TABLE II. Effect of Antivaccinial Rabbit Serum on Plaque Formation in Culture and Pock Production on Chorioallantoic Membrane of the Chick Embryo. An equal amount of serum was added to virus dilution (10^{-5}) and mixture incubated at 37° for one hour.

	Exp.	Control	Antiserum	% reduction
Plaques	1	28, 24 (.2 ml), 65 (.4 ml)	1, 2 (.4 ml)	95
	3	27, 24 (.2 ml)	10 (.4 ml)*	61
Pox	1	32, 22, 58, 61 (.05 ml)	2, 1, 1, 2 (.1 ml)	96
	2	24, 42, 40, 44, 38 (.05 ml)	3, 1, 1, 3, 1, 2 (.1 ml)	95

* Serum frozen-thawed 4×.

fresh nutrient fluid added. Using this modified technic a plaque to pock ratio close to 1:1 was obtained.

Discussion and summary. A simple technic is described whereby the production of plaques in chick embryo tissue cultures has been consistently obtained with vaccinia virus. The plaques are probably due to viral activity. This conclusion is supported by the fact that 1) the plaques are absent when the virus is absent and their number increases with the concentration of the virus, 2) the number of plaques produced in tissue culture correlates with the number of pox produced on the CAM of the developing chick embryo and 3) specific antibody reduces both the number of plaques formed in tissue culture and the number of pox formed on the CAM by 95%.

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Characteristic Individual Electrophoretic Patterns in Humans.* (20379)

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The electrophoretic analysis of serum and plasma proteins in normal individuals and in patients with a variety of diseases has been repeatedly described(1,2). Six to 9 different protein components can generally be recognized in the plasma at pH 8.6. It is customary to characterize these protein components by their electrophoretic mobility and to determine their relative amounts by measuring the surface area of the electrophoretic patterns. From the data thus obtained and from the value for total plasma protein, determined by chemical analysis, the absolute concentrations of the plasma protein components can then be computed. Consequently, the electrophoresis of human plasma is generally considered to be a method for quantitative determination of some of the protein constituents of blood. Many attempts have been made to correlate the values obtained by electrophoresis for the various protein components with disease, age, sex, and other conditions of the blood donor. In most instances the plasma protein composition appears to be rather independent of these factors. Only in a few cases are the electrophoretic plasma protein values more or less specific for a disease, such as p.e. in multiple myeloma. In the course of studies on plasma proteins in patients from 3 separate hospitals and in a number of normal individuals, 1250 electrophoretic plasma analyses of 440 individuals have been carried out. In many cases, analyses have been performed on the same

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person at different time intervals, ranging from several hours to more than 2 years. Our findings on the relationship between plasma protein composition and factors such as disease, nutrition and age will be reported elsewhere.

The purpose of the present paper is to document with data our view that the careful examination of the general and detailed morphological appearance of the electrophoretic patterns yields valuable information which cannot be obtained by calculating the relative amounts or the electrophoretic mobilities of the protein components alone.

Methods. 5 to 10 ml of fresh venous blood were added to 0.3 g of crystalline sodium citrate. The dissolution of the crystals in the blood was accelerated by gentle shaking. Immediately thereafter, the red cells were separated by centrifugation for 10 minutes at 2000 r.p.m. The plasma was stored in the refrigerator at +3°C until used for electrophoresis study, but generally not for more than 2 to 3 days. If it had to be kept for longer periods, it was shell-frozen at −70°C and then stored at −15°C. Two ml of citrated plasma were dialysed in Visking sausage casings for 48 hours in the refrigerator against 500 ml of a veronal-citrate buffer of pH 8.6 and of ionic strength $\mu = 0.1$ (containing 0.06 M diethylbarbiturate and 0.008 M citrate). The dialysed plasma was then diluted with 0.7 ml of distilled water when the total protein was 7%, and the solution was made up to 7.0 ml with the above mentioned buffer. For plasma with other total protein values, 0.1 ml of distilled water were added for each 1% of total protein. The purpose of the addition of distilled water to the dialysed and diluted plasma is to accomplish the total or almost total elimination of some of the known electrophoretic anomalies, such as the delta- and epsilon-salt gradients and other asymmetries between the patterns of the ascending and descending limb of the cell(3,4). The *electrophoretic analyses* were carried out in a 2.5 ml Tiselius cell of a Perkin-Elmer model 38 apparatus. The original protein-buffer boundary was displaced 9 mm into the limb of the cell, and photographically recorded by means of the Longsworth scanning method.

