THE EFFECT OF CERTAIN ION EXCHANGE RESINS ON THE PROTEIN FRACTIONS OF CHICKEN SERUM

by

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INTRODUCTION

Many methods, based on precipitation, have been used to separate the various components of protein mixtures (1). Before the influence of heat, solvents, change in pH, and dehydration upon proteins was understood, almost any of these methods was used for this purpose. When the effects of the environmental influences became known, the most useful methods of protein fractionation became limited to increase in ionic strength, decrease in dielectric constant, and decrease in ionic strength.

An increase in ionic strength includes the various methods devised to "salt out" the components. In this general method, ammonium sulfate is the most efficient salt, primarily because its great solubility in water permits the attainment of high ionic strengths. This permits a greater range of salting out than any other salt. Sodium sulfate has been widely used, and was first developed as an analytical method by Howe (2). For any specific ionic strength the salting out action of sodium sulfate is greater than ammonium sulfate; however, it is less soluble than ammonium sulfate. This low solubility seriously limits the use of sodium sulfate since it must be maintained at 37° C. in order to prevent precipitation of the salt. In the use of ammonium salts, the nitrogen present makes it impossible to use direct methods for nitrogen analysis to determine the protein concentration. In all salting out processes, the protein must be

freed from the salt precipitating it by time consuming dialysis, the protein then heat coagulated or separated in some other way.

The methods involving decrease in dielectric constant include protein electrolyte interaction in organic solvent-water mixtures. The most used combination in recent years has been the alcohol-water system. This system permits a balance between the solvent action of the electrolyte and the precipitating action of the alcohol-water mixture, and permits a wide range of conditions such that the solubility of the proteins under consideration may remain constant. Five independent variables; solubility of protein, electrolyte and ethanol concentrations, pH, temperature, and protein concentration may be maintained under accurate control.

A method dependent upon decrease in ionic strength is low temperature alcohol precipitation at low ionic strength. This method has the advantage of being more specific for an individual protein than the previous method and the alcohol may be readily removed by vacuum distillation at low temperature, without denaturing the protein. The protein can remain in the dry state for a long time without alteration, and the dangers of bacterial growth which besets dialysis is completely avoided.

In the latter two methods, alcohol and protein electrolyte interaction with alcohol-water mixtures, decrease in ionic strength by low temperature precipitation, the design and expense of the apparatus and equipment, and the necessity of obtaining and maintaining the correct temperatures are important

disadvantages. The addition of alcohol at an unduly high ethanol concentration might denature the protein.

The disadvantages of the methods described above were too many to be of value at this time.

In seeking a less complicated or time consuming method of decreasing the ionic strength of the serum, the use of ion exchange resins appeared promising. This investigation was initiated to determine the effect of certain ion exchange resins on the blood serum of the chicken, and to determine if they could be used to advantage in the fractionation of its various components. A need for a method of this kind became apparent during the studies concerning the effect of Newcastles disease on the changes occurring in serum proteins, and the calcium-protein relationships in the blood serum during egg production.

After this investigation had been started, Reid and Jones (3) published some preliminary findings on the fractionation of human blood plasma with ion exchange resins. His instructions were too general, however, and no attempt was made to duplicate them. As this paper was being written, Reid and Jones (4) published some revised techniques of their former work under nearly the same title. This paper will be more fully analyzed later in the discussion.

METHODS

Preparation of Serum

Chicken blood was allowed to stand at room temperature for about an hour to allow time for clotting and for the serum to partially separate. The clot-serum mixture was then subjected to mild centrifugation for fifteen minutes, the resulting serum poured into an Erlenmeyer flask, and stored at 5° C. until used. The maximum amount of serum was obtained by collecting approximately 20 ml of blood per serum tube. Upon centrifugation the tube contained about 50 per cent serum.

Nature of the Ion Exchange Resins (Amberlites)

Various amberlite* ion exchange resins were available in the cation and anion form. The cation exchange resins were furnished usually as the sodium salt, and contained a carboxylate or a sulfonate group ($RCOONa^+$ or RSO_3Na^+). When the resins were prepared for use the sodium ion was replaced by a hydrogen ion by regeneration with 5 per cent sulfuric acid. The anion exchange resins had active amine groups, and were usually available in the chloride salt form, RNH_3Cl^-. They were prepared for use by replacing the chloride ion with the hydroxyl ion, by regeneration with 4 per cent sodium hydroxide. The regenerated exchange resins were then used to remove salts from the blood serum as described subsequently.

*Amberlite, trade name, Rohm and Haas Company, Philadelphia, Penn.

In the batch method, the cation and anion exchange reactions were equilibrium reactions. The reaction of the resin with the serum salts could be illustrated as follows:

$$RSO_3H^+ + Na^+ \rightleftharpoons RSO_3Na^+ + H^+$$

This release of hydrogen ions into the serum decreased the pH of the serum.

The anion exchange resin reaction with the serum salts reacted as follows:

$$RNH_3OH^- + Cl^- \rightleftharpoons RNH_3Cl^- + OH^-$$

This release of the hydroxyl ions into the serum increased the pH of the serum.

By the column method, the cation and anion exchange resin reactions were the same, except that the reactions were not equilibrium reactions, but were reactions which were driven completely to the right.

Methods of Studying the Effect of Amberlite on the Serum Proteins

General Description of the Batch Method. This process consisted of mixing a weighed amount of ion exchange resin and a measured volume of serum in a beaker. This mixture was agitated and set aside for a few minutes to reach equilibrium. The serum was then decanted and the process repeated three more times. Variations of this process were used and they will be discussed in the appropriate experiments.

General Description of the Column Method. The column method

consisted of preparing 8 inch columns with ion exchange resins and passing serum through the columns. The effluent serum was collected at a rate of six drops per minute in 3 ml quantities in calibrated serum tubes. A constant rate of flow was obtained by maintaining the top level of the serum in the tube.

The information gained in the batch and column methods was useful as a guide in later experiments which dealt with pH adjustment and the precipitation of the serum proteins.

General Description of the Mixed Amberlite Phase. This phase consisted of mixing together four anion exchange resins with three cation exchange resins in 12 combinations, with 4 different volume proportions for each combination. The anion exchange resins were IRA 400, IR 410, IR 75, and IR 4B, and the cation exchange resins were IR 100, IRC 50, and IR 120. The milliliter proportions of anion exchange resin to cation exchange resin were 2 to 3, 1.5 to 3.5, 1.0 to 4.0, and 0.5 to 4.5. Each of the 48 mixtures were treated with 5 ml portions of serum and agitated. The pH was determined when equilibrium was established.

Decrease in Ionic Strength Method

This method involved the alternate addition of cation and anion exchange resin so that the pH of the serum was kept within known limits. This cycle of adjustment was completed some twenty times in order to remove serum salts. When the volume of resin became large, the serum was decanted and the process continued.

As the serum salts were removed the change of the pH of the serum as the exchange resins were added became more critical; as the anions of the serum became depleted, it made the pH adjustment impossible. The pH of the serum was then adjusted to 6.0 by adding the cation exchange resin where cloudiness and precipitation occurred. The mixture was then refrigerated for 1 hour at 5° C., centrifuged, and the resultant serum was used for further pH adjustment to 5.6, and 5.1. These pH values represented the iso electric points of γ, β, and α globulins respectively (5).

Analytical Methods

Introduction. The most practical methods available for this investigation were based on the Kibrick and Blonstein (6) procedure used in conjunction with the micro Kjeldahl (7) and the Biuret reaction method (8).

Kibrick and Blonstein Method. Kibrick and Blonstein devised a salt fractionation method for protein precipitation, using different concentrations of sodium sulfate. In the case of human serum this method agreed well with electrophoretic, methyl alcohol and immune serum methods of protein analysis. (8)

The fractionation was made by adding 0.5 ml of serum to 10 ml of 15.75, 19.90, and 27.20 per cent sodium sulfate solutions, at 37° C. About 10 mg of Hyflo Super-Cel were added and the mixtures were allowed to stand in the incubator at 37° C. for 1 hour. The precipitates were then filtered in covered funnels, in the

incubator with Whatman no. 50 filter paper, 9 cm in diameter.

Other helpful information contained in the above article is summarized here. The 15.75 per cent sodium sulfate concentration precipitated mainly γ globulin, leaving albumin, α , and β , globulins as the main components of the filtrate. The 19.90 per cent sulfate concentration precipitated γ , and β globulins leaving mainly albumin and α globulin in the filtrate. The 27.20 per cent sodium sulfate precipitated all the globulins, leaving albumin as the main component of the filtrate. The total serum protein components were considered to be albumin, α , β and γ globulins. The analysis of the filtrate from the precipitation caused by the 27.20 per cent sodium sulfate gave the total albumin in the serum. The filtrate analysis from the 27.20 per cent sodium sulfate subtracted from the filtrate analysis from the 19.90 per cent sodium sulfate gave the α globulin. The filtrate analysis from the 19.90 percent sodium sulfate subtracted from the filtrate analysis of the 15.75 per cent sodium sulfate gave the β globulin. The filtrate analysis of the 15.75 per cent sodium sulfate subtracted from the total protein analysis gave the γ globulin.

Five milliliter portions of the clear filtrates from the three sodium sulfate concentrations were used for analysis with the Biuret reaction, and a 1 ml portion containing 0.02 ml of the original serum was used in determining the total nitrogen by the micro Kjeldahl method*. The total nitrogen was used to calculate the total protein of the serum.

