

ELECTROPHORESIS STUDIES OF WHEY PROTEINS IN MILK  
FREED OF CASEIN BY THE USE OF SALT

by

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## INTRODUCTION

There are several known methods used for the isolation of whey proteins from milk. Harland and Ashworth (1) have outlined a salt-acid method which is perhaps one of the simplest and easiest. Their procedure involves the precipitation of casein by saturation of the skim milk with sodium chloride followed by precipitation of the whey proteins upon addition of hydrochloric acid to the resulting filtrate. Harland and Ashworth claimed that whey proteins prepared by this method were "relatively undenatured." However, it is possible that addition of hydrochloric acid to the noncasein filtrate might change the characteristics of the original whey protein molecule. It is feasible that any such change in the original whey protein should be indicated by an altered electrophoretic pattern of those proteins. Differences between the electrophoretic patterns for Harland and Ashworth whey proteins and for those obtained from whey proteins isolated by other methods should show possible changes in the whey proteins caused by precipitating reagents.

The usual procedure employed in the preparation of whey proteins for electrophoresis studies involves the precipitation of casein from skim milk by acid or rennet followed by the concentration of the casein-free proteins by lyophilization. Smith (2, 3) and Deutsch (4) carried out electrophoretic studies of whey proteins prepared by this method.

The proteins to be used in this study are Harland and Ash-

worth proteins and whey proteins prepared by a modified Harland and Ashworth procedure. In the latter method the whey proteins are concentrated by lyophilization of the noncasein filtrate rather than by acid precipitation. It is assumed that concentration of the whey by lyophilization is a much less drastic treatment than concentration by precipitation of the proteins by hydrochloric acid. Indeed, it is always observed that samples prepared by lyophilization are always much clearer than those obtained by the procedure of Harland and Ashworth. This suggests that acid-precipitated proteins might be slightly denatured and possibly not completely soluble in the buffer solution.

Comparison of the electrophoretic patterns using these two preparations with those of Smith and Deutsch should indicate any effect upon the normal whey proteins due to the salting out of the casein or due to the acid precipitation.

According to Harland and Ashworth, the nonprotein filtrate contained only nonprotein nitrogenous substances and was assumed to be completely free of whey proteins. In the salt-lyophilized method, this filtrate was still present in the sample. Electrophoretic patterns of the nonprotein filtrate should prove the presence or absence of any whey proteins not precipitated or of any nondialyzable, nonprotein nitrogenous substances in the nonprotein filtrate.

## MATERIALS AND METHODS

## Preparation of the Whey Proteins

Three gallons of pooled, whole, raw milk were obtained from the Kansas State College Dairy. The cream was separated from the skim milk with a Sharples Super Centrifuge. Two gallons of the skim milk were saturated with 2500 g of sodium chloride at 40° C. and placed in an oven regulated at this temperature. The salted milk was allowed to stand at this temperature with an occasional shaking for a period of 24 hours.

Some difficulty was encountered in filtering off the precipitated casein. Harland and Ashworth recommended the addition of supercell or filter aid to hasten the filtering. However, this tended to clog up the filter paper. After many attempts at securing a clear filtrate, the best results were obtained by the use of a 4 liter vacuum flask with 10 inch Buchner funnel. A mat of precipitated casein was carefully prepared on a Sargent No. 500 filter paper and suction applied. After a few minutes, a clear, pale yellow whey was obtained. The precipitated casein was discarded. As an added precaution, to insure complete removal of the casein, the filtrate was again filtered through a folded Whatman No. 2 filter paper without suction.

The noncasein filtrate was divided into two parts, A and B. Part A was adjusted to pH 2.0 by addition of 10 percent hydrochloric acid with rapid stirring and allowed to stand overnight at room temperature. A mat of precipitated whey proteins was

prepared on a Whatman No. 2 filter paper as before and the proteins filtered with vacuum.

The whey proteins were carefully collected from the filter paper and transferred to a cellophane dialysis sack with a minimum of distilled water. Proteins prepared by this method were similar to the Harland and Ashworth proteins and designated as I on the flow sheet, Fig. 1.

The nonprotein filtrate from A was dialyzed against distilled water at 4° C. until free of chloride ion. It was then lyophilized to one-fifteenth of its original volume and designated as II.

Part B was dialyzed against water at 4° C. until free of chloride ion and then lyophilized. This fraction was designated as III. Fractions I and III were diluted with veronal-citrate buffer of 0.088 ionic strength at pH 7.6 until the protein concentration was approximately 1.5 percent and dialyzed in two-liter bottles at 4° C. for 72 hours against three changes of buffer solution. Sample II was dialyzed without dilution in the same manner.

The final buffer solutions, against which the samples were dialyzed, were used as the solvents in the electrophoresis cells to form the boundaries for migration.

The veronal-citrate buffer was prepared by bringing a solution of 206.18 g of sodium barbital and 45 g of sodium citrate dihydrate to pH 7.6 by the addition of citric acid and diluting to 20 liters with distilled water.

