STUDIES ON THE FORMATION AND IONIZATION OF
THE COMPOUNDS OF CASEIN WITH ALKALI.

I. THE TRANSPORT NUMBERS OF ALKALI CASEINATE SOLUTIONS.*

BY DAVID M. GREENBERG AND CARL L. A. SCHMIDT.
(From the Department of Biochemistry and Pharmacology of the University of
California, Berkeley.)

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INTRODUCTION.

The ability of proteins to take up hydrogen ions from acid solutions and hydroxyl ions from alkaline solutions and the phenomena accompanying this, such as imbibition of water, have been explained from two very different viewpoints. Pure colloidal or adsorption theories (1, 2) have on the one hand received consideration and on the other hand what may be called chemical theories have been advanced to explain these phenomena. Although a great deal of experimental work which is favorable to the latter view has been brought forth, there is however no unanimity of opinion with respect to the groups within the protein molecule which enables it to combine with acids and with bases. It has been suggested by Loeb (3) and others (4-6) that this combination takes place through the agency of the free amino and the free carboxyl groups and the resulting compounds will therefore ionize to yield protein ions which will be positively or negatively charged depending on whether the protein is combined with an anion or a cation. A criticism of this theory lies in the fact that the proponents of this view have failed to show the presence within the protein molecule of a sufficient number of free amino or carboxyl groups in order to establish quantitative relationships.

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The theory proposed by Robertson disagrees with the above propositions and instead postulates that the $-\text{COHN}-$ groups of the protein molecule are the chief agencies which enable it to combine with both acids and bases and that complex protein ions are formed as shown by the following equations.

With acids,

$$\text{COH} = N^- + \text{HA} \rightarrow \text{COH} + N^-$$

(1)

With bases,

$$\text{COH} = N^- + \text{BOH} \rightarrow \text{COB} + N^-$$

(2)

**EXPERIMENTAL.**

From considerations of his theory Robertson concluded that the migration velocity of both the cation and anion of a protein salt should be equal; or in other words, the fraction of the current carried by each ion is one-half. Robertson (8) and later Haas (9) carried out experiments to test this hypothesis and concluded that their data confirmed the above hypothesis. The experimental method employed by Robertson and Haas consisted in electrolyzing casein solutions which had been placed in a two-compartment cell and then determining the amount of casein which was lost from each compartment. From theoretical considerations Robertson decided that if each ion carries one-half of the current the ratio of loss of casein from the anode compartment to that in the cathode must be as 2:1. Using solutions of potassium caseinate he, and Haas, did obtain this ratio. Robertson considered this as strong evidence in favor of this theory.

We decided that a direct determination of the transference numbers of protein solutions would be far preferable, and would give much stronger evidence for or against Robertson’s theory than the experi-
ments described by him. Robertson's method of experimentation
is not a standard method such as the well known methods for direct
transference determination; and moreover, since no attempt was
made to maintain a constant pH in the cathode compartment, and
since the ionic properties of protein solutions are a function of the
pH, comparable experimental data cannot be obtained when the pH
is allowed to vary.

Accordingly, an examination was made of the methods in use in
search of one which would suit our purpose. First, the moving
boundary method of Dennison and Steele (10) was tried, but being
unable to get a sharp boundary with protein solutions it was aban-
donned. We then tried the Hittorf method of direct analysis, and
were able to work out a procedure that gave satisfactory results.

Since the ionic properties of a protein are a function of the pH
it was necessary to employ a method which would maintain a con-
stant pH during the course of the experiment. This was accom-
plished by the use of non-polarizable electrodes. To be sure that
what we were measuring were the transport numbers of the casein
ions, it was very desirable to determine directly the transport num-
bersons of the alkali metal in the solution. This was accomplished by
working out a titration method of determining the amount of alkali
metal.