*Total nitrogen was converted to total protein by multiplying by 6.25 factor.

The Weichelbaum Biuret Method (7). Attempts have been made, without success, to use the Biuret reaction as a rapid method for determining protein in small amounts of blood serum and plasma. This determination is based on the fact that the Biuret reagent deepened in color in proportion to the amount of protein present. It was found necessary to change the 0.2 N sodium hydroxide, as indicated in the literature, to 2.0 N sodium hydroxide in order to get consistent results. This method of analysis was used to determine the protein content of the clear filtrates from the Kibrick Blonstein fractionation method, and the total protein in the original serum.

The Klett-Summerson photoelectric colorimeter, using a 540 millimicron wave length filter, was used in the Biuret determinations.

A standard curve was prepared by using 0.2 ml diluted serum containing 0.02, 0.0132 and 0.0066 ml each of the original serum and adding 5 ml water and 5 ml Biuret reagent. The blank was prepared by mixing 5 ml water and 5 ml Biuret reagent. After the reagent was added, the mixture was allowed to stand for 30 minutes and then the Klett-Summerson value was determined using the 540 millimicron filter. The blank was subtracted from the readings. The standard curve, Fig. 1, was constructed by plotting the colorimeter readings, which were logrithmic, against serum concentration.

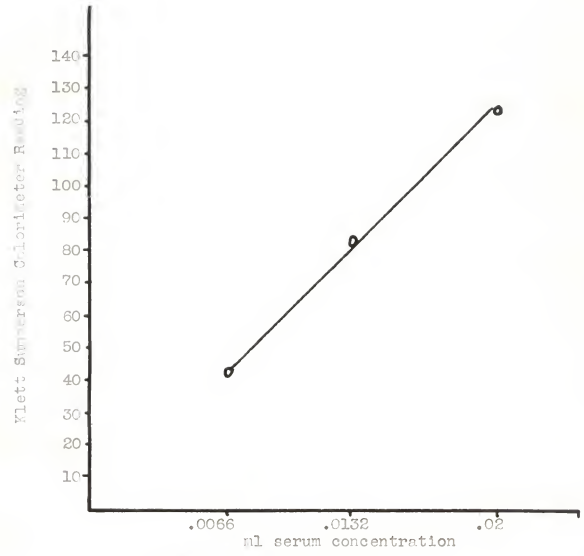


Fig. 1. Standard Curve

As shown by the curve, a straight line relationship was obtained. It was possible therefore to relate the serum and total protein content directly to the colorimeter reading.

Micro Kjeldahl Method. The method was used to analyze the original serum, resin treated serum, and the clear filtrates from the Kibrick Blonstein precipitations.

For total protein in a serum sample, 0.01 ml of the serum was diluted to 5 ml with water. One milliliter of this diluted serum containing 0.02 ml of the original serum, was used for the Kjeldahl digestion.

When this method was used with the Kibrick Blonstein filtrates, 1.0 ml of the clear filtrate, containing 0.05 ml original serum, from each sodium sulfate concentration was used for the Kjeldahl digestion.

Calculations

Weichelbaum Biuret Method. The total protein, and filtrates from the Kibrick Blonstein sodium sulfate concentration were determined by the Weichelbaum method. The colorimeter reading minus the blank, multiplied by the factor (slope of the line) from the standard curve, gave the total protein in each of the Kibrick Blonstein filtrates from the three sodium sulfate concentration. For convenience, let the analyzed total for the 27.20 per cent sodium sulfate filtrate be designated C; the 19.90 per cent filtrate be designated B; and the 15.75 per cent filtrate

be designated A. Then from the Kibrick Blonstein method under analytical methods, concentration C gave albumin; concentration B minus concentration C gave α globulin; concentration A minus concentration B gave β globulin; and total protein minus concentration A gave γ globulin. The values obtained from the Klett-Summerson readings were reduced by 20 per cent when reading total protein from the Kibrick Blonstein filtrates, as 0.024 ml of the original serum was analyzed as compared to 0.02 ml of the serum used to calibrate the standard curve by the Kjeldahl method.

Micro Kjeldahl. The following formula was used in the calculations for the total protein by the Kjeldahl method:

$$\frac{\text{ml std. acid}}{\text{ml std. acid react 1 mg N}} \times \text{dilution factor} \times \frac{100}{\text{ml dil. serum used}} \times K = \text{grams of protein per 100 ml serum.}$$

1. ml std. acid (variable)
2. 7.28 ml std. acid react 1 mg N (constant)
3. dilution factor $\frac{5}{0.1}$ (constant)
4. 100 to convert to 100 ml serum (constant)
5. K was 6.25 nitrogen conversion factor to protein (constant)

By substituting all the constants in the above formula a factor of 4.80 resulted. If the ml of acid used in the Kjeldahl titration were multiplied by this factor, the number of grams of protein per 100 ml of serum was obtained.

In the calculations for the analysis of the Kibrick Blonstein

filtrate, the only factor that changed from the above constants, was the dilution factor which became $\frac{10.5}{0.5}$ (constant). Substituting this new dilution constant and the other constants given above into the formula gave a new factor of 1.80. This factor nultiplied by the ml used in the titration gave total protein in the filtrates of Kibrick Blonstein concentrations. The actual protein fraction totals were obtained by subtraction as explained in the description of the Kibrick Blonstein method.

EXPERIMENTAL

Preliminary Experiments

The batch and column methods were used to determine the effect of the different ion exchange resins on the amount of the various protein fractions obtained by the Kibrick Blonstein method of analysis. In the batch experiments 13 ml samples of serum were adjusted to pH 9.0, pH 7.8, pH 7.5 and pH 7.0 by the addition of 0.2 N hydrochloric acid or 0.2 N sodium hydroxide, and then treated with 1 gram amounts of resins IRA 400, (regenerated) IR 100, (unregenerated) and IR 4B, (unregenerated). The regenerated form of IRA 400 was used to observe the effect the release of hydroxyl ions had on the apparent composition of the serum. The unregenerated forms of the cation and anion exchange resins were used to determine if the amberlite itself had any effect on the analysis of the serum components.

Table 1 summarizes the results obtained in this preliminary batch investigation.

Table 1. Effect of pH adjusted and resin treated serum.

Experiment	ml serum	pH adjusted to	1 g. resin number	resultant pH
1	13	9.0	IR 100	8.1
	13	7.8	100	7.65
	13	7.5	100	7.5
	13	7.0	100	7.3
2	13	9.0	IRA 400	10.9
	13	7.8	400	11.3
	13	7.5	400	11.3
	13	7.0	400	11.4
3	13	9.0	IR 4B	8.3
	13	7.8	4B	8.3
	13	7.5	4B	8.3
	13	7.0	4B	8.3

The final pH of the serum samples treated with IR 100 varied from 8.1 to 7.3 and in general indicated a tendency toward pH 7.5. The pH of the serum samples treated with IRA 400 were very high, about pH 11.0 or higher. In the case of all samples treated with IR4B the pH was close to that of the original serum (pH 8.3).

The analytical results of representative serum samples from each experiment, and of the original serum are summarized in Table 2.

Table 2. Analysis of pH adjusted and resin treated serums.

Component	Total Protein	Albumin	α Globulin	β Globulin	γ Globulin
serum	5.22	1.72	1.23	.52	1.75
IR100	5.18	1.60	1.35	.63	1.60
serum	4.25	1.61	1.34	.66	.64
IR 4B	4.19	1.58	1.35	.72	.54
serum	3.81	.72	.29	.21	2.59
IRA 400	3.81	.20	.16	.14	3.31

Amounts of protein are expressed in grams per 100 ml serum. IR100 represents serum treated with that resin.

The amounts of the total protein and protein components of the treated serums with unregenerated resins IR 100, and IR 4B varied but little from the untreated serum. The serum treated with regenerated IRA 400, however, showed a large drop in the albumin content, .72 to .20 g per 100 ml of serum. The α globulin dropped from .29 to .16 g, and the β globulin decreased from .21 to .14 grams. These decreases, however, were balanced by a large increase in the γ globulin fraction from 2.59 to 3.31 grams. Since the total protein in the serum remained the same before and after treatment with the resin, it is apparent that the high pHs resulting from the addition of IRA 400 changed the properties of the protein components.

A second series of preliminary experiments in which resin columns were employed, explored further the effect of the various regenerated amberlites on the final pH of the serum, and the apparent composition of the serum. The columns were prepared by

packing 8 inches of amberlite into 6 mm glass tubes. The serum was allowed to run slowly through the column and 3 ml samples of the effluent serum were collected. The pHs of these samples were determined and the variations obtained with IRA 400 are illustrated in Fig. 2. Actual values are found in Table A1. The composition of serum samples, selected on the basis of pH variation are summarized in Table 3.

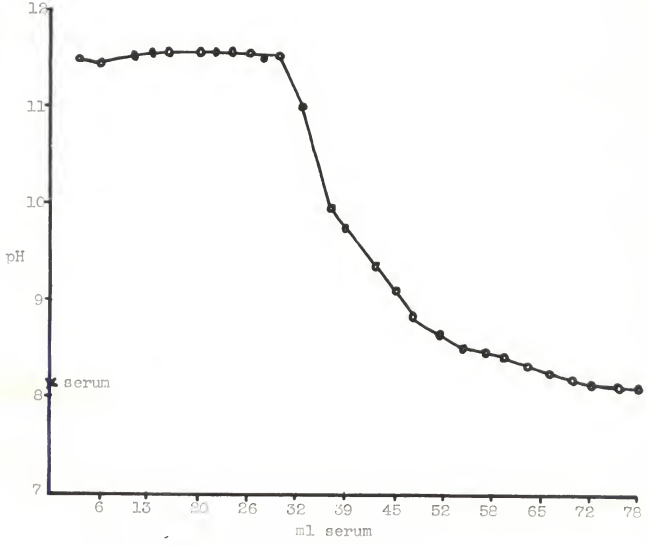


Fig. 2. IRA 400 Column Experiment.