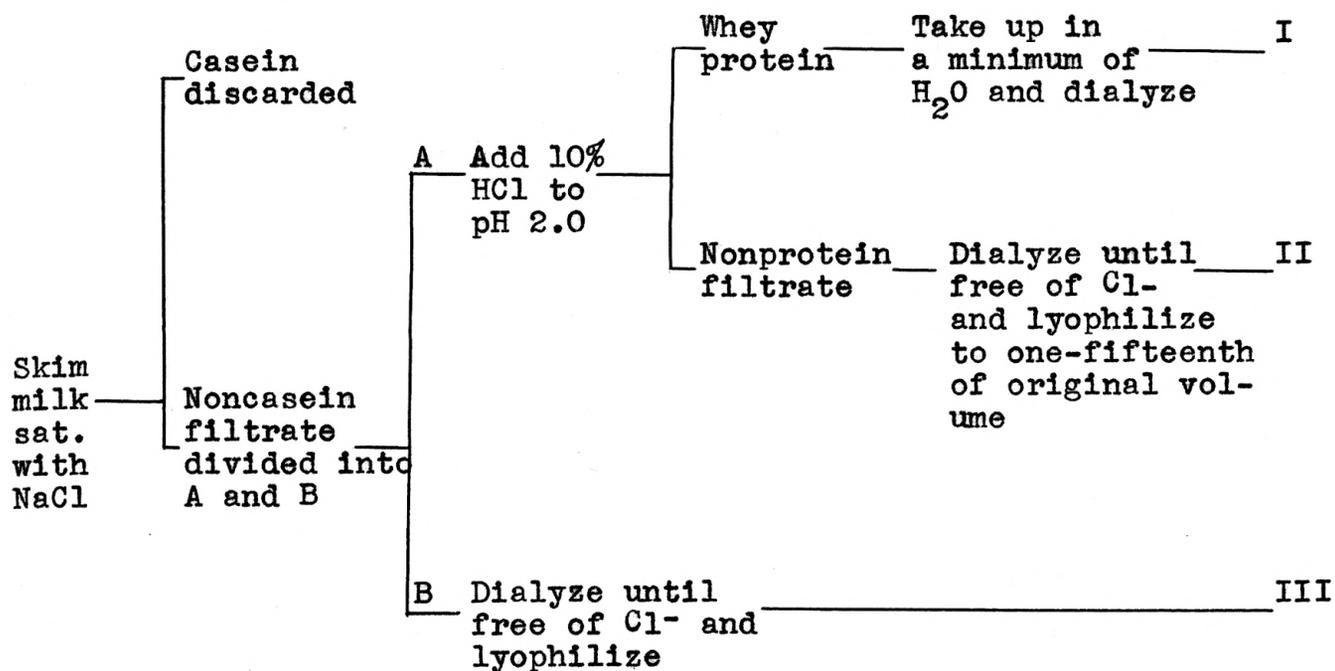


Fig. 1. Flow sheet for preparation of fractions of milk proteins.

## Electrophoresis Apparatus

The electrophoresis equipment used in this study was a modified Tiselius apparatus manufactured by the Klett Manufacturing Company, New York City. Description and operation of this apparatus has been extensively described elsewhere (5, 6, 7, 8, 9, 10, and 11) and will not be repeated in this thesis. The essential parts of the instrument, however, consisted of a lighthouse containing a mercury vapor lamp to serve as a light source, a constant temperature water bath equipped with a refrigeration unit capable of maintaining near zero temperatures, and a camera. These were all mounted on a channel iron optical bench secured on three cement piers set firmly through the floor of the laboratory and terminating in large footings in the basement subsoil, Fig. 2. The electrophoresis cells, Fig. 3, were clamped in a brass assembly rack which was placed in the water bath for an electrophoresis determination. The constant temperature bath was maintained at  $0.5^{\circ}$  C. for all determinations in this study.

A constant current of 20 ma for electrophoresis was supplied by the current regulator as modified by McColloch (11), Fig. 4.

## Focusing of Apparatus

As the electrophoresis laboratory at Kansas State College had recently been moved, it was necessary to completely reassemble and rewire the electrophoresis apparatus before any measure-

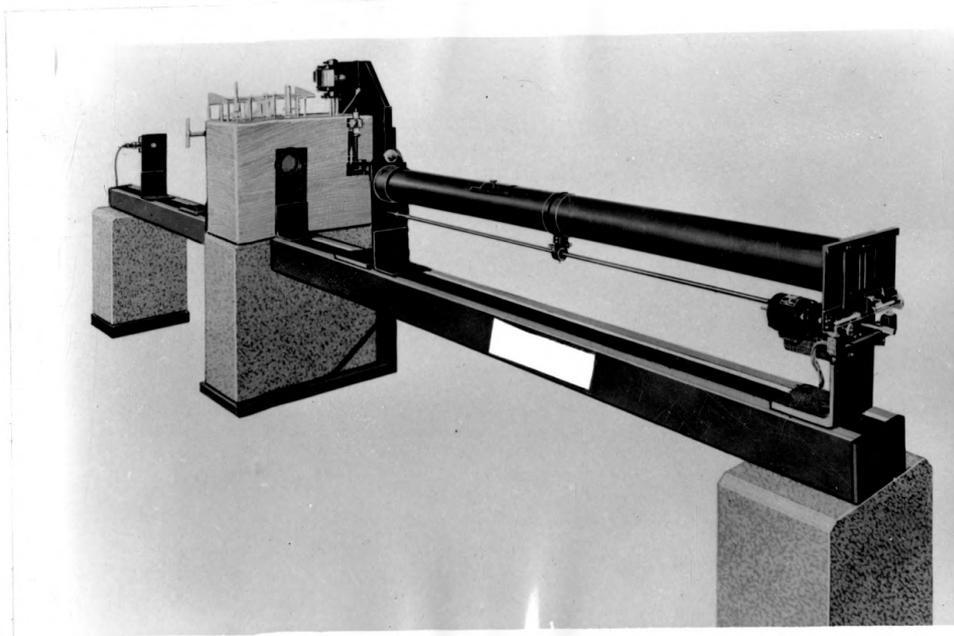


Fig. 2. The electrophoresis apparatus.