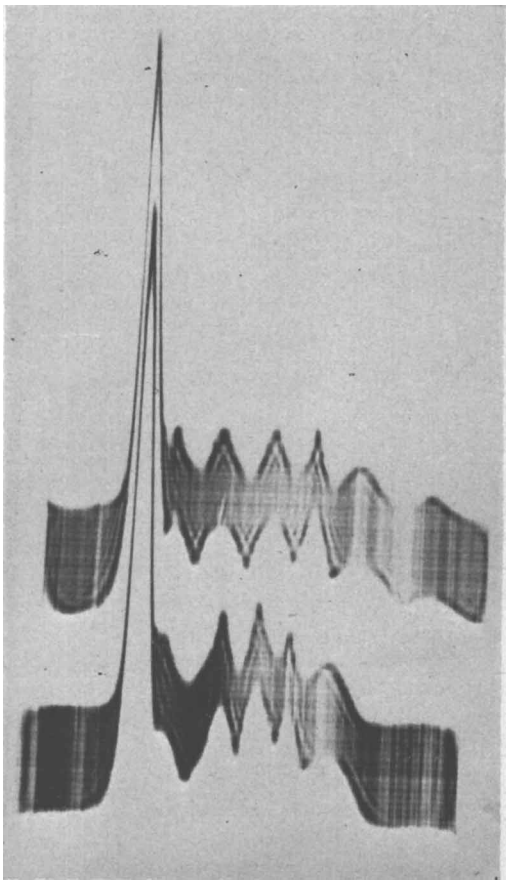

FIG. 1. Comparison of the electrophoretic patterns of two patients. Top patterns: patient S. Bottom patterns: patient M. The peaks represent from left to right: albumin, α_1-globulin, α_2-globulin, β-globulin, fibrinogen, γ-globulin, and a small remainder of the δ-salt anomaly (only in the top patterns).

The protein boundaries obtained after exposure to an electric field of 10.5 volt/cm (130 volt difference of potential between the two electrodes) for 70 minutes were photographed on the same film. The *relative amounts of the protein* components were determined according to the method of Tiselius and Kabat(5). Numerous determinations of the protein composition and of the electrophoretic mobilities from both the ascending and the descending boundaries have shown that under our experimental conditions (which are characterized in particular by the almost complete elimination of the salt anomalies through the addition of water) practically the same values are obtained from the patterns

TABLE I. Comparison of the Electrophoretic Patterns of 2 Patients.

	Patient	Albumin	α_1-glob.	α_2-glob.	β-glob.	Fibr.	γ-glob.
Relative amt of proteins*	S.	42.8	7.9	14.2	15.6	9.3	10.2
	M.	43.0	7.9	14.4	14.1	8.4	12.2
Electrophoretic mobilities†	S.	5.9	5.15	4.15	3.0	2.1	1.1
	M.	5.9	5.1	3.9	3.05	2.0	1.35

* In % of total protein. † In $cm^2\ sec^{-1}\ volt^{-1} \times 10^5$.

of either limb of the cell. As the ascending boundaries are known to yield a much better resolution of the proteins than the descending boundaries, only the patterns from the ascending boundaries are used in routine calculations of the protein composition, and all patterns reproduced in this paper are ascending boundaries.

Patients. S. (Fig. 1, Table I), 39 yr. ♀ with chronic myelogenic leukemia. M. (Fig. 1, Table I), 75 yr. ♀ with rheumatoid arthritis and arteriosclerotic heart disease. E. (Fig. 2, Table II), 47 yr. ♀ with rheumatoid arthritis. A.S. (Fig. 3, Table II), 37 yr. ♀ with cancer of breast. B.M. (Fig. 4), 49 yr. ♂ with hemolytic anemia. D. (Fig. 5), 47 yr. ♂ with rheumatoid arthritis.

Results. The general characteristics of the appearance of electrophoretic patterns. The following example illustrates the importance of thorough examination of the shape, the symmetry or asymmetry, and the width of each protein gradient, the distance between adjacent peaks and their tendency to separate from or to adhere to each other. In Fig. 1 the patterns of 2 patients are superimposed one on top of the other. The relative amounts of the plasma protein components and their electrophoretic mobilities, obtained from these patterns, are given in Table I.