The first 30 ml of the effluent serum had a pH of 11.3, the same pH as the serum treated with IRA 400 in the batch method. The pHs then dropped sharply and became the same as the original serum. The analysis of representative samples from the high pH portion of the curve, along with the analysis of serum treated by the batch method with IRA 400, are tabulated in Table 3.

Table 3. Protein analysis serum composition resin treated by batch and column

Method	Resin	Total protein	Albumin	α Globulin	β Globulin	γ Globulin
serum		5.20	0.70	1.85	0.90	1.75
column	IRA 400	4.50	0.02	0.60	1.25	2.63
serum		3.81	0.72	0.29	0.21	2.59
batch	IRA 400	3.61	0.20	0.16	0.14	3.31

Total protein in both the column and batch methods was found to decrease. The albumin content dropped sharply, especially in the column method where it was practically zero. In both methods of treatment α globulin decreased materially. The β globulin fraction showed an increase in the total in the column method, but a decrease in the batch method of treatment. Large γ globulin increases were shown, apparently due to the denaturation of the other protein fractions.

Other regenerated resins were investigated, using the column method, and the results of these analyses are given in Table 4.

Table 4. Analysis of serum in column experiments.*

pH	Resin	Total protein	Albumin	α Globulin	β Globulin	γ Globulin
	serum	5.25	.70	1.85	.90	1.80
11.5	IRA400	4.50	.00	.70	1.25	2.55
	serum	5.35	1.60	1.25	.80	1.70
12.1	IR 410	5.25	.15	.25	.55	4.30
	serum	5.65	1.85	1.35	.65	1.80
9.2	IR 4B	5.10	1.25	1.35	.70	1.80
	serum	4.10	1.35	.65	.55	1.55
12.3	IR 75	3.70	.40	.85	.80	1.65
	serum	3.95	1.86	.63	.55	.92
1.5	IR 100	3.84	.02	.02	.30	3.50
	serum	3.95	1.86	.63	.55	.92
4.2	IRC 50	3.95	.20	.10	.38	3.27

*Original serum totals are followed by treated total.

From an examination of the pH values and the analysis of the protein fraction totals in Table 4, certain relationships are evident. The serum-amberlite mixtures exhibiting the high and low pHs showed evidence of denaturation. The basic anion exchange resins IRA 400, IR 410, and IR 75 resulted in high pH values. Acidic cation exchange resins IR 100 and IRC 50 gave low pH values. The analytical results showed that, in general, the greater the pH variation the greater the discrepancy between the composition of the original serums and the treated serums. For example, IR 100 with a pH of 1.5 had only a slight total protein decrease from 3.95 to 3.84 grams per 100 ml serum, but the albumin and α globulin fractions practically disappeared going down from 1.86 grams to .02 gram, and .63 gram to .02 gram respectively. The alteration in the β globulin fraction was not

so severe, as the total drop was from .55 gram to .30 gram. Most of the altered proteins showed up in the analysis for the γ globulin. The γ globulin total increased from .92 gram to 3.50 grams.

Mixed Amberlite Experiments

The proper proportions the different cation and anion exchange resins, which would maintain a pH near neutral, in order to avoid denaturation of the serum proteins, was the next immediate problem. The batch and column methods indicated the amount of pH variation to be expected when the individual resins were added to the serum.

The following experiments were carried out to determine the pH of the original serum mixed with the ion exchange resin; the effect of mixing different proportions of two different ion exchange resins in the serum; and the comparative ability of the resin to change pH.

Resins IR 75 and IR 100 were lightly packed and leveled with a blunt end of a pencil and placed in a 15 ml graduated serum tube, in the following milliliter proportions: 4.5 and 0.5; 4.0 and 1.0; 3.0 and 2.0; 2.0 and 3.0; 1.5 and 3.5; 1.0 and 4.0; and 0.5 and 4.5. Each of 7 mixtures was dumped into a beaker containing 5 ml serum, mixed carefully, and the pH was read when the mixture came to equilibrium. The same procedure was followed for the following combinations: IR 410 and IR 100; IR 410 and IR 120; IR 4B and IR 100; IRA 400 and IRC 50; IRA 400 and IR 100; IRA 400

and IR 120; IR 75 and IR 100; IR 75 and IR 120; IR 4B and IR 120; IR 75 and IRC 50; IR 410 and IRC 50; and IR 4B and IRC 50. The results are graphically illustrated in Figs. 3 and 4, and the actual values in Tables A2 and A3.

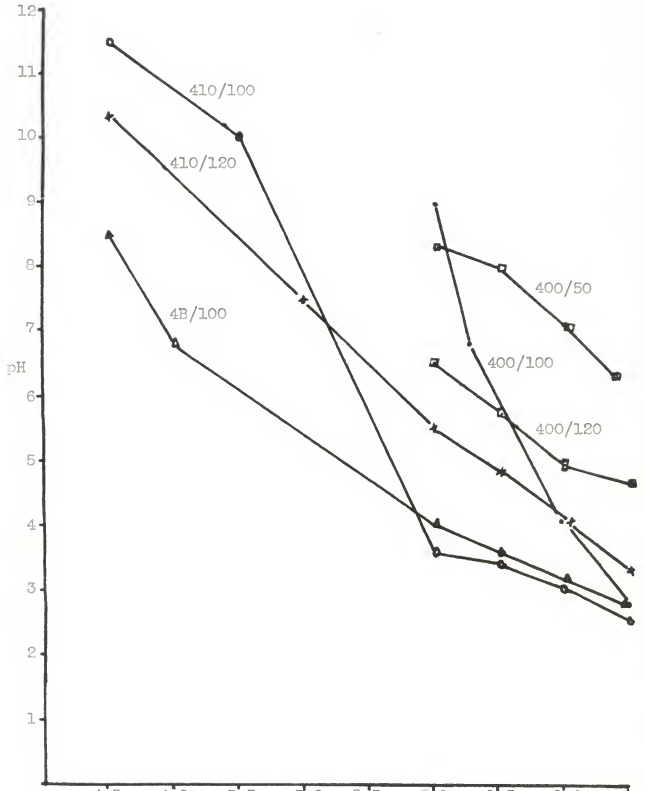


Fig.3. Mixed Amberlites.

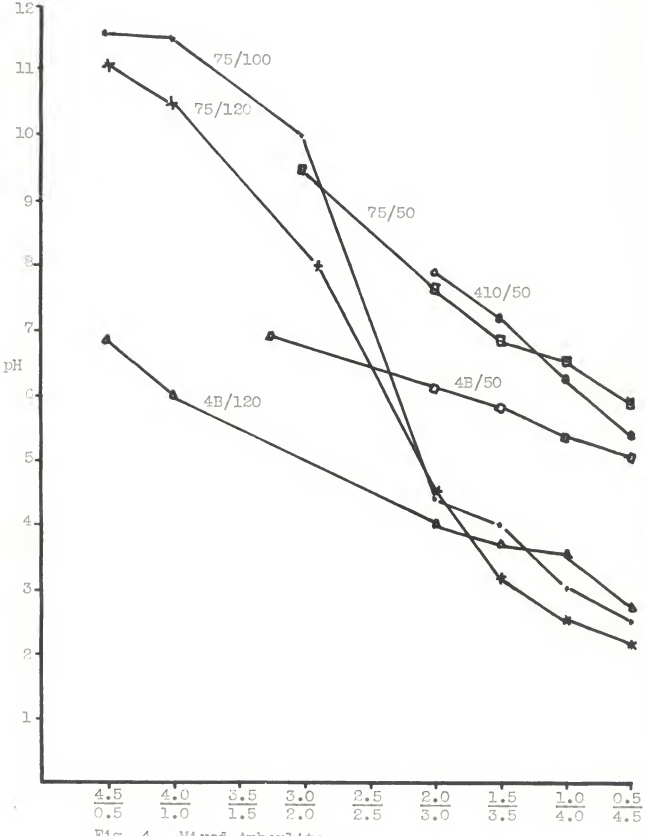


Fig. 4. Mixed Amberlites.

As shown in Fig. 4, when 4.5 ml of resin IR 75 and 0.5 ml of resin IR 100 were mixed with the serum, the pH was 11.6; and when 0.5 ml resin IR 75 and 4.5 ml resin IR 100 were mixed the pH was 2.5. Intermediate proportions of the mixed resins gave in between pH values.

The pH range important to this investigation was between 6.5 and 8.0, where the serum salts could be removed without denaturation of the proteins. Upon examination of Figs. 3 and 4, this pH range between 6.5 and 8.0 was obtained when 0.5 ml IRA 400 and 4.5 ml IRC 50 were mixed with serum. The resulting pH was 6.3. When 2.0 ml IRA 400 and 3.0 ml IRC 50 were used pH 8.0 resulted. This same range was obtained with the other exchange resin combinations when proportioned in mixtures according to Table 5.

Table 5. Various ion exchange combinations with proportions giving pH range 6.5 to 8.0.