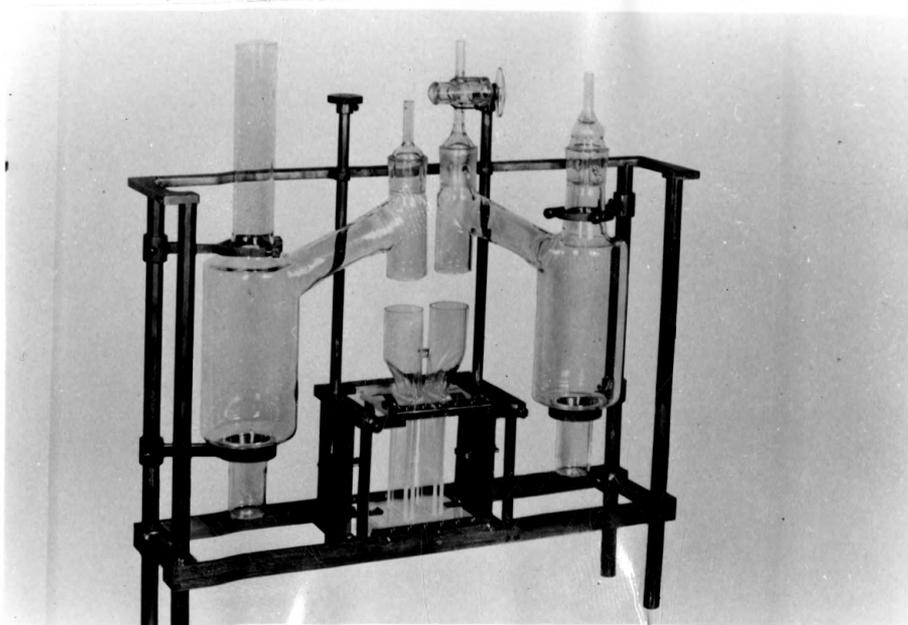


Fig. 3. The electrophoresis cell.

ments could be undertaken. This naturally necessitated the complete refocusing and aligning of the instrument. A very complete procedure for the focusing of the optical system was given by Longworth (10) and will only be briefly reviewed here. It must be emphasized, however, that this is one of the most important steps in making electrophoretic measurements. Improper focusing of the apparatus could be one of the greatest sources of errors and might result in unsymmetrical boundaries which lead to erroneous peak areas. Inaccurate mobility measurements could easily result from any deviation of the magnification factor of 1:1.

The water bath was filled with distilled water and the brass assembly rack, to which was clamped a ruled scale prepared in this laboratory, was placed into position approximately 4 inches from the schlieren lens. The iron beams making up the optical bench were carefully leveled and bolted securely to the cement piers. The mercury vapor lamp in the lighthouse was turned on and adjusted until the schlieren lens was fully illuminated.

Since the focal length of the schlieren lens was about 100 cm, the light source was placed along the optical bench at approximately this distance as was the camera on the other side of the water bath. With the cylindrical lens turned out of range in the camera barrel and with the diagonal slit removed from in front of the camera objective, the image of the ruled scale was observed on a ground glass plate placed in the photographic plate holder. The camera assemblage was moved along the optical

bench until the image on the ground glass was sharp and clear. A second ruled scale, identical with the one mounted in the water bath, was then placed in the photographic plate holder and the camera barrel adjusted in length by moving the back half of the camera until the ruled lines of the image of the scale in the water bath and the lines of the second scale in the plate holder exactly coincided. The camera was then clamped securely in place on the optical bench. Actual photographs were taken of the image of the ruled scale and compared with the original scale to make sure an exact 1:1 magnification was obtained with the apparatus.

The schlieren lens was focused following the directions as given by Longworth. The diagonal slit was placed in front of the camera objective and the cylindrical lens placed in position. When the schlieren lens was in exact focus, a vertical line was observed on the ground glass plate in the photographic plate holder. The light source was slid along the optical bench until the illuminated slit was vertical and then clamped in place. Improper positioning of the light source will result in the base lines of the electrophoretic patterns not being perpendicular with the line of symmetry of the peaks.

#### Procedure for Operating Direct Current Regulator

It was found during experimental work with whey proteins that the direct current regulator as set up following directions of McColloch failed to maintain the desired constant current. By using a modified operating procedure, however, it was pos-

sible to obtain the desired results. Simple changes in the wiring system were made by L. W. Brandt and will be presented in his forthcoming Ph.D. thesis. The revised method is given below. A schematic drawing of the direct current control panel is given by Fig. 4 and should be referred to in setting up the desired current.

The current regulator is plugged into a 110 v. A.C. transformer and given a few minutes to warm up. The potentiometer resistor box and slide wire dial,  $Kn_9$  and  $Kn_{10}$ , are set to read 1.0185 volts, which is the E.M.F. of the standard cell mounted in the control panel. Switch  $S_2$  is thrown into the "S.C." position and  $S_3$  in the "coarse" position. The galvanometer light is then snapped on by  $S_7$ . Balance the galvanometer on zero by adjusting the coarse and fine knobs,  $Kn_1$  and  $Kn_2$ .  $S_3$  is then thrown into the "fine" position and the balance refined using only the  $Km_2$  knob. Switches  $S_2$  and  $S_3$  are returned to the neutral position, the galvanometer light turned off with  $S_7$ , thus completing the balancing of the potentiometer against the standard cell voltage of 1.0185.