The cell which was used in the experiments is illustrated in Fig. 1.
It is a modified form of the type of cell used by Washburn (11) in his
very exact measurements of transference numbers. Essentially it
consists of three compartments of approximately 60 cc. each, with
stop-cocks which can be closed in order to keep the three portions
from mixing at the close of an experiment. This type of cell proved
very satisfactory. It is compact, the stop-cocks make it easy to
separate the three portions from each other, and the cross section is
large, a consideration which is very important since the protein solu-
tions have a rather high electrical resistance which otherwise makes
it difficult to pass sufficiently large currents through the solution.
For the cathode, an electrode of Pb covered with PbO₂ was used.
This keeps the pH constant in the cathode portion and no evolution
of hydrogen was observed. For the anode either a spiral of platinum
wire or a piece of platinum gauze was used. With an alkali caseinate
solution, on the passage of a direct current, casein is deposited on this platinum electrode giving a non-polarizable electrode that maintains the pH practically unchanged in the anode portion. This observation was first made by Robertson. The method, however, seems to be only applicable to proteins which are insoluble at their isoelectric points. We tried the method on gelatin solutions, adding small quantities of alkali from time to time to maintain an approximately constant pH but the results obtained were never concordant.

The casein used was made according to the procedure of Van Slyke and Baker (12) with some slight modification. After the casein was precipitated from the skimmed milk, it was put into tall glass cylinders and washed with distilled water by decantation several times a day for a period of 2 weeks or more. As a test for completeness of washing the wash waters were tested for chloride ion. After this the material was dried by first washing two or three times with 95 per cent alcohol, several times with absolute alcohol, and finally a number of times with absolute ether. In this way all but a trace of fat was also removed. Finally the ether was drained off and the casein was dried in an oven over $\text{H}_2\text{SO}_4$ at 40°C. A pure, snow-white, finely divided material was obtained. One lot was made by drying with acetone instead of ether, but the casein obtained was colored somewhat yellow and gave dark solutions which were more viscous than those in which the casein was obtained by drying with ether.
The object of using racemic casein and the method used for its preparation will be discussed in the third paper of this series. In all the transference and conductivity determinations on racemic casein, the sample with 13.5 per cent N was used. During the course of the work casein was prepared a considerable number of times, and while different samples gave some differences in the values which were obtained, especially of the electrochemical equivalent, still the results are as a whole in strikingly good agreement. An analysis of the ash of the casein gave 0.4 and 0.3 per cent, respectively, for one sample of the Van Slyke and Baker casein, and 0.6 and 0.5 per cent for the racemic casein.

The analysis for casein in these experiments was made by evaporating 10 cc. portions of the solution, drying at 100°C. in an electric oven, and weighing the residue. From this weight, the weight of alkali metal in the solution was subtracted. As a check on this method, in one experiment the casein was analyzed by determining the nitrogen; both methods gave the same results within the limits of experimental error. The method of determining the amount of alkali metal in solution consists simply in titrating with a strong acid to the isoelectric point of casein. We used 0.05 N trichloroacetic acid, which gives very good results. The casein acts as its own indicator. As the isoelectric point is approached the solution becomes very opaque, but there is no settling out of protein particles, probably

<table>
<thead>
<tr>
<th>Casein.</th>
<th>Base.</th>
<th>Time.</th>
<th>Q for electrode left standing.</th>
<th>Q for other electrode.</th>
<th>Loss per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.17</td>
<td>5.15 (CsOH)</td>
<td>1.5</td>
<td>1.74</td>
<td>1.72</td>
<td>0</td>
</tr>
<tr>
<td>1.93</td>
<td>5.00 (KOH)</td>
<td>2</td>
<td>2.09</td>
<td>2.06</td>
<td>0</td>
</tr>
<tr>
<td>1.96 (racemic casein)</td>
<td>7.47 (NaOH)</td>
<td>1</td>
<td>2.05</td>
<td>2.04</td>
<td>0.85</td>
</tr>
<tr>
<td>2.17 (racemic casein)</td>
<td>10.2 (KOH)</td>
<td>2</td>
<td>0.873</td>
<td>0.910</td>
<td>2.5</td>
</tr>
</tbody>
</table>