Combination		pH	Combination		pH
IRA 400	IRC 50		IR 410	IRC 50	
0.5	4.5	6.3	1.0	4.0	6.2
2.0	3.0	8.3	2.0	3.0	7.2
IRA 400	IR 100		IR 75	IRC 50	
1.75	3.25	6.8	1.0	4.0	6.5
2.0	3.0	9.0	2.0	3.0	7.7
IRA 400	IR 120		IR 4B	IRC 50	
2.0	3.0	6.5	2.0	3.0	6.1
2.75*	2.25	7.8	3.25	1.75	6.9
IR 410	IR 120		IR 75	IR 100	
2.0	3.0	5.6	2.0	3.0	4.4
3.0	2.0	7.5	3.0	2.0	10.0
			2.67*	2.33	7.0
IR 4B	IR 100		IR 75	IR 120	
4.0	1.0	6.8	2.0	3.0	4.5
4.5	0.5	8.5	3.0	2.0	8.0
IR 410	IR 100		IR 4B	IR 120	
2.0	3.0	3.5	4.0	1.0	6.0
3.5	1.5	10.0	4.5	0.5	6.8
2.9*	2.1	7.0			

*Amberlites mixed in given ml proportions will give pH 7.

The proportions from Table 5 were used as a guide in the following experiments when mixing cation and anion exchange resins in an attempt to obtain near neutral pH values when removing the salts.

After the concentration of the mixtures of the resins needed to obtain the desired pH values were determined, these values were used in an attempt to obtain the correct pH range for the removal of the serum salts.

Volumes of cation exchange and anion exchange resins, mixed in the proportions shown in Table 5, were added to the serum. During the first few additions of the resins, the serum assumed the expected pH, but as subsequent additions were made the pH became more difficult to control, and the nature of the protein changed. As the serum salts were removed, as will be shown in later experiments, the serum became more sensitive to pH change by the cation exchange resin, and less sensitive to the anion exchange resin. These facts explained, to a certain extent, the failure to maintain the desired pH by this method.

Alternate Addition of Ion Exchange Resin, Section 1

As stated previously, the attempt to mix cation and anion exchange resins in the correct proportions before mixing with the serum did not give the correct end pH. This may have been caused by such factors as excess moisture, packing, surface area, or reaction rate. As a result of this failure, it was necessary to change the procedure so that the pH would remain within the desired range, and to accomplish this the cation and anion exchange resins were added alternately. The pH of the serum was followed closely with a Beckman, model G, pH meter, so that the serum would

remain between pH 6.5 and 8.0. These experiments were carried out without conductance tests and with conductance tests, to determine the degree of salt removal. A description of the former procedure using IR 410, anion exchange resin, and IR 120, cation exchange resin, is described as follows:

A 40 ml serum sample was taken from the refrigerator and placed in a 100 ml beaker surrounded by an ice bath. This gave a temperature of about 8° C. during the removal of the salts and the subsequent removal of the proteins. With the electrodes of the Beckman pH meter in the serum, the pH of the serum was found to be 7.95. Small amounts of IR 120 were added with a weighing spatula, while the serum was carefully stirred. After equilibrium was reached, (about 30 seconds), the pH was found to have decreased. The serum was stirred and the pH readings taken about 4 times before additional IR 120 was added. This became standard procedure when adding other ion exchange resins in the subsequent experiments. The stirring was necessary since the resin fell to the bottom of the beaker and lost intimate contant with the serum. When stirring failed to produce a pH change, more exchange resin was added until pH 7.0 was reached. The anion exchange resin, IR 410, was added in a similar manner until pH 7.95 was reached. This cycle from pH 7.95 to pH 7.0 and back to pH 7.95 was repeated until the adjustment became extremely sensitive for the cation and more difficult for the anion exchange resin. At this point, the serum salts were considered to be adequately removed, and the serum was ready for the adjustment of the serum with the ion exchange resins to pHs 6.0, 5.6, and 5.1.

After the salt removal phase had been completed, the serum was refrigerated and then subjected to mild centrifugation for five minutes. From the supernatant a 3 ml sample, designated number 1, was taken for analysis. The remaining centrifugate was treated with cation exchange resin IR 120 until the pH reached 5.98. Again the serum mixture was refrigerated and centrifuged. From the supernatant sample number 2 was removed for analysis. To the remaining centrifugate, IR 410 was added to bring the pH back to 7.95, and then IR 120 to return it to pH 5.6. After refrigeration, the mixture was centrifuged and a 3 ml sample, number 3, was taken from the supernatant. The remaining centrifugate was treated with IR 120 to bring the pH to 5.10, refrigerated, centrifuged, and sample number 4 was taken from the supernatant.

If the pH value fell below that required and the anion resin failed to bring it back, a few drops of 0.2 n sodium hydroxide was added, with constant stirring, to reach the desired value.

Using the same experimental conditions explained above, the following exchange resin combinations were investigated: IRA 400 and IR 100; IR 4B and IR 100; IR 75 and IR 120. These three combinations were run at room temperature, about 30° C. At ice bath temperature, about 8° C. IR 410 and IR 120, and IRA 400 and IR 120, were the combinations investigated. Results of the analysis of the protein fractions of these combinations are shown in Plates I through VII. The actual experimental values are found in Tables A4 and A5. Since some of the experiments were conducted at room

temperature, and others in an ice bath, they will be discussed separately in this order.

PLATE I

The combination IRA 400 and IR 100, shown on Plate I, showed a steady decrease in total protein as the pHs were adjusted to 8.01, 5.98, and 5.10, the largest decrease being observed at pH 5.98. The albumin remained constant throughout the adjustment. The α globulin showed a regular decrease as the pH values were lowered. This was also true of the β globulin values. The γ globulin showed the biggest decrease at pH 5.98.

PLATE I

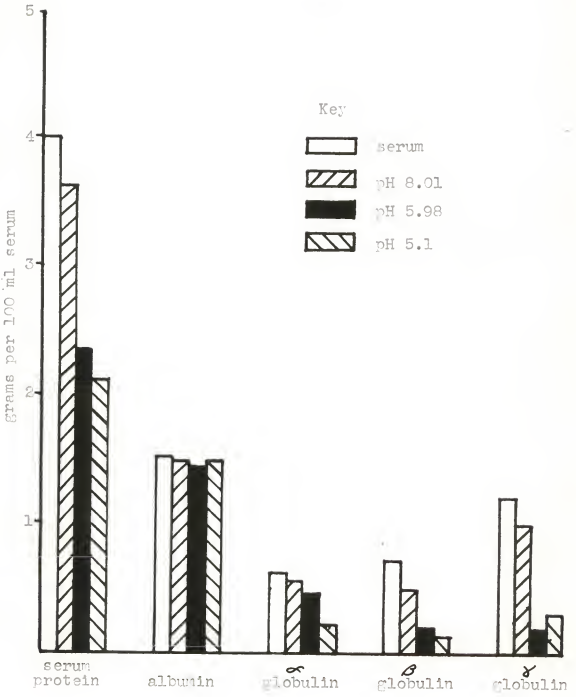

5
4
3
2
1
grams per 100 ml serum
Key
serum
pH 8.01
pH 5.98
pH 5.1
serum protein
albumin
α globulin
β globulin
γ globulin

PLATE II

As shown in Plate II, the resin combination IR 75 and IR 120 showed also a steady total protein decrease as the serum was taken through the pH adjustments from 7.95 to 6.05 to 5.6 to 5.05. The albumin remaining in the serum was approximately the same during the entire experiment. The α globulin showed a decrease at pH 7.95, after removal of the serum salts, but for the remainder of the pH adjustments the values were constant. The β globulin, except for a slight rise at pH 5.6, showed a steady decrease throughout the pH changes. The γ globulin showed a high and low level, the big change coming at pH 6.05.

PLATE II

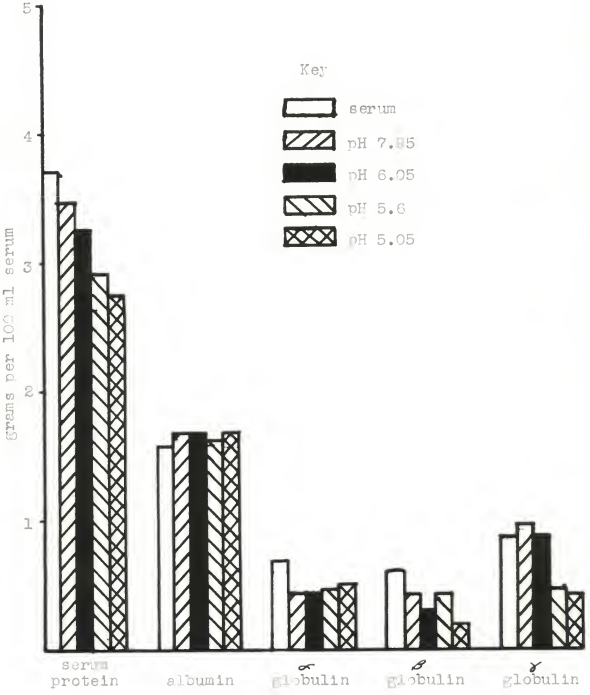

Key
serum
pH 7.95
pH 6.05
pH 5.6
pH 5.05
grams per 100 ml serum
5
4
3
2
1
serum protein
albumin
α globulin
β globulin
γ globulin

PLATE III

The Ir 4B and IR 100 ion exchange resin combination results are found on Plate III. A steady decrease in protein totals was noted as the pHs were adjusted to 7.3, 5.45, 5.90, and 5.10. Contrary to expectations the albumin showed a decreasing trend throughout the pH adjustments. The γ globulins also showed a stepwise decrease in values as the totals were taken at the individual pHs. This result differs materially from the other experiments. The α globulin showed a decrease in the first three adjustments, and remained constant after the fourth pH adjustment, while β globulins showed a small drop at pH 7.30 and then only slight variations during the rest of the experiment. The action of the strong cation exchange resin, IR 100, on the albumin could have denatured it slightly accounting for the decrease, while the denatured albumin appeared in the γ globulin fraction by our method of analysis. This same action was observed in the preliminary experiments and recorded in Table 4.

