Conclusions

All of the work reported so far is consistent with a picture of the binding process, for organic anions, which lays primary emphasis on the cationic groups in the protein. Thus the modified proteins described in this paper retain their affinity for anions so long as the change has not decreased the number of cationic loci. Acetylation, on the other hand, decreases the affinity of albumin for anions, just as the more drastic de-amination of gelatin reduces the number of bound dodecyl sulfates.[42]

In consideration of variations in binding ability of different proteins, a similar point of view, extended to include internal, competitive interactions, between functional groups of the protein, seems to be promising. As a minimum, it suggests a quantitative criterion, based on amino acid composition, for predicting whether a given protein will show significant binding properties. Applied in a general manner, it offers a model on the basis of which many phases of anion-protein interactions can be interpreted satisfactorily.

Acknowledgments.—These investigations were supported by grants from the Rockefeller Foundation and from the Office of Naval Research.

(42) I. I. Harris, K. G. A. Pankhurst and R. C. M. Smith, *Trans. Faraday Soc.*, **43**, 506 (1947).

Summary

The binding abilities of four fractions of bovine plasma, γ-globulin, β_2-globulin, α_2-globulin and crystallized albumin, have been compared. Only the latter showed significant binding properties. Among the crystallized native proteins which have been examined, including serum albumin, β-lactoglobulin, egg albumin, insulin, lysozyme, ribonuclease, pepsin, trypsin and chymotrypsin, only the first two formed complexes with the reference anion, methyl orange.

Conversion of the ϵ-ammonium groups of lysine to guanidinium groups did not alter the binding ability of serum albumin. On the other hand, acetylation of the ϵ-ammonium groups did reduce the degree of binding substantially.

The relative binding abilities of various native proteins can be correlated by a "binding index" based on the distribution in content of amino acids with the molecule. The index can be justified on a molecular basis in terms of internal interactions between the functional groups of the constituent amino acids of the protein.

EVANSTON, ILLINOIS RECEIVED NOVEMBER 19, 1948

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 1216, AND BAKER LABORATORY, CORNELL UNIVERSITY]

The Fractionation of Proteins by Electrophoresis-Convection. An Improved Apparatus and its Use in Fractionating Diphtheria Antitoxin

BY JOHN R. CANN, JOHN G. KIRKWOOD, RAYMOND A. BROWN AND OTTO J. PLESCIA[1]

Introduction

A method of fractionation of proteins in solution utilizing a combination of electrophoretic and convective transport of the components was suggested by Kirkwood[2] in 1941 and investigated experimentally by Nielsen and Kirkwood[3] several years later. The method is based upon the same principles as that of the Clusius column, except that horizontal electrophoretic transport instead of transport by thermal diffusion is superimposed on convective transport in a vertical convection channel. The fractionation scheme may be briefly described as follows. Two reservoirs connected by a vertical channel, of width sufficiently small to ensure laminar flow, contain a solution of the proteins to be fractionated. Upon application of a horizontal electric field, differential transport of the mobile components across the channel takes place, producing a horizontal density gradient depending upon the composition gradients. Under the action of gravity, the density gradient induces convective circulation in the channel with a velocity distribution qualitatively similar to that of the Clusius column. The result of the superposition of the horizontal electrophoretic transport and vertical convective transport is movement of the mobile components from the top reservoir to the bottom reservoir at rates depending on their mobilities, with a relative enrichment of the top reservoir with respect to the slow components and of the bottom reservoir with respect to the fast components. The mathematical theory of the transport has been worked out for representative operating conditions and will be presented in a future article. It has been used in the design of the improved fractionation cell to be described here.

In order to avoid contamination of the solution by electrolysis products, the walls of the convection channel are constructed of semi-permeable membranes, separated from the electrodes by buffer solution. The electric field across the channel is maintained by the electric current carried by the ions of the buffer electrolyte, to which the membranes are permeable. The exterior buffer solution is replenished by a cir-

(1) Present address: M. W. Kellogg Company Jersey City 3, New Jersey.

(2) J. G. Kirkwood, *J. Chem. Phys.*, **9**, 878 (1941).