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due to their still being electrically charged. On the addition of a small additional amount of acid, the isoelectric point is reached. At this point, whirling or stirring the solution causes the casein to coalesce into large particles which rapidly sink to the bottom leaving the liquid above nearly transparent. The effect is very striking and is an indicator which makes it easy to determine when the end-point is reached. This method seems applicable to all proteins which are insoluble at their isoelectric point. Good results were obtained with gliadin solutions. The method can probably be used also for determining the amount of acid in an acid-casein solution by titrating with an alkali. It has already been stated that during the passage of the current casein is deposited on the anode. Previous to this investigation, Robertson was the only one who had quantitatively investigated the phenomenon. He interpreted it as follows:

"We have seen that the casein anion, which is free from base, must migrate to the anode. There it may be presumed to react with water, liberating oxygen and free casein, which combines with the excess of base until the proportion of base to casein in the film in immediate contact with the anode falls to that which obtains at 'saturation' of the base with casein. Any additional casein thus migrating into the film in contact with the anode must be precipitated as uncombined casein. . . . Hence the electrochemical equivalent which is actually measured in solutions of all reactions is that of casein at 'saturation' of the base with the protein."

We studied the electrode deposition of casein in connection with our transference determinations and have come to quite a different conclusion. Pauli (5) also criticized Robertson's conclusions and experimental methods, and from theoretical considerations arrived at a belief which is very much in accord with our own results. From our experimental work we concluded that the amount of casein deposited is strictly proportional to the amount of current which passed through the solution; i.e., Faraday's law holds for these solutions.

An examination of Table II shows that \( Q \), the electrochemical equivalent, is the same for solutions with equivalent amounts of alkali per gm. of casein, and is independent of the alkali used (Na, K, Rb, Cs), or the size and shape of the electrodes. However, instead of \( Q \) being constant and being the electrochemical equivalent at
saturation of base with casein, we found that $Q$ does vary with the amount of alkali in solution. We found that $Q$ obeys the relationship:

$$Q \times B = K$$

**TABLE II.**

*Transport Numbers (Average Values at 30°C.)*

(a) Sodium Caseinate Solutions.

<table>
<thead>
<tr>
<th>Time</th>
<th>Casein</th>
<th>$B$ approx.</th>
<th>pH</th>
<th>$Q$</th>
<th>$K$</th>
<th>Casein transferred per millifaraday</th>
<th>$T_{\text{casein}}$</th>
<th>$T_{Na}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td>per cent</td>
<td>cc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5-3</td>
<td>1.75-3.0</td>
<td>5.6 6.6</td>
<td>1.73</td>
<td>9.68</td>
<td>0.784</td>
<td>0.453 0.561</td>
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<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.5</td>
<td>7.0 7.0</td>
<td>1.34</td>
<td>9.40</td>
<td>0.610</td>
<td>0.455 0.540</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>1.7</td>
<td>8.25 7.6</td>
<td>1.04</td>
<td>8.60</td>
<td>0.450</td>
<td>0.430 0.510</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Potassium Caseinate Solutions.

<table>
<thead>
<tr>
<th>Time</th>
<th>Casein</th>
<th>$B$ approx.</th>
<th>pH</th>
<th>$Q$</th>
<th>$K$</th>
<th>Casein transferred per millifaraday</th>
<th>$T_{\text{casein}}$</th>
<th>$T_{Na}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2-3</td>
<td>5.6 6.5</td>
<td>1.82</td>
<td>9.09</td>
<td>0.650</td>
<td>0.359 0.660</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.25-4.0</td>
<td>2.5-3</td>
<td>6.25 6.9</td>
<td>1.47</td>
<td>9.17</td>
<td>0.534</td>
<td>0.363 0.636</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-2.5</td>
<td>2-2.5</td>
<td>8.0 7.6</td>
<td>1.12</td>
<td>9.00</td>
<td>0.390</td>
<td>0.349 0.657</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>2.0</td>
<td>10.0 9.4</td>
<td>0.92</td>
<td>9.20</td>
<td>0.350</td>
<td>0.382 0.654</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) Rubidium Caseinate Solutions.