PLATE III

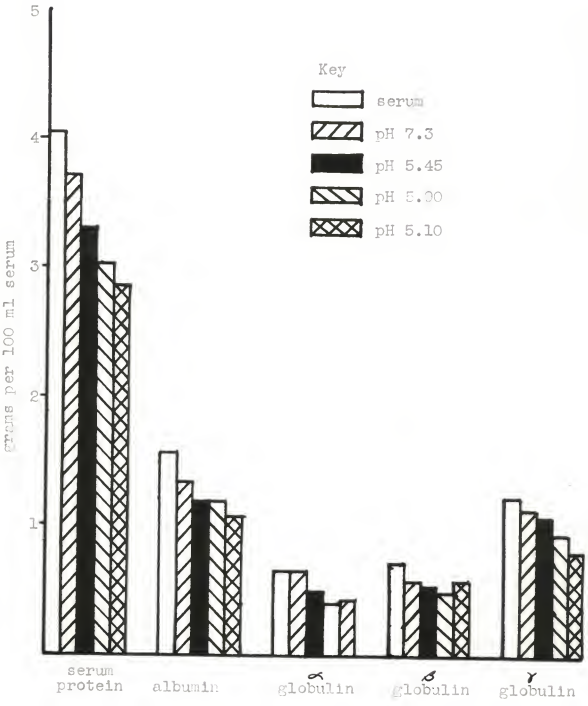

Key
serum
pH 7.3
pH 5.45
pH 5.30
pH 5.10
grams per 100 ml serum
5
4
3
2
1
serum protein
albumin
α globulin
β globulin
γ globulin

PLATE IV

The experiments conducted in an ice bath employed IR 410 and IR 120 in three separate experiments, and IRA 400 and IR 120 in a single experiment.

In Plate IV, the results of the first experiment are illustrated. It was observed as in the room temperature experiments that there was a steady decrease in the total protein as the pH of the serum was reduced from 7.95 to 5.98 to 5.60 and to 5.10. As expected the albumin remained fairly constant. The α globulin showed little or no change throughout the adjustments. The β globulin decreased steadily. The γ globulin, as in previous experiments, decreased sharply at pH 5.98, and remained at the low values throughout the adjustment phase.

PLATE IV

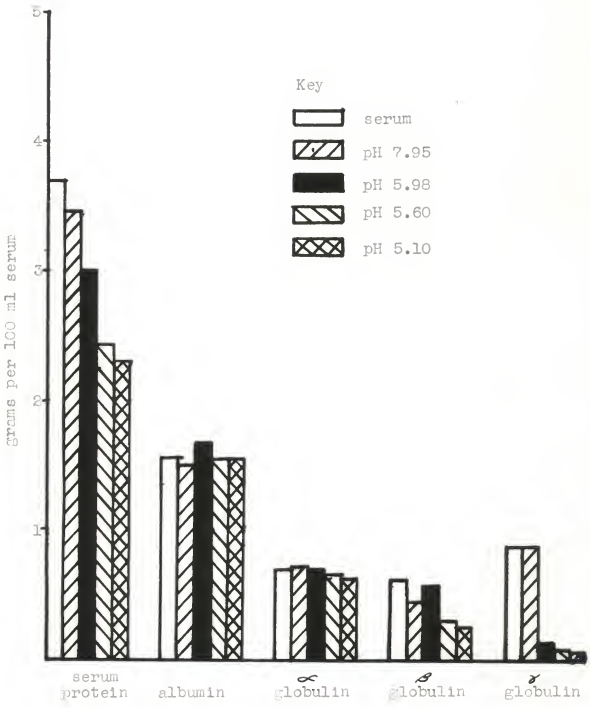

Key
serum
pH 7.95
pH 5.98
pH 5.60
pH 5.10
grams per 100 ml serum
5
4
3
2
1
serum protein
albumin
α globulin
β globulin
γ globulin

PLATE V

The second experiment with combination IR 410 and IR 120 is found recorded on Plate V. This experiment was found in agreement with the first experiment with the following exception:

In the second experiment, Plate V, there was a much larger protein drop at pH 6.0. Albumin showed a stepwise decrease with the pH adjustment, while in the first experiment, Plate IV, it was steady. The α globulin showed a large decrease at pH 6.0, while in the first experiment, Plate IV, it was steady.

PLATE V

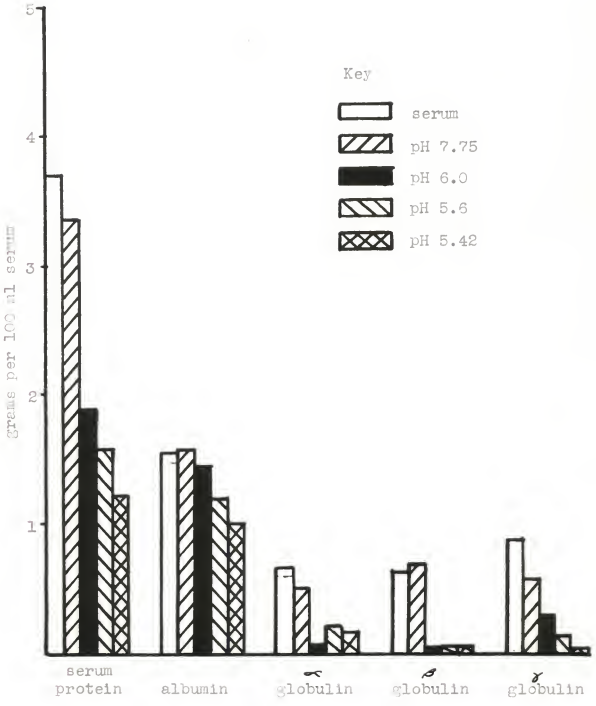

Key
serum
pH 7.75
pH 6.0
pH 5.6
pH 5.42
Grams per 100 ml serum
5
4
3
2
1
serum protein
albumin
α globulin
β globulin
γ globulin

PLATE VI

The third experiment of this group of three experiments, recorded on Plate VI, differ little from the first experiment, Plate IV. The main exception found in the third experiment was the β globulin, which showed a step like decrease in total, while the first experiment was steady in value.

PLATE VI

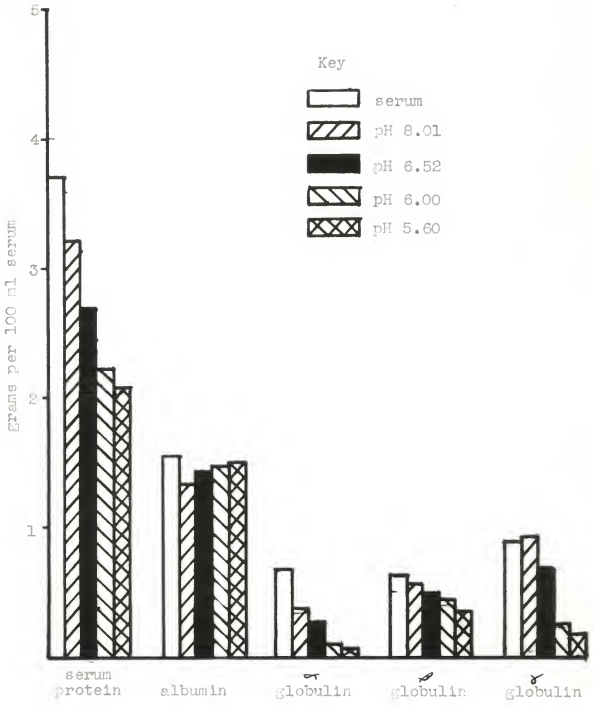
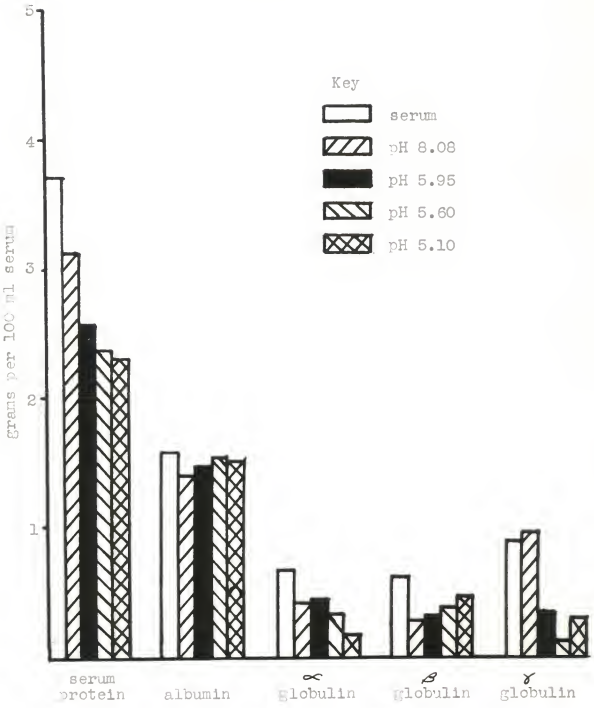

Key
serum
pH 8.01
pH 6.52
pH 6.00
pH 5.60
grams per 100 ml serum
5
4
3
2
1
serum protein
albumin
globulin
globulin
globulin

PLATE VII

The fourth experiment, conducted in an ice bath, used a combination IRA 400 and IR 120. Graphed results are found on Plate VII. The total protein values showed a regular decrease through the pH adjustment from 8.08 to 5.95 to 5.6, while at pH 5.10 the value decreased very slightly. Albumin, as expected, remained in the serum in fairly constant amount. The α globulin showed stepwise reduction in total except at pH 5.95, where it remained practically constant. The β globulin showed a decrease in value at pH 8.05. The γ globulin, as expected, showed a sharp decrease in total at pH 5.95, with minor fluctuations at pH 5.60 and pH 5.10.