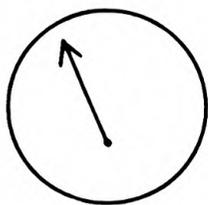
The power switch  $S_4$  is thrown to the "on" position as is the dummy load switch  $S_8$ . Snap  $S_1$  to the "ma" position. The milliammeter dial mounted in the instrument panel is adjusted to read 200 (20 ma) by manipulating  $Kn_3$  and  $Kn_4$ . This roughly sets up a current of 20 ma in the dummy load of 10,000 ohms. The potentiometer is set at 1.0000, using  $Kn_9$  and  $Kn_{10}$ , and  $Kn_8$  snapped to the 5 position. The galvanometer light  $S_7$  is turned on,  $S_2$  is thrown into the "E.M.F." position, and  $S_3$  into the "coarse" posi-

tion. The galvanometer is then balanced at zero using the current regulator controls,  $Kn_3$  and  $Kn_4$ . Switch  $S_3$  is changed to "fine" and final adjustments made with the fine adjustment,  $Kn_4$ .  $S_2$  and  $S_3$  are returned to neutral and  $S_7$  turned off. Some trouble may be encountered in the balancing of the galvanometer by  $Kn_3$  and  $Kn_4$ . The galvanometer tends to become stuck on one side or other of the scale. This may be remedied, however, by gently tapping the face of the galvanometer while adjusting  $Kn_3$  during the zeroing procedure. It is also advantageous to shut off the refrigeration unit and stirring motor for the water bath as the vibrations set up by these motors tend to jar the galvanometer from zero.

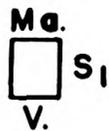
The power switch  $S_4$  and the dummy load switch  $S_8$  are turned off and the leads connected to the cell electrodes.

When it is desired to start the determination, switch  $S_4$  is turned on and the timing device started by  $S_6$ . It is now necessary to adjust the current to the new resistance of the electrophoresis cells. The milliammeter dial is set to read 200 by adjusting  $Kn_3$  and  $Kn_4$ , thus roughly setting the current. The galvanometer light is turned on and switches  $S_2$  and  $S_3$  placed in the E.M.F. and "coarse" positions. The galvanometer is quickly balanced by adjusting  $Kn_3$  and  $Kn_4$ ,  $S_3$  is snapped into the "fine" position, and final adjustments made with  $Kn_4$ . Thus a current of 20 ma is set up through the electrophoresis cells. This has been checked several times with a type K potentiometer, giving consistent readings of 20 ma.

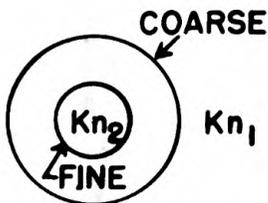
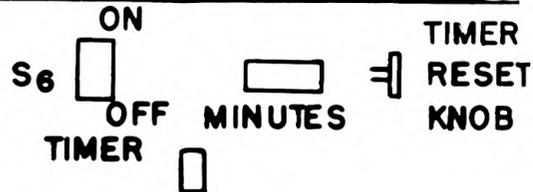
The above procedure establishes a current of 20 ma for elec-



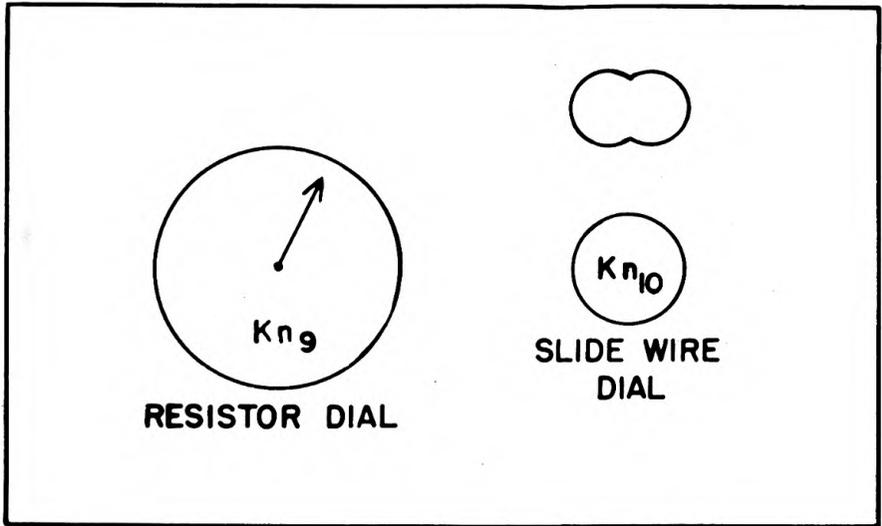
VOLT-MILLIAMETER



GALVANOMETER DIAL

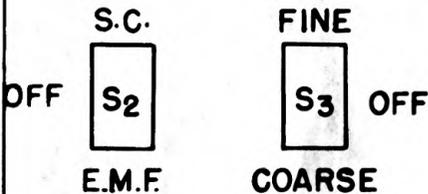


POTENTIOMETER BALANCE

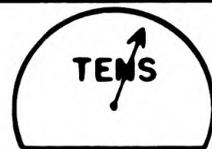


RESISTOR DIAL

SLIDE WIRE DIAL

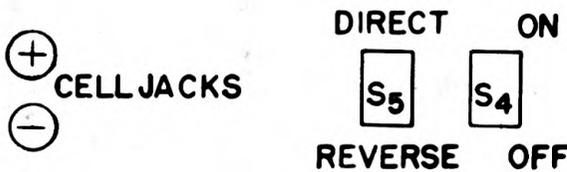


Kn7



Kn8

SHUNT RESISTORS



CURRENT COARSE



Kn3

CURRENT FINE



Kn4

MILLIAMETER SHUNT



Kn5

VOLTMETER RESISTOR



Kn6

(Mounted In Back)