<table>
<thead>
<tr>
<th>Time</th>
<th>Casein</th>
<th>$B$ approx.</th>
<th>pH</th>
<th>$Q$</th>
<th>$K$</th>
<th>Casein transferred per millifaraday</th>
<th>$T_{\text{casein}}$</th>
<th>$T_{Na}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>1.8</td>
<td>5.6 6.5</td>
<td>1.76</td>
<td>9.86</td>
<td>0.625</td>
<td>0.355 0.645</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(d) Cesium Caseinate Solutions.

<table>
<thead>
<tr>
<th>Time</th>
<th>Casein</th>
<th>$B$ approx.</th>
<th>pH</th>
<th>$Q$</th>
<th>$K$</th>
<th>Casein transferred per millifaraday</th>
<th>$T_{\text{casein}}$</th>
<th>$T_{Na}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-2.5</td>
<td>2.0</td>
<td>5.5 6.5</td>
<td>1.65</td>
<td>9.10</td>
<td>0.551</td>
<td>0.334 0.666</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(e) Sodium Racemic Caseinate Solutions.

<table>
<thead>
<tr>
<th>Time</th>
<th>Casein</th>
<th>$B$ approx.</th>
<th>pH</th>
<th>$Q$</th>
<th>$K$</th>
<th>Casein transferred per millifaraday</th>
<th>$T_{\text{casein}}$</th>
<th>$T_{Na}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-3.5</td>
<td>2.0</td>
<td>5.4 5.75</td>
<td>1.63</td>
<td>8.83</td>
<td>0.635</td>
<td>0.390 0.610</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(f) Potassium Racemic Caseinate Solutions.

<table>
<thead>
<tr>
<th>Time</th>
<th>Casein</th>
<th>$B$ approx.</th>
<th>pH</th>
<th>$Q$</th>
<th>$K$</th>
<th>Casein transferred per millifaraday</th>
<th>$T_{\text{casein}}$</th>
<th>$T_{Na}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.25-3.0</td>
<td>2-2.5</td>
<td>6.0 5.5</td>
<td>1.46</td>
<td>8.76</td>
<td>0.465</td>
<td>0.318 0.680</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5-2.5</td>
<td>1.6</td>
<td>8.65 6.4</td>
<td>0.985</td>
<td>8.50</td>
<td>0.355</td>
<td>0.360 0.610</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$B = \text{cc. 0.1 n. alkali per gm. casein.}$

$Q = \text{electrochemical equivalent per millifaraday.}$

*Lack of space prevents publication of the data in detail.*
in which \( Q \) is the electrochemical equivalent per millifaraday, \( B \) is the number of cc. of 0.1 \( N \) alkali per gm. of casein, and \( K \) is a constant. In other words, the electrochemical equivalent is inversely proportional to the amount of alkali which has combined with the casein. In the solutions with which we worked, the total amount of alkali can be considered as combined with the casein without making any appreciable error. An examination of Table II shows that while \( B \) has been varied between 4.8 cc. of 0.1 \( N \) alkali per gm., to over 10 cc. of 0.1 \( N \) alkali the value of \( K \) remains essentially the same.

It is true that the values of \( K \) which were obtained with solutions of higher alkali content are somewhat lower than the average values of \( K \), obtained when \( B \) is between 5 and 6 cc. of 0.1 \( N \) alkali per gm. This is to be accounted for by a dissolving off of part of the casein from the electrode during the course of the experiments. The higher the alkalinity, the more rapid this re-solution. Robertson noticed this re-solution and attempted to determine the amount lost by re-solution from the electrode in the solutions with which he worked. We also made some attempts to determine the loss of casein from the electrode. To maintain the conditions as nearly as possible the same as during the passage of the current, we electrolyzed samples of the same solution in each of two cells, and at the end of the electrolysis we let the electrodes stand in one cell, just as it was, for a period of several hours; while in the other cell we removed the electrode, and washed and dried it at once. In this way, from the difference in \( Q \) we could tell how much casein was lost from the electrode. The results are given in Table I. It is to be seen that in low concentrations of alkali, between 5 and 6 cc. of 0.1 \( N \) alkali per gm., the loss is negligible. In higher concentrations of alkali the loss does become significant.