(3) L. B. Nielsen and J. G. Kirkwood, THIS JOURNAL, **68**, 181 (1946).

culation system at a rate sufficient to prevent electrolysis products from reaching the membranes.

In the fractionation apparatus employed in the exploratory investigations of Nielsen and Kirkwood[3] the channel containing the protein solution was formed by the annular space between two cellulose tubes. The upper and lower ends of the annular space were connected to glass reservoirs. The experimental results obtained with this apparatus were not entirely reproducible from run to run, because of the difficulty in maintaining the membranes in a rigid configuration. Furthermore, there was some tendency for the membranes to stretch and lose their shape in the course of a series of runs. It was also found that runs made with different sets of membranes could not be compared without an appreciable margin of error because of difficulty in reproducing the width of the annular space between the membranes. The purpose of this report is to describe the construction and operation of a new apparatus designed to eliminate these disadvantages.

Our method is related to the Pauli[4] electrodecantation effect, which has been used by Pauli and Stamberger[5] to concentrate colloidal solutions and by Gutfreund[6] to stratify protein solutions. It appears that our electrophoresis-convection column bears roughly the same relation to the Pauli electro-decantation effect that the Clusius column bears to the Soret effect. The advantages of utilizing the principle of the Clusius column in convective electrophoresis are too clear to require further comment.

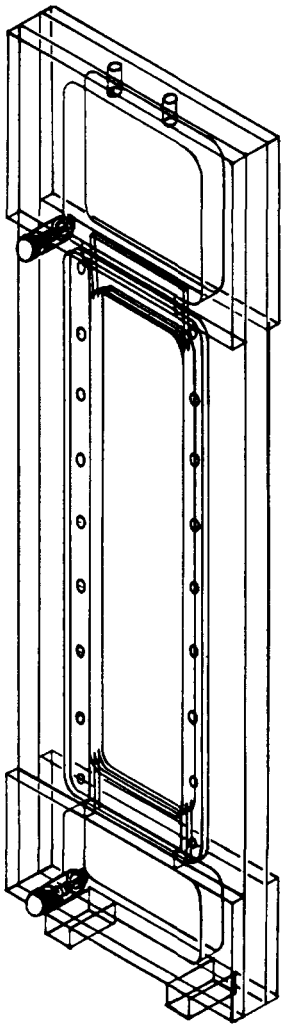

Fig. 1.—Cell block assembly, electrophoresis–convection apparatus.

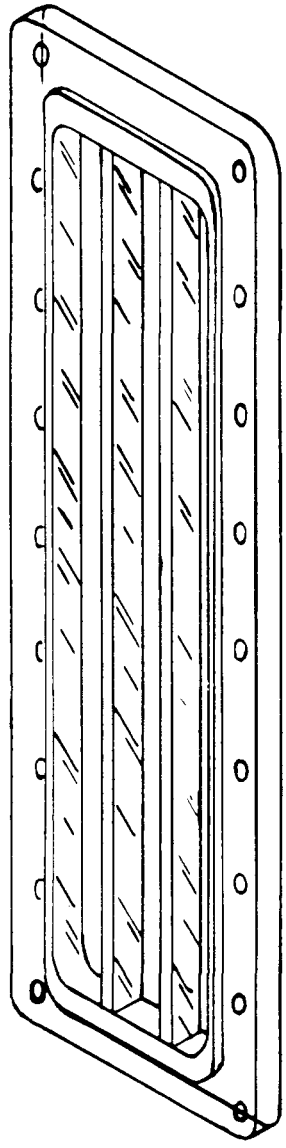

Fig. 2.—Face plate assembly, electrophoresis–convection apparatus.

Construction of the Electrophoresis–Convection Apparatus

The apparatus described here may be considered as being composed of five principle parts: (1) the fractionation cell consisting of a cell block, two face plates and semi-permeable membranes; (2) the box housing the fractionation cell and electrodes; (3) the electrode assemblies; (4) the buffer circulating system; and (5) the power pack. On assembly the cell consists schematically of a narrow vertical channel connecting upper and lower reservoir. That portion of the channel effective in fractionation is formed by the rectangular space between two sheets of semi-permeable membrane. The cell is immersed in buffer solution to within about an inch above the bottom of the upper reservoir. The electrode assemblies are placed in the box on opposite sides of the cell. During operation electrolysis occurs which tends to change the *p*H of the buffer solution. To counteract this, buffer is circulated vertically around the cell.