It is obvious that these 2 patients yield electrophoretic patterns of quite different appearance, although there is a remarkable similarity between the relative amounts of their albumin, their α_1-globulin and their α_2-globulin. The difference in the mobilities of the α_2-globulins is by no means the only one found between these 2 patterns. In order to illustrate our method of examining electrophoretic patterns, the main features distinguishing the patterns of S. from those of M. are indicated: 1) There is a definite separation of the albumin from the α_1-globulin peak in S. but only an incomplete separation of these components in M. 2) The shape of the α_2-globulin peak of S. is symmetrical. The α_2-globulin peak of M., however, is asymmetrical showing a small amount of a protein component with a mobility slightly exceeding that of the main α_2-globulin peak, but not separating well from the latter. 3) The peak of α_2-globulin for S. is much broader than that

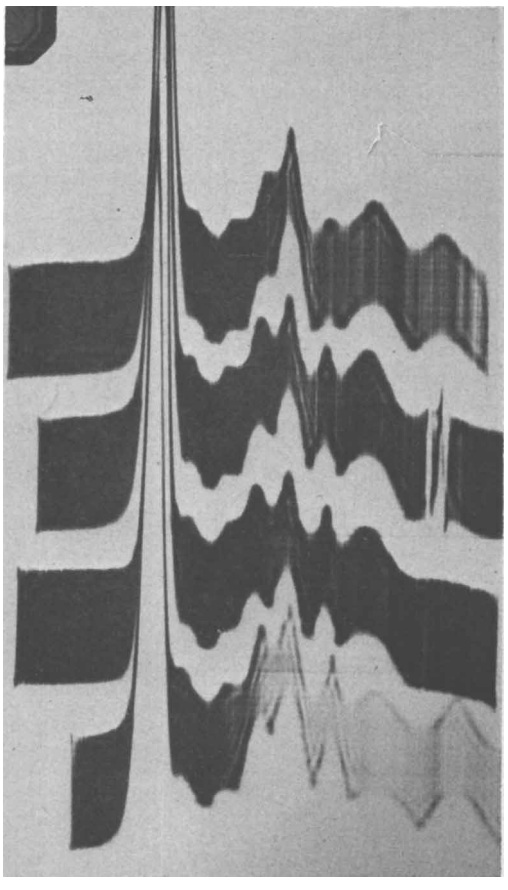

FIG. 2. Electrophoretic patterns of patient E. at different times. Dates from top to bottom: June 30, July 7 and 17 and Oct. 6, 1950. The peaks represent from left to right: albumin, α_1-globulin, α_2-globulin, α_3-globulin, β-globulin, fibrinogen, γ-globulin and remainder of δ-anomaly.

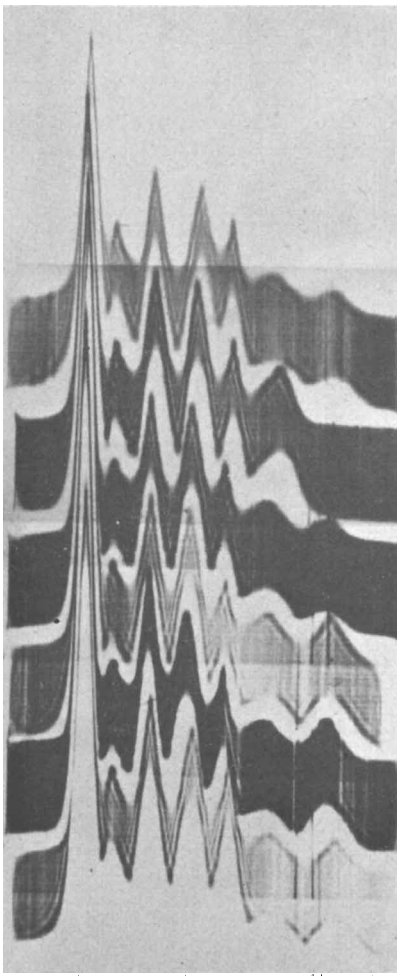

FIG. 3. Electrophoretic patterns of patient A.S. at different times. Dates from top to bottom: May 23, 26, 28, and 30, June 7 and 11, 1951. The peaks represent from left to right: albumin, α_1-globulin, α_2-globulin, β-globulin, fibrinogen, γ-globulin, and remainder of δ-anomaly.

of M.