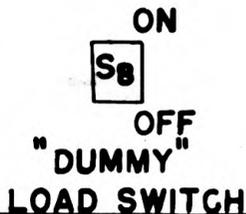


Fig. 4. A schematic drawing of electrophoresis control panel.

trophoresis. It is possible though, by using different settings of  $Kn_8$ ,  $Kn_9$ , and  $Kn_{10}$ , to obtain any current desired. As an example, assume it is desired to supply a current of 50 ma instead of one of 20 ma. In this case  $Kn_8$  would be set to read 2 instead of 5 in carrying out the procedure above. Or say a current of 40 ma is required. In this case  $Kn_8$  would be set at 2 with  $Kn_9$  and  $Kn_{10}$  reading .8000. These currents are calculated from a modification of ohms law:

$$I_{ma} = \frac{(E.M.F.) (100)}{(\text{Dial setting})} \quad (1)$$

where E.M.F. is the potentiometer setting as given by  $Kn_9$  and  $Kn_{10}$  and the dial setting is merely the number to which the resistance box,  $Kn_8$ , is set.

It is very important that the indicated dial reading should never be used as an accurate current reading in calculating mobilities. If it should ever be necessary to measure an unknown current being supplied by the unit, the potentiometer should always be used to accurately measure that current. To accomplish this,  $S_2$  is thrown to the "E.M.F." position,  $S_3$  to "coarse,"  $S_7$  to "on," and knobs  $Kn_9$  and  $Kn_{10}$  adjusted until the galvanometer balances on zero. Switch  $S_3$  is then thrown to "fine" and final adjustment made with  $Kn_{10}$ . Switches  $S_2$  and  $S_3$  are then returned to neutral and the galvanometer light turned off by  $S_7$ . The actual current through the cells is now given by equation 1. In no case should the milliammeter dial reading be taken as the correct current passing through the cells.

### Assembling of Cells

In preparation of an electrophoretic run, the refrigeration unit was turned on approximately 6 hours in advance of the experiment to allow the constant temperature bath to reach the required  $0.5^{\circ}$  C. This time can be greatly shortened by placing ice, frozen from distilled water, in the water bath.

The three sections of the electrophoresis cells were carefully cleaned in a lukewarm soapy water solution, utilizing pipe cleaners to assure complete removal of any vaseline-mineral oil lubricant that might be adhering to the inside of the cell channels. The cells were assembled in a cold room maintained at  $4^{\circ}$  C. so as to eliminate any chance of breakage of the cells due to sudden immersion into the water bath at  $0.5^{\circ}$  C. Cell joints were carefully coated with the vaseline-mineral oil mixture to insure a perfect seal and to allow the cells to slide easily into place. The center section was placed upon the bottom plate and all air carefully worked out from between the two sections. This is very important in preventing any air bubbles from forming at the joints between the cell channels. The bottom plate was filled with protein solution by means of a 5 ml hypodermic syringe fitted with a 10 inch needle, and then displaced to the left until the channels were cut off. The top section was then placed on the center section, again carefully working out all air from between the two plates. This was easily done if the consistency of the vaseline-mineral oil mixture was correct. Too

stiff a mixture also increases the chances of cell breakage due to excess pressure upon the cells. The assembled cell was placed in the brass assembly rack and loosely clamped into place. The right channel of the center cell was then filled with protein solution and all bubbles carefully removed by the long 10 inch needle fitted to the hypodermic syringe. Gentle agitation will generally remove these bubbles adhering to the cell walls. It was very important that all bubbles be removed because, if one became dislodged during a run, the bubble was sure to pierce and destroy the boundary. The left channel was rinsed three times with cold distilled water and then three more times with excess cold buffer solution to completely remove all traces of protein solution. It was then filled with buffer solution and all bubbles removed as before. The top section was pushed to the left until the channels were closed and the cell clamped solidly into position. Excess protein solution remaining above the right channel was removed and the right section rinsed carefully as before, then filled with buffer. The top section of the cell was connected with the cell reservoirs by means of the rubber sleeves and the complete cell assembly filled with buffer solution up to the ground glass joint receptacles. The silver electrodes which had previously been coated with silver chloride, as described by McColloch, were then placed in the reservoir wells. The ground glass caps were carefully coated with the vaseline-mineral oil lubricant and placed in position; the three way stopcock placed over the right channel; and the other two "nippled" caps placed in their proper receptacles, Fig. 3. By

means of the long needled hypodermic syringe, 30 ml of saturated sodium chloride solution were forced down through the electrodes so as to continually coat the anode electrode during electrophoresis. The cells were then "topped off" with buffer solutions so that buffer levels in both reservoirs were exactly even.

Fingerprints were wiped from the optically flat cell surfaces with a clean soft cloth saturated with acetone and the assembly rack placed in the water bath.

The compensator syringe mounted on the side of the water bath was filled with extra buffer solution and the rubber tube from this syringe was connected to the horizontal connection on the three way stopcock. The stopcock was turned so that the syringe was connected to the vertical outlet, and then buffer solution was forced through the tube by the driving wheel on the compensator. This expelled all air from the system. The stopcock was then turned until the vertical outlet was connected to the cell reservoirs and all air forced from the top of the stopcock. The stopcock was turned until the compensator was connected to the cell reservoirs.

By means of the rack and pinion gears, the center section was moved to the left into line with the top and bottom sections. This joined the U-tube and created the boundaries.

Buffer solution was then slowly forced into the right cell reservoir by slowly turning the driving wheel mounted on the compensator assembly. This caused the right boundary to be propelled downward and the left boundary to move upward into a posi-

tion about one-half an inch into the center section.