The material used in the construction of the fractionation cell and its housing must meet certain requirements. It must be electrically non-conducting, exhibit no swelling when in contact with aqueous solutions, possess dimensional stability and be readily machined with high precision. Although there is something to be desired in its machineability, lucite appears to be the material most suitable for this purpose. Consequently, it was chosen as the construction material. When necessary the lucite was cemented with Dupont Cement H-94.

The fractionation cell[6a] is shown to scale in Figs. 1 and 2. The cell block, Fig. 1, consists of an upper and lower reservoir between which is a vertical rectangular slot. The reservoirs are connected to this central slot by means of narrow vertical channels passing through the body of the block. The capacities of the upper and lower reservoirs are 100 and 50 ml., respectively. Both reservoirs are supplied with valves for sampling, and the top reservoir is open to the atmosphere. A recess is milled around the periphery of the rectangular slot on both faces of the cell block. The cell block is supplied with two small legs.

(4) M. Adolf and W. Pauli, *Biochem. Z.*, **152**, 360 (1924).
(5) P. Stamberger, *J. Colloid Sci.*, **1**, 93 (1946).
(6) H. Gutfreund, *Biochem. J.*, **37**, 186 (1943).
(6a) Full working drawings are available for loan.

Both face plates, Fig. 2, are of identical design. The face plate is essentially a frame which fits into the recess around the periphery of the central slot of the cell block. Around the inner periphery of the back side of the frame is a shoulder constructed so as to fit into the central slot of the cell block. The face plates clamp the sheets of semi-permeable membranes in place against the bottom of the recess. Since the membranes form the face-walls of the effective portion of the channel, the height of the shoulder controls the channel wall separation. In the cell described here the wall separation is 0.037 inch. A $^1/_{64}$ in. clearance is allowed between the sides of the shoulder and the central slot and the sides of the frame and the recess. The face plate is supplied with two longitudinal ribs, which act as supports for the membranes. The one face plate and the cell block are drilled to receive brass machine screws, the holes in the face plate being counterbored. Since the heads and tips of the screws must be electrically insulated and since, in any case, metal screws distort the electric field across the channel walls, the use of screws made of insulating material is being investigated. Plastic or hard rubber screws are recommended. The other face plate is drilled and tapped for the screws. The holes in the face plates correspond to those in the cell block.

The effective portion of the channel connecting upper and lower reservoirs is formed as follows: Visking Corporation cellulose sausage casing, No. 133, is soaked overnight in distilled water. Sheets of semi-permeable membrane are made by cutting along one edge of the casing and unfolding. The wet membranes are applied to both faces of the cell block and pressed along the bottom of the peripheral recess of the central slot by insertion of the face plates into the recess. This produces a gasketed seal. One edge of the plates is bolted snugly to the block by means of three screws. The membranes are now stretched and the free edge of the plate forced back into the recess, exercising care to keep the membranes taut. The remaining screws are put into place, all the screws being tightened sufficiently to prevent leakage. The excess membrane is removed and the heads and tips of the screws electrically insulated with Fenox, supplied by the Bakelite Corporation. In order to prevent drying out of the membranes the cell is filled with distilled water and stored in a container of water.