PLATE VII


Key
serum
pH 8.08
pH 5.95
pH 5.60
pH 5.10
grams per 100 ml serum
5
4
3
2
1
serum protein
albumin
α globulin
β globulin
γ globulin

These results may be summarized as follows: There was a drop in protein value in all experiments at pH 8 after the alternate cation and anion exchange resin treatment, and there were decreases in total protein with each subsequent pH adjustment. In general, the albumin remained at a fairly constant value, except for one room temperature experiment, IR 4B and IR 100, which was observed to have a gradual reduction in total albumin content with each pH adjustment. This same reduction was noticed in an earlier preliminary experiment when strong cation exchange resin IR 100 reacted with the serum (Table 4). Closely related to this was the higher than normal globulin totals in the same experiment. Otherwise, the globulin fractions in the remainder of the experiments showed their largest decreases at about pH 6.0, and their totals remained low thereafter. There were general decreasing trends in the globulin and the globulin fractions with each pH adjustment.

Alternate Addition of Ion Exchange Resin, Section 2

The preceding experiments were conducted upon the basis that the serum salts were almost completely removed. However no experimental data was presented to support this conclusion. In the following section, evidence is presented, in the form of conductance tests, which indicates a very high serum salt removal.

In the conductance tests, it was found necessary to adjust the pH of the serum from 8.0 to 7.0 to 8.0, 22 times before the resistance of the serum salts increased sharply, (Table A7). On the 22nd adjustment, the resistance was 1180 ohms, whereas (in experiment 1) on the 25th adjustment it had risen to 6450 ohms. The conductivity of 6450 ohms is 1.5×10^{-4} reciprocal ohms which shows (Fig. 5) that the conductivity is equivalent to a solution of sodium chloride of ionic strength of .0025. As the pH of the serum was adjusted to pH 6.0, 5.6, and 5.1 the conductance decreased to $.31 \times 10^{-4}$ reciprocal ohms as shown in Table 6.

Table 6. Conductance measurements for serum adjustments.

Exp. no.	pH adjustment	ohms resistance	rho X10-4 conductance
1	8.0	6450	1.55
	6.0	11900	.84
	5.6	18400	.54
	5.1	32400	.31
2	8.0	6480	1.55
	6.0	11890	.84
	5.6	17200	.58
	5.1	27100	.37

The low conductance shown in Table 6, indicated a sodium ionic strength of .001, or less was attained in the final pH adjustment.

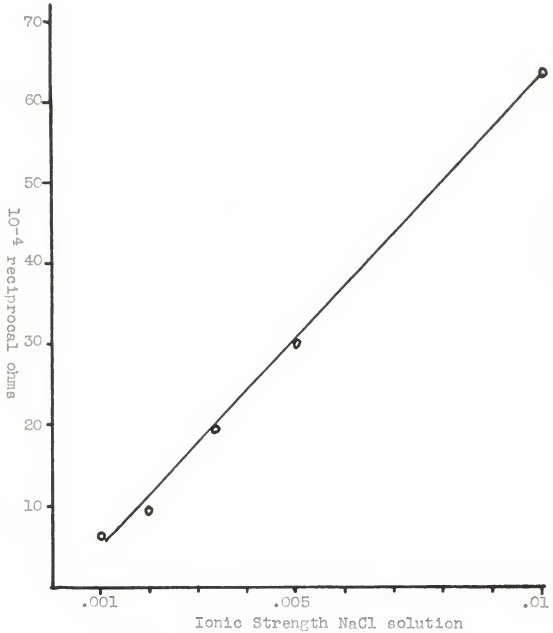


Fig. 5. Conductance Curve.

A description of the experimental procedure using IR 75, anion exchange resin, and IR 120 cation exchange resin, for the section 2 experiments is described as follows.

A 75 ml serum sample was transferred to a 150 ml beaker, and placed in an ice bath. This gave a temperature of about 8° C. which was maintained during the removal of the serum salts, followed by the adjustment to the individual pHs, and the conductivity experiments. The pH of the original serum was found to be 8.0. Small amounts of IR 120 were added with a weighing spatula, and stirred with a glass rod. Within 30 seconds, the pH meter gave a constant value, and the pH was found to have decreased. Stirring was repeated until the pH decrease approached zero, and then more resin IR 120 was added. This was repeated until pH 7.0 was reached. Then anion exchange resin, IR 75 was added until pH 8.0 was reached. This cycle pH 8 to pH 7 and back to pH 8 was repeated until the pH of the serum became more sensitive to the cation exchange resin, and the anion exchange resin failed to change the pH significantly. At this time a few drops of 0.2 N sodium hydroxide were added with constand stirring to adjust the serum pH to 8.0. The serum was then placed in an ice bath, and conductance tests were made at 0.5° C. When the resistance measured more than 6000 ohms at the climax of a sharp increase, it was decided that sufficient serum salts had been removed. Each pH adjustment and conductance measurement took about 15 minutes to complete.

The third part of the experiment consisted of adjusting the

nearly salt free serum to pHs 6.0, 5.6, and 5.10. Before this adjustment, the serum was centrifuged at slow speed for five minutes in cold tubes, and a 3 ml sample (no. 1) was taken from the supernatant for analysis. The centrifugate was then adjusted, as above, to pH 6.0 with resin IR 120. The serum mixture was again centrifuged as above, for five minutes. From the supernatant a 3 ml sample (no. 2) was taken for analysis. As before, the pH of the serum mixture was adjusted to pH 5.60, centrifuged and sample number 3 was taken for analysis. Finally the pH of the serum mixture was adjusted to 5.1, it was then centrifuged and the final 3 ml sample (no. 4) was taken.

The experiment was repeated with another 75 ml serum sample, using the above procedure. The results of the protein analysis of the original serum, treated serum, and serum from the pH adjustments was determined by the Kibrick Blonstein method in conjunction with the Biuret reaction with Klett Summerson colorimeter, using a 540 millimicron filter. Results are found on Plates VIII and IX, and Table A6.

PLATE VIII

The results of experiment 1 are given in Plate VIII. At pH 8.0, there was a large difference in the total serum protein before and after alternate treatment with cation and anion exchange resins. The adjustments to pHs 6.0, 5.6, and 5.1 found smaller and nearly equal decreases in total protein. A noticeable drop was made in the albumin fraction between the treated and untreated serum at pH 8.0; the remaining totals varied a little. The α globulin made 2 decreases, one between the treated and untreated serum, and the second at pH 6.0. The β globulin made a 2 step decrease in total at pH 6.0, and 5.6. The γ globulin had a large total drop at pH 8.0, when the serum was analyzed after treatment with ion exchange resin. The second drop was to zero protein at pH 6.0.

PLATE VIII

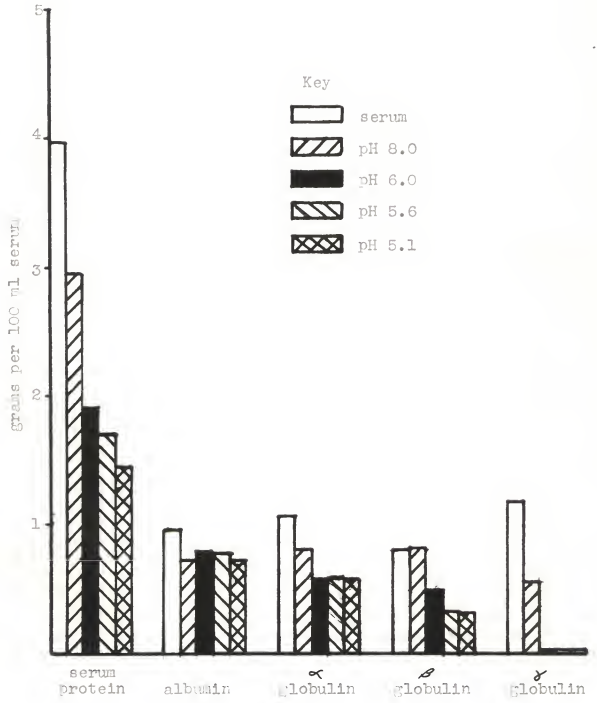
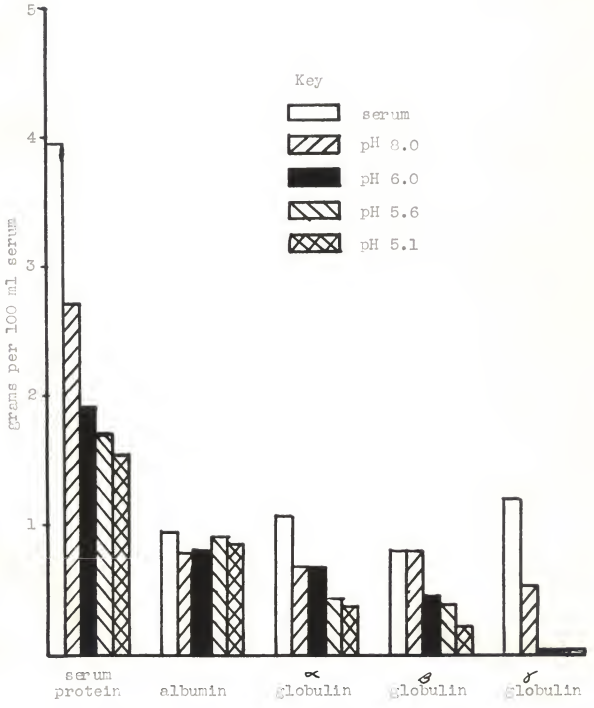