It is very important that the boundaries be moved very slowly as any rough or jerky motions tend to distort the boundary and, in some cases, possibly rupture them. As a general rule it should require approximately 5 minutes to move the boundaries one-half inch into the center section of the cell.

The cells must be carefully lined up in the water bath so as to get a full view of the boundaries on the photographic plate. This was done by placing the brass disk with the two adjustable parallel vertical slits into position just in front of the photographic plate and, with both sides open in front of the schlieren lens, the assembly rack was moved until the center cell was directly in line with the optical path. This could be seen by the full illumination of the vertical slits.

The leads were connected to the electrodes and the experiment started as described in the section under operation of the control panel.

It depends upon the charge on the protein molecule just which electrode is used as the anode and which is used as the cathode. In the preceding outline the boundary in the left channel must be the ascending boundary so as to provide the full length of the cell for protein splitting. Only by a trial run can the proper connection for the leads be determined for a given protein solution. In the case of whey proteins which acted as anions above pH 4.8, the anode was always connected to the left electrode to have proper direction of migration.

Pictures were taken at 30 minute intervals until splitting was complete, using the schlieren diaphragm method. However, progress of the splitting was followed by mounting a diagonal slit in front of the camera objective and placing the cylindrical lens in the camera barrel.

Some practice in the formation of boundaries was necessary before actual experimentation of whey proteins could begin. Boundaries were formed of sodium chloride against water and, although these boundaries could not be migrated, much knowledge in the manipulation of the instrument was attained thus making repeated successful runs of protein solutions possible.

#### Measurement of Peak Areas

According to the theories of electrophoresis, the area of a given component of an electrophoretic pattern is directly proportional to the actual concentration of that fraction. Thus, theoretically, it should be easy to measure the total area of a given pattern, measure the area of a particular fraction, and then the ratio of the two would represent the actual percentage of the component in the protein solution. However, the problem is not as simple as this. It is very seldom possible to have resolution such that all fractions are widely separated into single symmetrical peaks. Instead, most patterns are a rather complicated picture of symmetrical peaks and half split off fractions. It is thus necessary to make some sort of arbitrary separation of the fractions in order to measure them individually.

There are two general methods that can be used in the divi-

sion of a pattern. The procedure of Tiselius and Kabat (12) separates the fractions by drawing an ordinate from the lowest point between two adjacent peaks, while use of the method of Svedberg and Pedersen (13) involves division of the pattern into a series of symmetrical curves. Naturally, it can be seen that both of these theories have their disadvantages; however, results obtained using both methods were in remarkably good agreement. Longsworth (9), in working with human blood plasma, obtained results that differed as much as 33 percent, especially when measuring the larger components. Results obtained in this laboratory for whey proteins, however, were much closer than this, Table 1. There seems to be no advantage in using one method over the other. The Tiselius and Kabat method is by far the easier of the two. It is left entirely up to the discretion of the worker, when using the Svedberg and Pedersen procedure, of just where the symmetrical curves should be placed and how far they should extend.

To facilitate the measurement of the peak areas, the patterns were enlarged approximately four times by projecting them on a sheet of 10 x 12 bond paper. The enlarged images were traced off and component areas measured with a planimeter. Patterns obtained for the ascending boundaries were used for all calculations.

A micro-Kjeldahl method as described by Niederl and Niederl (14) was used to determine the nitrogen concentration of each of the samples. Protein concentrations were calculated using a conversion factor of 6.38.

Table 1. Areas of electrophoresis components from whey proteins.

Component	% of Total Area						
	Harland and Ashworth: salt - acid	Tiselius: & Kabat	Svedberg & Pedersen	Salt - Lyophilized	Tiselius: & Kabat	Svedberg & Pedersen	Smith: Deutsch Tiselius & Kabat
1	3.0		2.9	7.9		7.5	6 7.0
2	8.1		6.6	3.8		2.6	4
3	15.3		13.3	17.3		16.8	16 17.5
4	8.5		8.4	8.8		8.5	9 18.0
5	59.6		63.1	55.4		58.7	58 53.7
6	5.5		5.7	6.8		5.9	7 3.8

## RESULTS AND DISCUSSION

The electrophoretic patterns of whey proteins prepared by both methods contained six well-defined peaks in relatively the same position, Plates I and II. The patterns showed no gross differences in the nature of whey proteins prepared by either method. The original assumption assumed the salt-acid method of whey protein preparation was a much more drastic treatment of those proteins than the salt-lyophilized procedure. This could not be substantiated by electrophoresis of the two preparations. Therefore, from this evidence, it is assumed the Harland and Ashworth method does produce "relatively undenatured" whey proteins, at least, "electrophoretically undenatured."

There were, however, differences in the relative concentra-

tions of several components. The apparent complete reversal in the relative concentrations of the 1 and 2 components is interesting. E. L. Smith (2, 3) identified these as immune lactoglobulins present in all normal milk. Components 1 and 2 were identified as euglobulin and pseudoglobulin, respectively.

When the Harland and Ashworth salt-acid method (Table 1, column 2) was used, components 1 and 2 constituted, respectively, 3 and 8.1 percent of the total whey proteins. However, components 1 and 2, as prepared by the salt-lyophilized method (column 4), were respectively 7.9 and 3.8 percent of the whey proteins. These figures were obtained using the Tiselius and Kabat method of component division. Similar results were obtained using the Svedberg and Pedersen method, Table 1. The values for salt-lyophilized proteins (column 4) agreed closely with those of Smith (column 6) whose method of preparation also involved lyophilization of the noncasein filtrate. As reported by Smith, 10 percent of the whey proteins were immune lactoglobulins, 6 percent being euglobulin, and 4 percent pseudoglobulins.