In Fig. 2 are given curves for \( K \) plotted against \( B \) (cc. of 0.1 \( N \) alkali per gm.), for casein and racemic casein. The values of \( K \) as plotted are averages, and are uncorrected with respect to re-solution of casein from the electrode. It is seen that the experimental points fall on both sides of the theoretical curve which appears to be a straight line parallel to the abscissa. It will be noticed that when the alkalinity rises above pH 7 the points fall below the theoretical curve. This probably indicates a small amount of re-solution of the
casein. The magnitude, however, is well within the maximum experimental error.

We can obtain a very interesting check on the value of $K$ from an entirely different source. Cohn and Hendry (13) have published a very accurate study of the solubility relations of casein in NaOH. They found that at saturation with casein it takes 4.76 cc. of 0.1 N NaOH to dissolve 1 gm. of casein, which gives an equivalent weight of casein of 2,100. From the values of $K$ which we have determined, the equivalent weight of casein can also be calculated at the value of 4.76 cc. of 0.1 N alkali per gm. of casein; and doing so we obtain $1,000 \times Q = \left(\frac{9.6}{4.76}\right) 1,000 = 2,015$ gm. This is a good agreement considering the great difference in the methods which have been employed.

Having now discussed the meaning of the electrode deposit of casein, we can again take up the determination of transference numbers. The electrode deposit is an important quantity in these determinations, as it is a measure of the current which has passed
through the solution. Since our knowledge of the equivalent weight of casein is otherwise very uncertain, calculation of the transference

TABLE III.
Calculation of Transference Experiments.

Sample A.
2.85 per cent casein + 5.6 cc. 0.1 N NaOH per gm. Temperature 30°C. Time 2.5 hrs.
Current 2.55 cc. 0.1 N Ag.

Anode Portion = 76 Gm.
Electrode deposit.
3.6240
3.1695
0.4545 gm.

Q (electrochemical equivalent) = 0.4545/0.255 = 1.78 gm. per millifaraday.

pH of solutions.
Original = 6.45
Middle = 6.50
Anode = 6.50
Cathode = 6.45

Analysis (10 Gm. Samples Used).

<table>
<thead>
<tr>
<th></th>
<th>Anode</th>
<th>Middle</th>
<th>Original</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.2540</td>
<td>12.7550</td>
<td>15.0190</td>
<td>17.9913</td>
</tr>
<tr>
<td>13.0000</td>
<td>12.4998</td>
<td>14.7300</td>
<td>17.7035</td>
</tr>
<tr>
<td>0.2540</td>
<td>0.2552</td>
<td>0.2890</td>
<td>0.2878</td>
</tr>
</tbody>
</table>

Mean conc. = 2.546 per cent. Corrected for Na = 2.515 per cent.

Anode Gain of Casein.
(2.845 - 2.515) 0.76 = 0.250 gm.
0.4545 - 0.250 = 0.2045 gm.

Transfer of casein per millifaraday.*
0.2045/0.255 = 0.802 gm.

Anode Loss of Na.
(15.9 - 13.9) 0.76 = 1.52 cc. 0.1 N Na

T_{casein} = 0.802/1.78 = 0.45
T_{Na} = 1.52/2.55 = 0.596

Total ...................... (0.45 + 0.596) = 1.046

Alkal Analysis (Titration with Trichloroacetic Acid).

<table>
<thead>
<tr>
<th>Amount.</th>
<th>Sample</th>
<th>0.05 N acid.</th>
<th>0.1 N per 100 gm. sample.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Middle.</td>
<td>12.8</td>
<td>15.9</td>
</tr>
<tr>
<td>40</td>
<td>Anode.</td>
<td>11.1</td>
<td>13.9</td>
</tr>
</tbody>
</table>

* Since each cc. of 0.1 N Ag liberated in the coulometer represents 0.1 millifaraday then 2.55 cc. is equivalent to 0.255 millifaraday.
numbers of the casein ion, assuming some value for the equivalent weight, would give doubtful results. But knowing the change in concentration of casein in the anode portion, and the weight of casein deposited on the anode, the transference numbers of the casein ion can easily be calculated.