Key
serum
pH 8.0
pH 6.0
pH 5.6
pH 5.1
grams per 100 ml serum
5
4
3
2
1
serum protein
albumin
α globulin
β globulin
γ globulin

PLATE IX

The results of experiment 2 are given in Plate IX. The total protein values showed a large decrease at pH 8.0, when the serum was largely salt free, with nearly equal decreases as the pHs were lowered to 6.0, 5.6, and 5.1. A noticeable drop was made in the albumin total at pH 8.0; the remaining totals varied a little. There were two decreases in value of the α globulin, one between treated and the untreated serum, at pH 8.0, and the second at pH 5.6. The β globulin showed a 3 step decrease in totals at pHs 6.0, 5.6, and 5.1. The γ globulin, as in experiment 1, had a large total decrease at pH 8.0, and the second drop was to zero protein at pH 6.0.

PLATE IX


Key
serum
pH 8.0
pH 6.0
pH 5.6
pH 5.1
grams per 100 ml serum
5
4
3
2
1
serum protein
albumin
α globulin
β globulin
γ globulin

The use of the conductivity measurements to evaluate the removal of the serum salts resulted in a complete removal of γ globulin. Better duplication of the results was probably due to the reproduction of the same conditions of ionic strength prior to the pH adjustments and to the isoelectric points of the individual fractions. It is very possible that a lower ionic strength resulted from the use of the conductivity measurements.

DISCUSSION

In seeking a less complicated or time consuming method of decreasing the ionic strength of chicken serum so that the various components could be separated by adjusting the serum to their isoelectric points, the use of ion exchange resins employed in this investigation appears feasible.

The method as finally evolved consisted of treating the serum with cation and anion exchange resins, alternately, keeping the pH within certain definite limits to avoid denaturation of the protein, and then precipitating the components of the serum by adjusting it to their isoelectric points. In order that the experimental conditions could be more nearly duplicated, the ionic strengths of the remaining serum salts were determined by conductivity measurements.

The only other investigators reporting work of this type are Reid and Jones (3,4). In their preliminary report (3) they

did not give any detailed procedure. In a later report (4) too, which was published while this manuscript was in preparation, it was difficult to determine exactly what these investigators were using, since they used the words, blood plasma and blood serum interchangeably. In the present investigation the ionic strength of the original serum salt, as evaluated by conductivity measurements and calculated on the basis of sodium chloride concentrations, was approximately 0.01. In Reid's work the ionic strength of the original human serum determined in the same manner was 0.20. It may be that this difference was due to the fact that Reid and Jones were using human serum or plasma. It has been shown by Brandt (9) that the amounts of the protein components in chicken serum are different than those found in human serum. In some of the preliminary experiments of this work, the results were complicated by the fact that the serums of laying and non-laying hens were mixed. This may have caused some of the variations found in these preliminary investigations because Brandt, Clegg, and Andrews (10) indicated that there was a great difference in the amounts of the protein fractions in laying and non-laying hens. In the final series of experiments this difficulty was eliminated by employing the blood serum of cockerels or non-laying hens.

An examination of the results reported by Reid and Jones (4) indicated that they did not obtain a clear cut removal of the various components from what, in this investigation, is called the supernatant serum. This present investigation

indicated a rather successful removal of globulin from the serum, and also indicated that the protein remaining in the last supernatant was chiefly albumin. Reid and Jones were attempting to prepare extremely pure fractions for medical use. In the present investigation an attempt was being made to remove the various components progressively, so that the supernatant could be used in the future to study calcium/calcium proteinate equilibrium in chicken serum.

In view of the differences in the time needed for the individual ion exchange resins to come to equilibrium with the serum, work should be done to compare reaction rates of different resins so that two ion exchange resins may be selected with the same reaction rate for the alternate addition method. This would make for better pH control. Those amberlites which do not change the pH of the solution significantly should be investigated to determine whether or not these particular resins could be used to attain the low ionic strength needed for these separations. Other methods of analysis, such as the electrophoretic method, or those dependent on miscible organic solvents should be explored to determine if the salt fractionation method in this study is as dependable with chicken serum as results indicate that the Kibrick and Blonstein method is for human serum. Finally, since complete removal of the various protein components was not obtained at the pH values used at the isoelectric points which were for human protein, it may be possible that the chicken protein fractions had slightly different isoelectric points. Work should be done to explore this possibility.

SUMMARY

The use of ion exchange resins to remove the inorganic ions from the blood serum of the chicken is possible. Lowering the ionic strength of the serum in this way allows a practical separation of the protein components.

The use of conductivity measurements to evaluate the ionic strength of the serum, as the salts are removed, has been shown to be a definite advantage.

The removal of the γ globulin at a pH of 6.0, and an ionic strength of less than 0.001 can be considered quite satisfactory.

The α globulin and the β globulin removal was not as good. The α globulin showed a definite decrease as the serum salts were removed before the pH was adjusted to the isoelectric point. When the pH was adjusted to the various isoelectric points the removal was less consistent. The β globulin had its main decrease at pH 6.0 with minor decreases with the subsequent pH adjustments.

The total albumin remaining in the supernatant, after the pH adjustments, compared well with the albumin in the original serum.

ACKNOWLEDGMENT

The author wishes to thank Dr. R. E. Clegg, Department of Chemistry, for aid in selection of this problem and for assistance in interpreting the data. He is grateful to the Poultry Department for their cooperation in furnishing the chickens which furnished the blood used in the experimental work.

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APPENDIX

Table A1. pH of serum samples using IRA 400 in column experiments.

Sample number	ml through column	pH	Sample number	ml through column	pH
1	3	11.50	14	42	9.30
2	6	11.40	15	45	9.10
3	9	11.52	16	48	8.88
4	12	11.52	17	51	8.60
5	15	11.55	18	54	8.50
6	18	11.60	19	57	8.45
7	21	11.60	20	60	8.40
8	24	11.60	21	63	8.30
9	27	11.58	22	66	8.27
10	30	11.53	23	69	8.16
11	33	11.00	24	72	8.15
12	36	9.95	25	75	8.12
13	39	9.75	26	78	8.12

Table A2. Effect of mixed amberlite combinations and varied proportions on chicken serum.

IR 410	IR 100	pH	IRA 400	IRC 50	pH
4.5*	0.5	11.5	2.0	3.0	8.3
3.5	1.5	10.0	1.5	3.5	8.0
2.0	3.0	3.55	1.0	4.0	7.1
1.5	3.5	3.45	0.5	4.5	6.3
1.0	4.0	3.05			
0.5	4.5	2.55			
IR 410	IR 120	pH	IRA 400	IR 100	pH
4.5	0.5	10.3	2.0	3.0	9.0
3.0	2.0	7.5	1.75	3.25	6.8
2.0	3.0	5.5	1.0	4.0	3.8
1.5	3.5	4.8	0.5	4.5	3.3
1.0	4.0	4.1			
0.5	4.5	2.8			
IR 4B	IR 100	pH	IRA 400	IR 120	pH
4.5	0.5	8.5	2.0	3.0	6.5
4.0	1.0	6.8	1.5	3.5	5.8
2.0	3.0	4.0	1.0	4.0	4.9
1.5	3.5	2.6	0.5	4.5	4.7
1.0	4.0	3.2			
0.5	4.5	2.8			

*Volume of resin in ml.

Table A3. Effect of mixed amberlite combination and varied proportions on chicken serum.

IR 75	IR 100	pH	IR 75	IRC 50	pH
4.5*	0.5	11.6	3.0	2.0	9.5
4.0	1.0	11.5	2.0	3.0	7.7
3.0	2.0	10.0	1.5	3.5	6.8
2.0	3.0	4.4	1.0	4.0	6.5
1.5	3.5	4.0	0.5	4.5	5.8
1.0	4.0	3.0			
0.5	4.5	2.5			
IR 75	IR 120	pH	IR 410	IRC 50	pH
4.5	0.5	11.0	2.0	3.0	7.7
4.0	1.0	10.5	1.5	3.5	7.2
3.0	2.0	8.0	1.0	4.0	6.2
2.0	3.0	4.5	0.5	4.5	5.3
1.5	3.5	3.2			
1.0	4.0	2.5			
0.5	4.5	2.1			
IR 4B	IR 120	pH	IR 4B	IRC 50	pH
4.5	0.5	6.8	3.25	1.75	6.9
4.0	1.0	6.0	2.0	3.0	6.1
2.0	3.0	4.0	1.5	3.5	5.8
1.5	3.5	3.7	1.0	4.0	5.3
1.0	4.0	3.6	0.5	4.5	5.0
0.5	4.5	2.7			

*Volume of resin in ml.