It was desired to further compare results obtained in this study with other workers. Deutsch, working with pooled, aged, cow's milk, obtained electrophoretic patterns that appeared similar to the results obtained in this laboratory. However, no data were given in the paper of component areas, therefore a typical pattern published by Deutsch was placed in an enlarger and the image traced off. Component areas were measured by the method of Tiselius and Kabat. The results are shown in Table 1, column 7. It was impossible to distinguish more than one immune

fraction. Components 4 and 5 were also poorly resolved and therefore could be measured only roughly. However, component 4 of Deutsch was certainly larger than component 4 of Smith or of this study.

According to Rowland (15), and confirmed by Harland and Ashworth, 5.64 percent of the total nitrogen in milk is present in the form of nonprotein nitrogen. In the preparation of whey proteins using the salt-acid method, all of this was present in the nonprotein filtrate. However, by the salt-lyophilized method, only the casein was discarded, the noncasein filtrate being lyophilized in making Sample III. Thus any nondialyzable nitrogenous substances in the nonprotein filtrate would still be present in Sample III. It was first thought the increase in concentration of the euglobulins in the salt-lyophilized method could be due in part to these substances. However, Sample II, as prepared for electrophoresis, produced only minute peaks on the photographic plate. Repeated trials of Sample II, when special precautions were taken in the filtering of all samples, produced no boundaries at all, thus indicating that nothing was present in the sample that could be demonstrated by electrophoresis.

If the nonprotein nitrogenous substances in the nonprotein filtrate were all nondialyzable, instead of minute peaks as shown in Plate III, there would have been obtained, as calculated from Rowland's figure 5.64, for Sample II a peak approximately one and one-half times as large as the 5 component in Figure 2,  $\gamma$ -lactoglobulin.

The small observed peaks, Plate III, could have been due

either to a small amount of nondialyzable, nonprotein, nitrogenous material or to a small amount of whey proteins not filtered from the nonprotein filtrate or precipitated by the addition of the acid to the noncasein filtrate. For this reason a sample was prepared of nonprotein filtrate, and, instead of lyophilizing it to one-fifteenth of its original volume, it was concentrated to one-seventy-fifth of its original volume. A single symmetrical boundary, Plate IV, was obtained whose mobility checked exactly with that of  $\beta$ -lactoglobulin of Sample III. From this evidence, it is very possible the small peaks obtained for Sample III were due not to nondialyzable, nonprotein nitrogenous substances, but to the presence of  $\beta$ -lactoglobulin that had never been precipitated by the addition of the acid or to poor filtration.

It is possible then, from this evidence, that the apparent concentration reversal of the 1 and 2 components could have been due to some chemical action of the hydrochloric acid upon the immune lactoglobulins, resulting in a change of their electrophoretic behavior.

Smith's data were given for electrophoresis using a veronal buffer of ionic strength .1 and pH 8.6. Different pH values and buffers could account for some of the discrepancies between the peak concentrations as reported by Smith and values obtained by these experiments. Repeated trials, however, using a veronal-citrate buffer of ionic strength .088 and pH 8.6 failed to bear out this contention.

## SUMMARY

1. Electrophoretic patterns were obtained from whey proteins. These proteins were prepared by two different procedures, namely, salt-acid and salt-lyophilized.

2. Six well-defined components were split off by electrophoresis of whey protein by both methods.

3. There seems to be no qualitative difference in whey proteins prepared by those methods.

4. A reversal in the relative concentrations of the euglobulin and pseudoglobulin was found as prepared by the two procedures.

5. This reversal of concentration could have been due to action of hydrochloric acid upon the immune lactoglobulin as shown by electrophoresis of the nonprotein filtrate.

6. Relative percentages of component areas agreed rather closely with those obtained by Smith and by Deutsch.

7. Electrophoretic patterns of the nonprotein filtrate gave no apparent evidence of nonprotein nitrogenous compounds. They did, however, indicate traces of unprecipitated or unfiltered  $\beta$ -lactoglobulin.

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**APPENDIX**

### Description of Plates I to IV

All photographs of electrophoretic patterns presented in this thesis represent typical data obtained from extensive experimentation. In all cases, pictures represent whey proteins migrating anodically to the right from the "salt-boundary,"  $\delta$ . Exposures during a given experiment are numbered in chronological order. The protein fractions are numbered to facilitate the identification of the components for Table 1.

### EXPLANATION OF PLATE I

Electrophoresis of whey proteins using sodium chloride-hydrochloric acid method of fractionation. 0.87 percent protein.