Constancy of electrophoretic patterns in the same person. Fig. 2 and 3 show the patterns obtained in 2 patients. In each figure several patterns obtained from the same patient on different days are superimposed. In Table II the relative amounts of plasma proteins, as calculated from the patterns, are given. It is obvious that the general appearance of the electrophoretic patterns in the same individual remains remarkably constant over long periods of time, although the relative concentrations of the protein components may vary considerably during this time. Differences such as observed in Fig. 1 do not appear in the same person at different times. This *constancy* in the aspect of the electrophoretic patterns has been observed in a great number of individuals. Temporary deviations, however, have been noticed in a number of cases under the influence of various factors, such as abscess formation, ACTH treatment(6), and others. In most of these cases the patterns return to their original appearance after a lapse of time.

Individual electrophoretic patterns. Upon comparing the electrophoretic patterns from different individuals with each other it becomes obvious that it is hardly possible to find two persons whose patterns have the same characteristic features. This phenomenon is so pronounced that in many cases it is possible to identify individuals by their electrophoretic plasma patterns. Fig. 4 and 5 show 2 examples of patients with characteristic individual patterns.

Such individual patterns have been observed in a great number of other cases. In some individuals the plasma always contained an α_3-globulin which separated well from the α_2- and β-globulins, such as in patient E. (Fig. 2). On the other hand, plasma of some other persons never showed the presence of an α_3-globulin, as in patients B.M. and D.

TABLE II. Relative Amounts of Plasma Proteins in % of Total Protein. Patients E. (top) and A.S. (bottom).

Date	Albumin	α_1-glob.	α_2-glob.	α_3-glob.	β-glob.	Fibr.	γ-glob.
6/30	49.4	3.4	5.0	9.0	14.8	5.8	12.6
7/ 7	49.1	4.5	4.9	9.5	12.8	6.6	12.6
17	47.9	5.7	5.6	9.2	12.8	7.0	11.8
10/ 6	44.0	5.7	5.5	10.1	15.7	8.3	10.7
5/23	31.4	10.0	19.0		18.4	11.6	9.6
26	30.7	7.9	21.0		17.6	10.2	12.6
28	34.5	9.2	19.3		19.0	9.0	9.0
30	38.1	8.4	19.8		17.9	7.9	7.9
6/ 7	36.8	8.5	20.9		18.0	9.3	6.5
11	34.3	9.7	18.8		17.4	12.6	7.2

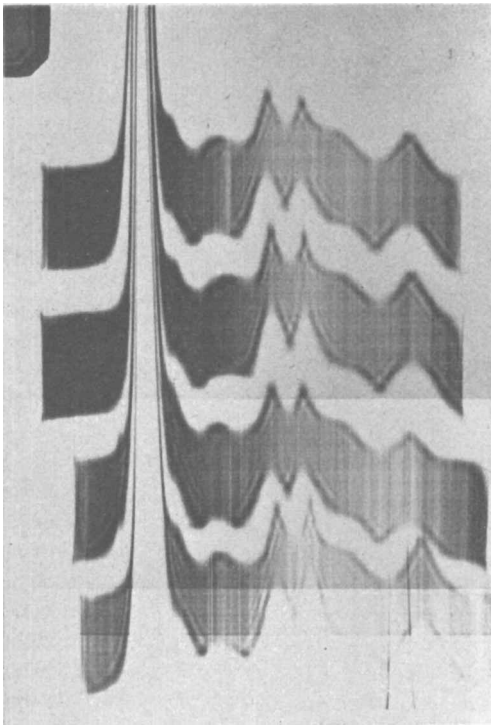

FIG. 4. Electrophoretic patterns of patient B.M. Dates from top to bottom: Dec. 7 and 9, 1950, Jan. 5 and 17, 1951.

(Fig. 4 and 5). In other individuals the α_3- and β-globulins did not separate from each other, yielding consistently one single, relatively high gradient at their place. Sometimes, the electrophoretic patterns of two persons were apparently very similar to each other, but a close examination of their structure always revealed characteristic differences, distinguishing the two individuals from each other, *e.g.* a patient (Y.) with seborrheic dermatitis produced electrophoretic patterns which were almost identical to those of patient D. (Fig. 5), with the difference, however, that all the patterns of Y. exhibited a much more pronounced α_1-globulin than those of D.

Our findings on the relationship between electrophoretic patterns and certain diseases will be reported later. It should be emphasized, however, that there is no evidence in our material for the existence of electrophoretic patterns characteristic for any of the diseases mentioned in this paper. Indeed, three patients with rheumatoid arthritis (Fig. 1, M.; 2, E.; and 5, D.) have entirely different electrophoretic patterns.