Table A4. Protein analysis alternate addition resin experiments at room temperature.

Resins used	pH	Total Protein	Albumin	α Globulin	β Globulin	γ Globulin
IRA400/ IR 100	serum	4.02[2]	1.52	.63	.73	1.18
	8.01[1]	3.54	1.44	.57	.49	1.04
	5.98	2.35	1.47	.49	.18	.21
	5.10	2.12	1.29	.21	.13	.29
IR 75/ IR 120	serum	3.70	1.55	.66	.62	.87
	7.95	3.44	1.67	.39	.41	.97
	6.05	3.25	1.67	.41	.29	.88
	5.60	3.18	1.62	.44	.46	.44
	5.05	2.96	1.67	.49	.16	.41
IR 4B/ IR 100	serum	4.02	1.52	.63	.73	1.18
	7.30	3.67	1.34	.64	.57	1.12
	5.45	3.28	1.18	.50	.54	1.06
	5.90	2.99	1.18	.39	.49	.93
	5.10	2.86	1.06	.41	.59	.80

(1) Serum salts have been removed.
(2) Protein in grams per 100 ml serum.

Table A5. Protein analysis alternate addition resin experiments ice bath temperature.

Resins used	pH	Total protein	Albumin	α Globulin	β Globulin	γ Globulin
	serum	3.70[1]	1.55	.66	.62	.87
IR 410/ IR 120	7.95[2]	3.48	1.49	.70	.43	.86
	5.98	2.99	1.67	.68	.53	.11
	5.60	2.45	1.55	.64	.29	.00
	5.10	2.32	1.55	.61	.26	.00
	serum	3.70	1.55	.66	.62	.87
IR 410/ IR 120	7.75	3.32	1.55	.52	.69	.56
	6.00	1.87	1.44	.00	.06	.27
	5.60	1.58	1.21	.21	.05	.11
	5.42	1.19	1.03	.15	.03	.00
	serum	3.70	1.55	.66	.62	.87
IR 410/ IR 120	8.01	3.22	1.34	.37	.56	.95
	6.52	2.67	1.44	.27	.50	.69
	6.00	2.38	1.47	.10	.40	.22
	5.60	2.06	1.49	.06	.35	.16
	serum	3.70	1.55	.66	.62	.87
IRA400/ IR 120	8.05	3.09	1.44	.43	.27	.95
	5.95	2.54	1.47	.46	.33	.28
	5.60	2.38	1.55	.34	.37	.12
	5.10	2.35	1.49	.15	.44	.27

[1] Protein in grams per 100 ml serum.
[2] Serum salts have been removed.

Table A6. Protein analysis alternate addition resin experiments ice bath temperature with conductance tests.

Resins used	pH	Total protein	Albumin	α Globulin	β Globulin	γ Globulin
	serum	3.97[1]	.96	1.04	.78	1.19
IR 75/ IR 120	8.0[2]	2.92	.74	.80	.83	.55
	6.0	1.89	.83	.64	.49	.00
	5.6	1.70	.77	.64	.32	.00
	5.1	1.47	.74	.61	.32	.00[3]
	serum	3.97	.96	1.04	.78	1.19
IR 75/ IR 120	8.0	2.72	.77	.67	.77	.51
	6.0	1.89	.83	.64	.45	.00
	5.6	1.67	.93	.42	.38	.00
	5.1	1.54	.90	.38	.21	.00[4]

(1) Protein in grams per 100 ml serum.
(2) Serum salts have been removed.
(3) End of experiment 1.
(4) End of experiment 2.

Table A7. Serum salt removal conductivity measurements.

pH adjustment no.	Resistance ohms	Conductance rho	pH adjustment no.	Resistance ohm	Conductance rho*
1	145	69	1	149	69
2	148	68	2	153	65
3	156	64	3	162	62
4	165	61	4	172	58
5	172	58	5	164	61
6	178	56	6	170	59
7	181	55	7	177	56
8	191	52	8	187	54
9	202	50	9	192	52
10	214	47	10	198	50
11	222	45	11	208	48
12	241	42	12	221	45
13	312	32	13	283	35
14	328	31	14	300	33
15	348	29	15	317	32
16	391	26	16	332	30
17	431	23	17	375	27
18	502	20	18	410	24
19	584	17	19	445	22
20	680	15	20	498	20
21	875	11.4	21	580	17
22	1180	8.5	22	710	14
23	1620	6.2	23	740	13
24	3620	2.8	24	1010	9.1
25	6450	1.5**	25	1240	8.1
			26	1510	6.6
			27	2050	4.9
			28	3880	2.6
			29	6480	1.5

* 10^{-4} reciprocal ohms.

** Last measurement first experiment.

Table A8. Weights of ion exchange resin used in serum salt removal for each adjustment, experiments 1 and 2.

Adjustment no.	Weight in grams IR120	IR75	Adjustment no.	Weight in grams IR120	IR75
1	0.43	0.85	1	0.41	0.70
2	0.42	0.72	2	0.41	0.76
3	0.48	0.80	3	0.43	0.81
4	0.46	0.82	4	0.51	0.97
5	0.42	0.76	5	0.38	0.70
6	0.39	0.82	6	0.40	0.76
7	0.41	0.85	7	0.47	0.74
8	0.42	0.85	8	0.40	0.67
9	0.41	0.72	9	0.38	0.65
10	0.40	0.80	10	0.35	0.75
11	0.34	0.65	11	0.51	0.90
12	0.38	0.78	12	0.40	0.68
13	0.29	0.56	13	0.29	0.64
14	0.24	0.38	14	0.33	0.53
15	0.17	0.38	15	0.28	0.43
16	0.34	0.60	16	0.26	0.49
17	0.27	0.66	17	0.47	0.65
18	0.53	0.72	18	0.15	0.66
19	0.25	0.67	19	0.25	0.32
20	0.25	0.61	20	0.27	0.67
21	0.42	0.75	21	0.38	0.72
22	0.18	0.61	22	0.41	0.79
23	0.36	0.74	23	0.17	0.48
24	0.46	1.26	24	0.38	0.79
25	0.48	0.93*	25	0.22	0.31
total	9.24	18.29	26	0.15	0.40
			27	0.26	0.52
			28	0.45	1.37
			29	0.95	0.66*
			total	10.72	19.52

*Several drops of 0.2 N sodium hydroxide were used in both experiments to adjust the pH to 8.0.
Experiment 1 on left.

THE EFFECT OF CERTAIN ION EXCHANGE RESINS ON THE PROTEIN FRACTIONS OF CHICKEN SERUM

by

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This investigation was initiated to determine the effects of certain ion exchange resins on the blood serum proteins of the chicken, and to determine if ion exchange resins could be used to advantage in the fractionation of the various serum components. The use of ion exchange resins for this purpose proved feasible and resulted in a separation of the protein components.

Preliminary experiments with unregenerated cation and anion exchange resins were carried out to determine if the amberlite had any effect on the serum, and a regenerated resin was used to observe the effect of the release of hydroxyl ions on the apparent composition of the serum. Analysis by the Kibrick and Blonstein method in conjunction with the micro Kjeldahl method, showed that the total protein and protein components of the treated serums with the unregenerated resins varied little from the untreated serum. The serum treated with unregenerated resin showed a large drop in the albumin content and a corresponding increase in the γ globulin. Since the total protein in the serum remained the same before and after treatment of the resin, it was apparent that the high pHs resulting from the addition of the resin changed the properties of the protein components.

Other regenerated resins were investigated. The basic anion exchange resins resulted in high pH values, and the acidic cation exchange resins gave low pH values. As was shown by the analytical results the serum amberlite mixtures exhibiting high and low pHs showed evidence of denaturation. The greater the pH variation

the greater the discrepancy between the composition of the original and the treated serum. The albumin and α globulin fractions practically disappeared, and the β globulin drop in total was not so severe. Most of the altered proteins appeared in the analysis for the γ globulin.

The next immediate problem was the preparation in the proper proportions of the different cation and anion exchange resins to maintain near neutral pH values in order to avoid denaturation of the serum proteins. Twelve combinations of cation and anion exchange resins were mixed with the serum in varying proportions. The results of those proportions and combinations giving near neutral pHs were noted, and were used as a guide in subsequent experiments, in which mixed cation and anion exchange resins were used in an attempt to obtain near neutral pH values when removing serum salts. As the serum salts were removed the serum became more sensitive to the cation exchange resin, and less sensitive to the anion exchange resin resulting in low pH values and the nature of the protein was changed.

As a result of this failure, it was necessary to change the procedure so that the pH would remain within the desired range, and to accomplish this the cation and anion exchange resins were added alternately. In addition, conductance measurements were used to determine the degree of salt removal. The better evaluation of the serum salts; the more complete removal of the γ globulin; and better duplication of results

were advantages exhibited by the experiments carried out with conductance measurements.

In summary, the removal of the γ globulin at pH 6.0, and at an ionic strength of less than 0.001 was considered rather successful. The α and α' globulin removal was not as good. The α globulin showed a definite decrease as the serum salts were removed, and before the pH was adjusted to the isoelectric points the removal of the α globulin was less consistent. The β globulin had its main decrease at pH 6.0, and minor decreases with subsequent pH adjustments. The total albumin remaining in the supernatant, after the pH adjustments, compared well with the albumin in the original serum.