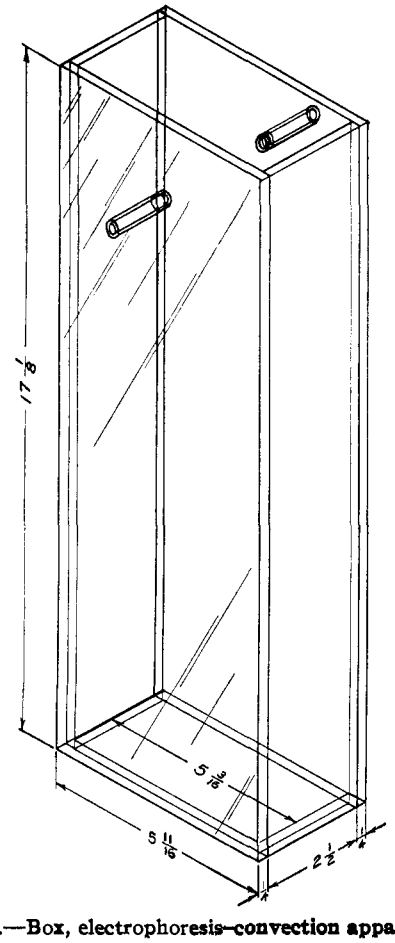

Fig. 3.—Box, electrophoresis-convection apparatus.

The box which houses the fractionation cell and the electrode assemblies is shown to scale in Fig. 3. The inside dimensions are such that a snug fit is obtained between the sides of the box and the edges of the cell block. This minimizes loss of electric field by leakage around the cell. The capacity of the box is sufficient to allow the cell to be surrounded by about 2.5 liters of buffer solution. The box is supplied with buffer inlet and outlet tubes.

The electrode assemblies are also shown to scale in Fig. 4. Originally each electrode assembly consisted of three graphite rods mounted in a lucite holder. However, contamination of the solutions by the deterioration of the anode and the inhomogeneity of the electrical field produced by the arrangement warranted changing to the platinum electrodes described here. Each assembly consists of 2 *mil* platinum foil mounted in a lucite frame. The dimensions of the platinum foil correspond to those of the inner periphery of the face plates. The dimensions of the frames are such that the strips of platinum and the effective channel of the cell are aligned when the cell and electrode assemblies are housed in the box. Each frame is supplied with a binding post, contact with the platinum foil being made with No. 18 platinum wire. Since platinum occludes large quantities of electrolytic hydrogen, it is advisable to alternate the polarity of the electrodes from run to run.

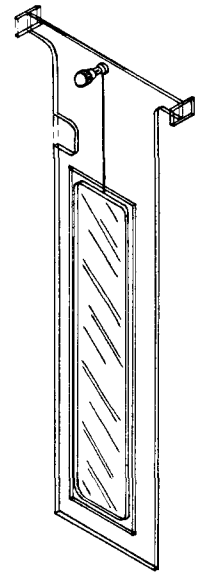

Fig. 4.—Electrode assembly, electrophoresis - convection apparatus.

The buffer circulating system was designed to counteract the change in *p*H of the solutions due to the accumulation of electrolysis products about the electrodes during operation. A buffer flow of about 30 liters an hour is sufficient to maintain the *p*H of the solutions constant to within 0.1 of a *p*H unit at a current density of 2.5 amp. per sq. dm. Circulation is by gravity flow. The buffer flows from a twelve-liter aspirator bottle into the box housing the cell and electrodes. It is then circulated vertically in the box so as to pass down the front and up the back of the cell, the small legs of the cell block permitting the flow of solution underneath the cell. The circulated buffer is discharged into a twelve-liter flask. A centrifugal pump periodically pumps the circulated buffer back into the aspirator bottle. It is desirable to operate under conditions of field strength and current density which do not lead to electrolytic decomposition of the buffer anions. Under these conditions, the original *p*H of the buffer solution is restored on mixing, and the buffer may be recycled in the circulation system without replenishment. The pump is activated as follows. One branch of a mercury manometer is inserted into the side of the aspirator bottle so as to measure the hydrostatic pressure. A flexible rubber sack filled with air and sealed onto the end of the manometer tube separates the buffer solution from the mercury. When the hydrostatic pressure decreases to some predetermined value the pump is activated through

an electronic relay[7] by means of a system of three platinum contacts in the open branch of the manometer. The relay is self-energized through one of the contacts thus preventing short cycling of the pump.

The use of silver, silver chloride electrodes, which would eliminate the necessity for buffer circulation, has been considered.

The power pack consists of a potentiometer connected across a 110 volt direct current line. A 35-ohm slide wire resistor, maximum capacity of 5 amp., is used for the potentiometer. The voltage drop across the cell and the electrolyzing current are measured with a standard voltmeter and ammeter.