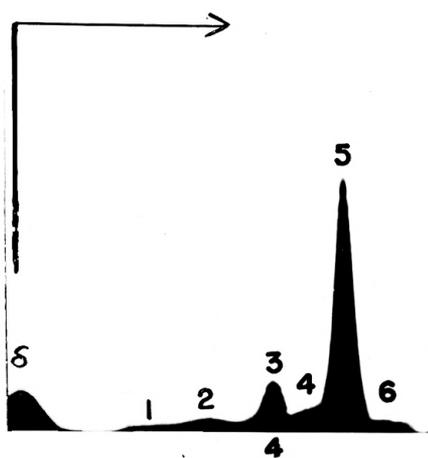
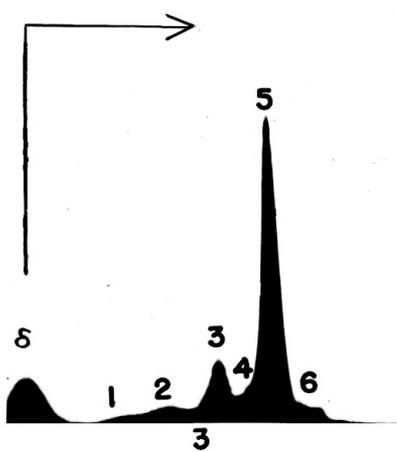
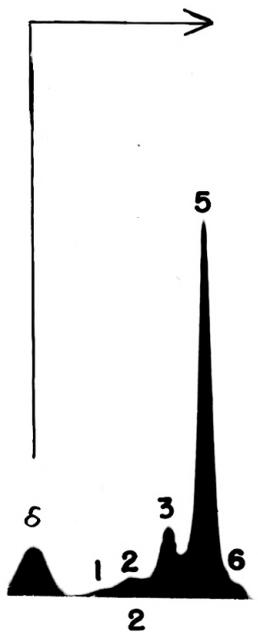
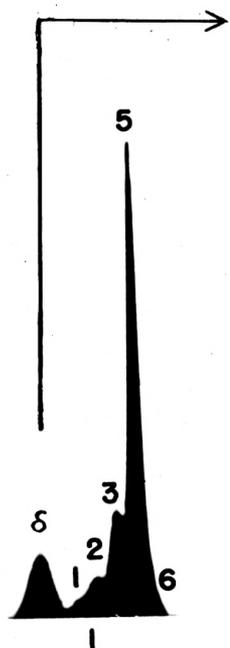
Exposure 1. Boundary at 38 minutes.

Exposure 2. Boundary at 68 minutes.

Exposure 3. Boundary at 98 minutes.

Exposure 4. Boundary at 128 minutes.

## PLATE I



## EXPLANATION OF PLATE II

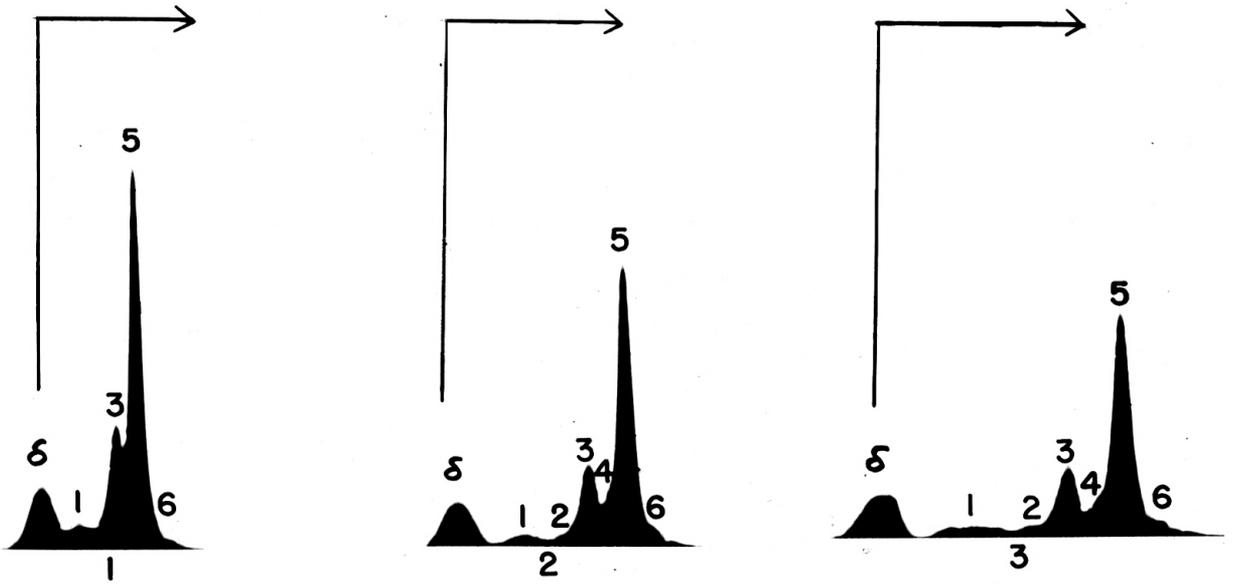
Electrophoresis of whey proteins using the sodium chloride-lyophilization method of fractionation. 0.815 percent protein.

Exposure 1. Boundary at 38 minutes.

Exposure 2. Boundary at 68 minutes.

Exposure 3. Boundary at 98 minutes.

PLATE II

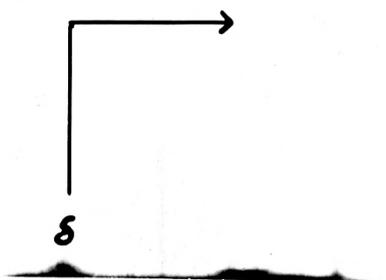


EXPLANATION OF PLATE III

Electrophoresis of lyophilized nonprotein filtrate concentrated fifteen times. 0.035 percent protein.

Exposure 1. Boundary at 90 minutes.

## PLATE III



#### EXPLANATION OF PLATE IV

Electrophoresis of lyophilized nonprotein filtrate concentrated seventy-five times. .415 percent protein. Components were identified by comparing their mobilities with the mobilities of the components of a known sample. No. 1, immune lactoglobulins; No. 2, identified as No. 3 fraction of normal whey proteins (see Plate I); No. 3,  $\beta$ -lactoglobulin.

Fraction No. 4 could not be identified. It was, however, a very fast moving fraction and had migrated out of view at the end of 120 minutes.

- Exposure 1. Boundary at 60 minutes.
- Exposure 2. Boundary at 90 minutes.
- Exposure 3. Boundary at 120 minutes.
- Exposure 4. Boundary at 150 minutes.

PLATE IV

