lewis, and J. Clark: Am. J. Med. Sci., 1945, *210*, 81.
48 MacFarlane, A. S.: Biochem. J., 1935, *29*, 1175.
49 Moore, D. H.: J. Biol. Chem., 1945, *161*, 21.
50 Moore, D. H., L. Levin, and J. H. Leathem: J. Biol. Chem., 1944, *153*, 349.
51 Moore, D. H., and J. Lynn: J. Biol. Chem., 1941, *141*, 819.
52 Moore, D. B., P. S. Pierson, F. M. Hanger, and D. H. Moore: J. Clin. Invest., 1945, *24*, 292.
53 Moore, D. H., J. Van der Scheer, and R. W. Wyckoff: J. Immunol., 1940, *38*, 221.
54 Oncley, J. L.: Abstracts Am. Chem. Soc. Meeting, April 1946, p. 34.
55 Pappenheimer, A. M. Jr., H. P. Lundgren, and J. W. Williams: J. Exper. Med., 1940, *71*, 247.
56 Pedersen, K. O.: [In] The Svedberg Jubilee Volume, p. 490, Upsala, 1945.
57 Pedersen, K. O.: Thesis. Printed in Sweden by Almqvist and Wiksells Boktryckeri-A.-B., Upsala, 1945.
58 Perlmann, G. E., W. W. L. Glenn, and D. Kaufman: J. Clin. Invest., 1943, *22*, 627.
59 Perlmann, G. E., and D. Kaufman: J. Am. Chem. Soc., 1945, *67*, 638.
60 Pillemer, L., and M. C. Hutchinson: J. Biol. Chem., 1945, *158*, 299.
61 Rutstein, D. D., F. H. Clarke, and L. M. Taran: Science, 1945, *101*, 669.
62 Van der Scheer, J., and R. W. G. Wyckoff: Proc. Soc. Exper. Biol. & Med., 1940, *45*, 634.
63 Van der Scheer, J., R. W. G. Wyckoff, and F. H. Clarke: J. Immunol., 1940, *39*, 65.
64 Van der Scheer, J., R. W. G. Wyckoff, and F. H. Clarke: J. Immunol., 1941, *40*, 173.
65 Van der Scheer, J., R. W. G. Wyckoff, and F. H. Clarke: J. Immunol., 1941, *40*, 39.
66 Schick, B., and J. W. Greenbaum: J. Pediat., 1945, *27*, 241.
67 Scudder, J.: Chapt. XVI [in] *Blood Substitutes and Blood Transfusion*, edited by Stuart Mudd and Wm. Thalhimer, C. C Thomas, 1942.
68 Seibert, F. B.: Bact. Rev., 1941, *5*, 69.
69 Seibert, F. B., and J. W. Nelson: Am. Rev. Tuberc., 1943, *47*, 66.
70 Seibert, F. B., and J. W. Nelson: J. Biol. Chem., 1942, *143*, 29.

71 Seibert, F. B., and J. W. Nelson: Proc. Soc. Exper. Biol. & Med., 1942, *49*, 77.
72 Seibert, F. B., and J. W. Nelson: J. Am. Chem. Soc., 1943, *65*, 272.
73 Seibert, F. B., K. O. Pedersen, and A. Tiselius: J. Exper. Med., 1938, *68*, 413.
74 Seibert, F. B., and D. W. Watson: J. Biol. Chem., 1941, *140*, 55.
75 Shapiro, S., V. Ross, and D. H. Moore: J. Clin. Invest., 1943, *22*, 137.
76 Sharp, D. G., M. H. Hebb, A. R. Taylor, and J. W. Beard: J. Biol. Chem., 1942, *142*, 217.
77 Shedlovsky, T., and J. Scudder: J. Exper. Med., 1942, *75*, 119.
78 Shipley, R. A., K. G. Stern, and A. White: J. Exper. Med., 1939, *69*, 785.
79 Stenhagen, E.: Biochem. J., 1938, *32*, 714.
80 Stern, K. G.: Ann. N. Y. Acad. Sci., 1939, *39*, 147.
81 Stern, K. G.: Unpublished experiments.
82 Stern, K. G.: Unpublished experiments with B. O. Kramer, A. Sobel, and S. P. Gottfried.
83 Stern, K. G., and N. Fell: Unpublished experiments.
84 Stern, K. G., and N. Fell: Unpublished experiments.
85 Stern, K. G., M. Reiner, and R. H. Silber: J. Biol. Chem., 1945, *161*, 731.
86 Stern, K. G., and R. H. Silber: Unpublished experiments.
87 Stern, K. G., A. Rottino, and D. Suchoff: Unpublished results.
88 Svensson, H.: Kolloid Ztschr., 1939, *87*, 181.
89 Svensson, H.: Kolloid Ztschr., 1940, *90*, 141.
90 Svensson, H.: J. Biol. Chem., 1941, *139*, 805.
91 Svensson, H.: Thesis. Arkiv f. Kemi., 1946, *22A*, No. 10.
92 Taylor, H. L., and A. Keys: J. Biol. Chem., 1943, *148*, 379.
93 Thompson, K. W., and J. Melnick: Endocrinol., 1941, *28*, 723.
94 Tiselius, A.: Nova Acta Soc. Sci., Upsala, 1930, *4*, 7.
95 Tiselius, A.: Biochem. J., 1937, *31*, 1464.
96 Tiselius, A.: Trans. Faraday Soc., 1937, *33*, 524.
97 Tiselius, A.: Svenska Kemisk Tidskrift., 1938, *50*, 58.
98 Tiselius, A.: Harvey Lectures, 1940, *35*, 37.
99 Tiselius, A., and F. L. Horsfall Jr.: Arkiv Kemi Mineral. Geol., 1939, A *13*, No. 18.
100 Tiselius, A., and E. A. Kabat: J. Exper. Med., 1939, *69*, 119.
101 Zeldis, L. J., and E. L. Alling: J. Exper. Med., 1945, *81*, 515.
102 Zeldis, L. J., E. L. Alling, A. B. McCoord, and J. P. Kulka: J. Exper. Med., 1945, *82*, 157.
103 Zeldis, L. J., E. L. Alling, A. B. McCoord, and J. P. Kulka: J. Exper. Med., 1945, *82*, 411.