Operation of the Electrophoresis-Convection Apparatus

The apparatus is operated as follows: The lower reservoir and channel of the cell are filled with the buffered protein solution to be fractionated, exercising care to eliminate all air bubbles from the channel. The cell is immersed in buffer solution contained in the box to within about one inch above the bottom of the upper reservoir. The remainder of the protein solution is now put into the upper reservoir. A total volume of 110 to 150 ml. of protein solution may be used depending upon whether or not one wishes to fill the upper reservoir to capacity. The electrode assemblies are placed in the box on either side of the cell making certain that they are parallel to the cell-faces. By making one electrode positive and the other negative a relatively homogeneous electric field can be set up across the channel containing the protein solution. Buffer is now circulated vertically around the cell. The temperature of the system is regulated in a constant temperature cold-room operating at 4°. More than one fractionation may be conducted at the same time by cascading the desired number of units.

The nominal field strength in the solutions is determined from measurements of the specific conductances of the solutions and from the average current density.

At the conclusion of the fractionation the top fraction is withdrawn from the upper reservoir by means of a long hypodermic needle and syringe. This operation is performed while the cell is still in the box and surrounded by buffer solution. The bottom fraction, which includes the solutions from both the lower reservoir and the channel, is drawn off through the lower-reservoir valve.

Depending upon the *p*H employed, fractionation of a heterogeneous protein can be effected by two modes of operation. In the first method the *p*H of the solution is such that all the components are either on the alkaline or acid side of their isoelectric points. Under these conditions the components are differentially transported out of the upper and into the lower reservoir, the fractionation depending upon the difference in mobilities of the constituent proteins. The greatest separation of components is obtained by so choosing the operating time that half of the major component is transported out of the upper reservoir.

In the alternative method of operation one of the constituents of the heterogeneous protein is immobilized by operating at its isoelectric point. The mobile components are transported out of the upper and into the lower reservoir, leaving the immobilized component in the upper reservoir. When applicable this procedure is by far the more efficient one.

The successive separation of the components of a protein mixture can be effected by the isoelectric procedure as follows. The component with the most alkaline or acid isoelectric point is first separated from the others in several successive stages by operating at its isoelectric point. The composite of the top cuts of these stages is further processed to purify the desired constituent. The bottom cut of the last of these stages is a concentrate of the mobile components. The process is repeated until the mixture has been resolved.

Fractionations by the isoelectric method have generally been carried out at a field strength of 1 to 4 volts/cm. for ten to thirty hours, depending upon the mobilities of the proteins. The concentration of the proteins may be as high as 4 g. per 100 ml.

Fractionation of Horse Diphtheria Antitoxin Pseudoglobulin

In the investigation of Nielsen and Kirkwood[3] artificial binary mixtures of proteins were fractionated by operating at a *p*H on the alkaline side of the isoelectric points of the constituents. Both components were transported out of the upper and into the lower reservoir, the separation depending upon the difference in mobilities of the two proteins. As pointed out above, immobilization of one of the components of the mixture by operation at its isoelectric point would have yielded greater efficiencies of fractionation. In this case only the mobile component is transported out of the upper and into the lower reservoir, leaving the immobile one in the upper reservoir. It appeared desirable to demonstrate this manner of operation by fractionating horse diphtheria antitoxin pseudoglobulin, which consists of at least three components of appreciably different isoelectric points.

Material.—Electrophoretic analysis of the horse diphtheria antitoxin pseudoglobulin[8] in barbital buffer, *p*H 8.6 and ionic strength 0.1, at a concentration of 2 g./100 ml. yielded 8% α-globulin (-3.72×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$), 56% β_2-globulin (-2.44×10^{-5}), and 36% γ-globulin (-1.10×10^{-5}). In phosphate buffer of ionic strength 0.1 the γ-globulin was isoelectric at *p*H 6.54. At this *p*H the mobilities of the α-globulin and β_2-globulin were -2.4×10^{-5} and -1.1×10^{-5}, respectively.