Patients as well as normal individuals show this diversity of the electrophoretic plasma patterns. The most characteristic features distinguishing normal individual patterns from each other are in general found in the α_1-, α_2- and α_3-globulins.

Summary. 1. The detailed examination of the contours and fine architecture of the electrophoretic patterns of human plasma in different individuals may reveal considerable dissimilarities between persons, even in those cases where the relative amounts of the plasma protein components, calculated from the electrophoretic patterns, are similar. The careful examination of the electrophoretic patterns, of the shape, symmetry or asymmetry, and width of each protein peak and of the tendency of adjacent protein peaks to separate from or to approach each other is considered to be of great importance to characterize a given pattern. 2. The general features and the appearance of the electrophoretic patterns in one and

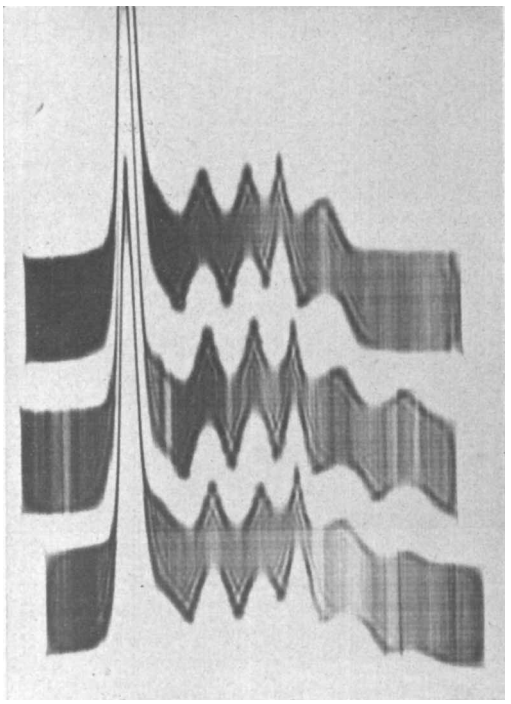

FIG. 5. Electrophoretic patterns of patient D. Dates from top to bottom: April 14, Sept. 2, 1950, and June 1, 1951.

the same person usually remain constant over long periods of time, although the plasma protein composition may vary considerably during this period. 3. Characteristic individual electrophoretic patterns of the plasma proteins have been observed in numerous cases of both normal individuals and patients with various diseases. The distinguishing characteristics encountered in individual electrophoretic patterns are independent of sex and age; in healthy persons they are confined mainly to the α-globulins; with only a few exceptions they are not related to the disease state in patients.

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Growth Promotion in Carnivorous Protozoa by Substituted Purines.* (20380)

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(Introduced by George W. Kidder.)

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Tetrahymena pyriformis(1) has been extensively used as a favorable animal microorganism for nutritional and biochemical studies(2-4). When *Tetrahymena* has been used as food for carnivorous protozoa an interference with their normal growth and reproduction has been noted. By the addition of small concentrations of naturally occurring substances to the culture medium, however, (together with the food organism) it has been possible to obtain satisfactory growth of several species of carnivorous protozoa. In the case of the hypotrichous ciliate, *Stylonychia pustulata,* in bacteria-free cultures growth was proportional to the number of *Tetrahymena* supplied as food, but failed immediately if a supplement, extracted from yeast or other plant materials, was omitted from the medium. The supplement was never obtained from animal tissues and attempts to substitute known vitamins were unsuccessful(5-7). Chemical properties of the supplementary factor, such as solubility in aqueous, but not in organic solvents, thermostability and photosensitivity were established. By chromatographic methods concentrates were produced which were active in dilutions as low as 0.1 μg per ml(8,9). In the case of the suctorians, *Tokophrya infusionum* and *Podophrya collini*† the interference with growth was manifested differently. The adult suctorians captured *Tetrahymena* readily with their tentacles and sucked out the cytoplasm of the ciliate. There was apparently no immediate need for a supplement in the medium as long as the number of food ciliates was limited. Thus Rudzinska was able to maintain *Tokophrya* for several years by carefully controlling the supply of *Tetrahymena*(10). It has long been known, however, that after abundant feeding on ciliates a large percentage of suctorians changed into giant forms which failed to reproduce normally(11). Under such conditions there was invariably a decline in the growth rate with eventual loss of the cultures. In bacteria-free cultures of suctorians feeding on *Tetrahymena* small concentrations of yeast

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† We wish to thank Dr. Harold E. Finley of Howard University for sending us the original culture of this organism.