Electrophoretic Analysis.—The moving boundary technique of Tiselius[9] as modified by Longsworth[10] was used in the electrophoretic analysis. Electrolysis of the protein in barbital buffer, *p*H 8.6 and ionic strength 0.1, was allowed to proceed for four hours at a field strength of 4 volts/cm.

Mobilities were calculated from measurements of the displacement from the initial boundary of the constituent boundaries as suggested by Longsworth and MacInnes.[11]

For the purpose of determining the total protein concentration, a factor relating concentration to area unit was obtained by planimetric integration of tracings of enlarged electrophoretic diagrams of solutions of known concentration.

The apparent concentrations of the components of the pseudoglobulin and its fractions were determined by finding the ratio, in each case, of the component area to the total area, exclusive of the ϵ-boundary. The areas were measured on projected tracings of the descending patterns with a planimeter, resolution into components being carried out by the method of Pedersen.[12] The difference between the apparent and true distribution of the electrophoretic components[13] was considered negligible compared to the changes in distribution effected by fractionation. By extrapolation of apparent concentrations to zero protein concentration, the true relative concentration of γ-globulin in the pseudoglobulin was found to be 39%. The apparent concentrations of γ-globulin at protein concentrations of 0.9 and 2.0 g./100 ml. were 38 and 36%, respectively.

Results

Fractionation experiments were carried out in phosphate buffer, *p*H 6.5 and ionic strength 0.1

(7) R. C. Hawes, *Ind. and Eng. Chem. Anal. Ed.*, 11, 222 (1939.)

(8) The Department of Physical Chemistry, Harvard Medical School, kindly supplied the horse diphtheria antitoxin pseudoglobulin. This material was prepared at the Massachusetts Antitoxin Laboratory.

(9) A. Tiselius, *Trans. Faraday Soc.*, 33, 524 (1937).

(10) L. G. Longsworth, *Chem. Rev.*, 30, 323 (1942).

(11) L. G. Longsworth and D. A. MacInnes, THIS JOURNAL, 62, 705 (1940).

(12) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, London, 1940, p. 296.

(13) (a) L. G. Longsworth, *J. Phys. and Colloid Chem.*, 51, 171 (1947); (b) S. H. Armstrong, Jr., M. J. E. Budka and K. C. Morrison, THIS JOURNAL, 69, 416 (1947).

and 0.05. In these experiments the α-globulin and β_2-globulin were transported out of the upper and into the lower reservoir, leaving the immobilized γ-globulin in the upper reservoir. The extent of separation depended upon the time of operation. Samples withdrawn from the reservoirs at the conclusion of the runs were dialyzed against water to remove salts. When necessary, concentrations were adjusted by lyophilization. The solutions were then dialyzed against 150 volumes of barbital buffer, *p*H 8.6 and ionic strength 0.1, at 4° for seventy-two hours with three changes of buffer and analyzed electrophoretically. A series of representative runs is presented in Table I, where

E = nominal field strength in volts/cm.
t = duration of run in hours
C_i = initial protein concentration in g./100 ml.
f_t = top separation factor
Γ = ionic strength

TABLE I

(a) CONDITIONS FOR FRACTIONATION OF PSEUDOGLOBULIN

Run	*p*H	Γ	E, v/cm.	t, hr.	C_i, g./100 ml.	f_t
1	6.6	0.1	1.0	5	2	1.4
2	6.5	.1	2.0	10.2	2	2.2
3	6.4	.05	6.0	13.9	1	2.2
4	6.4	.05	6.0	14		
5	6.5	.1	6.0	14	0.6	2.4

(b) DISTRIBUTION OF ELECTROPHORETIC COMPONENTS INTO FRACTIONS

Run	Cut	α-Globulin	β-Globulin	T-Component	γ-Globulin
Pseudoglobulin		8	56		36
1	Top	4	51		45
	Bottom	11	58		31
2	Top	0	44		56
	Bottom	14	56		30
3	Top		44[a]		56
4	Top		50[a]		50
	Bottom	14	63	9	14
5	Top		26		74
	Bottom	6	60	28	6

[a] Includes α-globulin.

The efficiency of separation is expressed in terms of the top separation factor defined by the relation

$$f_t = \frac{x_\gamma}{X^0_\gamma} \frac{1 - x^0_\gamma}{1 - x_\gamma} \qquad (1)$$

$$x_\gamma = \frac{C\gamma}{\sum_i C_i}$$

x_γ^0 and x_γ are the initial and final ratios of γ-globulin concentration to total protein concentration in the upper reservoir, respectively.

Runs 1 and 2 were carried out to determine the extent of separation to be expected for given operating conditions. It was found that by an appropriate choice of field strength and time the relative concentration of γ-globulin in the upper reservoir could be increased by more than 50% in a single operation. Thus, electrolysis of a 2% solution ionic strength 0.1, at a field strength of 2 volts/cm. for ten hours yielded a top cut containing 0.6 g. material of composition 56% γ-globulin and 44% β_2-globulin.

The separation factor of 2.2 obtained in run 2 indicated the practicability of separating "pure" γ-globulin from the pseudoglobulin by a multistage batch fractionation. Such a two-stage fractionation was carried out as shown schematically in Fig. 5. The data are presented in Table I, runs 3, 4 and 5. The electrophoretic patterns presented in Figs. 6 and 7 follow the course of fractionation.

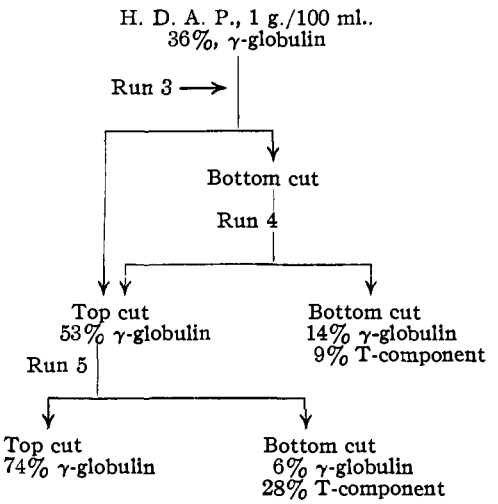

Fig. 5.—Schematic fractionation of horse diphtheria antitoxin pseudoglobulin.

The material obtained from the upper reservoir of the first stage, run 3, analyzed 56% γ-globulin (1.44×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$) and 44% β_2-globulin (-2.55×10^{-5}). The small percentage of α-globulin is included in the analysis for β_2-globulin. Figures 6a and 6b represent the pseudoglobulin and the first γ-globulin fraction, respectively. The solution withdrawn from the lower reservoir was further fractioned for γ-globulin in run 4, the resulting top cut being comparable in composition to that of the first fraction. The bottom cut of this run is indicated by the electrophoretic pattern shown in Fig. 6c. It will be noted that removal of the γ-globulin revealed the presence of another component (-2×10^{-5}), which was masked in the electrophoretic pattern of the pseudoglobulin. This is presumably the T-component of van der Scheer, Wyckoff and Clarke.[14,15]

The starting material for the second stage of fractionation, run 5, was a composite of the top cuts of runs 3 and 4. This stage yielded a top cut which analyzed 74% γ-globulin ($-1.53 \times$

(14) J. van der Scheer, R. W. G. Wyckoff and F. H. Clarke, *J. Immunol.*, **39**, 65 (1940).

(15) If the nomenclature of Hess and Deutsch [THIS JOURNAL, **70**, 84 (1948)] were to be adopted, the T-component and γ-globulin would be designated as γ_1- and γ_2 globulin, respectively.

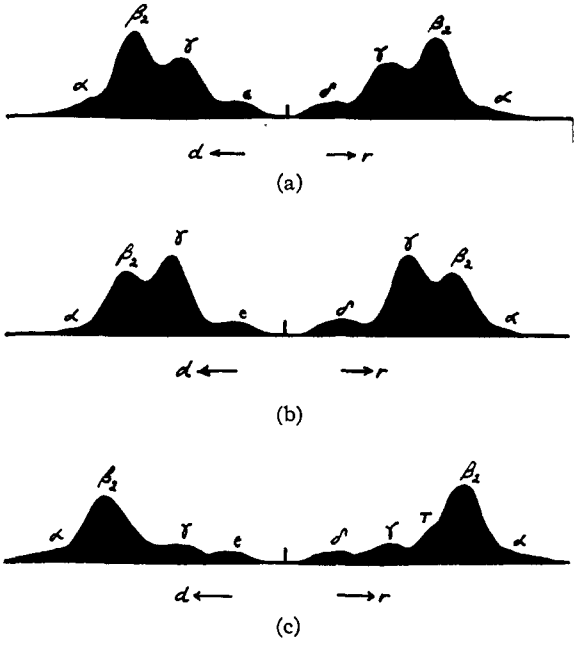

Fig. 6.—Electrophoretic patterns of pseudoglobulin fractions: a, original pseudoglobulin; b, top cut run 3; c, bottom cut run 4.

10^{-5}) and 26% β_2-globulin (-2.76×10^{-5}). The electrophoretic pattern of this material is shown in Fig. 7a. The electrophoretic pattern presented in Fig. 7b reveals that the T-component was concentrated in the bottom reservoir which contained 6% γ-globulin (-0.9×10^{-5}), 28% T-component (-1.7×10^{-5}), 60% β_2-globulin (-2.86×10^{-5}) and 6% α-globulin (-3.9×10^{-5}).

The field strength and time employed in the two-stage fractionation were 6 volts/cm. and fourteen hours, respectively. Yet, the efficiencies of separation were no greater than that obtained in run 2 at 2 volts/cm. and ten hours. This is probably due to a combination of two factors. Firstly, osmosis occurred in runs 3, 4 and 5 but not in runs 1 and 2. Osmosis increased the volume of the protein solution in the cell, the volume of osmoid varying from 15 to 25% of the initial volume of solution depending upon the operating conditions. The influx of water through the membranes re-inforces the upward convection and counteracts the downward convection thereby decreasing the rate of transport out of the upper reservoir. It was found that osmosis increases with increasing field strength and decreases with increasing ionic strength. Although theory predicts only a slight dependence on initial protein concentration, efficiencies of

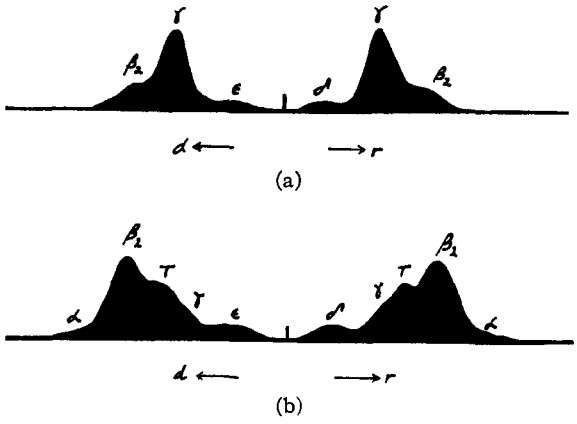

Fig. 7.—Electrophoretic patterns of pseudoglobulin fractions: (a) top cut run 5; (b) bottom cut run 5.

separation have been observed to be markedly lower at the lower protein concentrations. This may be due to the lower viscosity of these solutions and failure to achieve conditions of laminar flow in the channel at the necessary field strengths.

Discussion

The results of these fractionations demonstrate that with the proper choice of field strength, ionic strength and duration of run, high efficiencies of separation of the components of a protein mixture can be achieved. Thus, five successive stages of fractionation of pseudoglobulin in phosphate buffer, *p*H 6.5 and ionic strength 0.1, at a field strength of 2 volts/cm. for ten hours would effect separation of 97% pure γ-globulin.

Not only does this method effect an efficient separation of the major components of a heterogeneous protein on a macro scale, but it makes available a convenient means of concentrating those components which are originally present in such quantities as to be masked in the electrophoretic pattern.

Summary

This article describes the construction and operation of an improved electrophoresis-convection apparatus for the fractionation of protein mixtures. The method is based upon the same principles as that of the Clusius column, except that horizontal electrophoretic transport instead of transport by thermal diffusion is superimposed on convective transport in a vertical convection channel.

The value of electrophoresis-convection as a tool in protein fractionation is demonstrated by its use in the fractionation of diphtheria antitoxin.

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