The methods of analysis used have already been described. It is only necessary to add further that the amount of current was determined with the aid of a silver titration coulometer, the dissolved silver being titrated with 0.025 N thiocyanate solution using ferric alum as an indicator. It is to be observed in Fig. 1 that both electrodes are at the bottom of the solution, although the solutions near both the anode and cathode become more dilute, which must give rise to some convection. The cathode is placed at the bottom since otherwise the PbO₂ would fall away from the Pb. For the anode it was actually found to be an advantage to have a small amount of convection, as this renews the solution in the neighborhood of the electrode and keeps the anode solution quite homogeneous. We tried putting the anode at the top and found that the casein in the neighborhood of the electrode was soon exhausted; a considerable evolution of gas commenced and the solution became acid.

In Table II the averages of the results are given. The transference numbers are calculated from the anode portions only. In the cathode, while the pH remained constant, the presence of Pb-PbO₂ led to some complicating reaction which affected the transference, so that the values calculated from the cathode portion were not concordant. To illustrate how the calculations of the transference numbers were made, a sample note-book page is given in Table III and the calculations are shown.

The transport numbers for the casein are obtained from the electrode deposit and the total gain of casein in the anode portion. The transport number of the cation is calculated from the determination of the quantity of electricity and the loss of cation in the anode portion. It is to be seen from the tables that the sum of the two is always close to unity. This favors the view that we are really measuring the transport numbers. In these solutions the alkalinity was always negligibly small and no correction was needed.
According to Robertson's theory, the transport number of both anion and cation, as we have stated before, should each be one-half. In the tabulated results it is seen that we did not obtain this figure. Instead, the values of the transport numbers vary according to the mobility of the cation which was combined with the casein. That this is so is evident from the following considerations. The transport number of an ion, is given by the equation

\[
\text{transport number} = \frac{\lambda_0 \text{anion}}{\lambda_0 \text{anion} + \lambda_0 \text{cation}},
\]

where \(\lambda_0\) is the mobility or equivalent conductivity at infinite dilution. We have the data for the transport numbers from our experiments, and data for the mobility of the cations used can be obtained from collected tables of physical constants such as the Landolt-Börnstein Tabellen (14). With the aid of these we can solve for the mobility of the anion, which in this case will be the casein ion. Now if the transport numbers vary according to the mobility of the cation used, the value calculated for the mobility of the casein ion should be the same in all cases. The results of these calculations are given in Table IV.

\[^{1}\text{Robertson (7), p. 180.}\]
The transference numbers of the casein solutions containing between 5 and 6 cc. of 0.1 N alkali per gm. casein are used for the calculations, as more determinations were carried out with these. The values of the mobilities at 18°C. for Na⁺, K⁺, Cs⁺, and Rb⁺, and their temperature coefficients were taken from the Landolt-Börnstein Tabellen,² and from them the values of the mobilities for 30°C. were calculated. It is to be seen there is a surprisingly good correspondence in the values which we obtained for the mobility of the casein ion, especially since all the errors are thrown on the values for the mobility of the casein ion. The value obtained for the mobility of the racemic casein is about 20 per cent less than that of ordinary casein. Why this should be we have for the present no explanation.

Using these results we can now explain why Robertson (8) and Haas (9) obtained the values which they found in their migration experiments. It is to be remembered that, using two-compartment cells and electrolyzing casein solutions, they found the ratio of loss from the anode compartment to that of the cathode compartment to be in the ratio of 2:1. They used only potassium caseinate solutions. We have found that the transport number of the caseinate ion in potassium caseinate solution is 0.363. As a first approximation we will call it ½. During the passage of the current a certain amount of casein will be deposited on the anode electrode, which will be a measure of the current. At the same time there will be a migration of casein from the cathode to the anode. Now if we call the casein deposited a, the amount moving from the cathode to the anode will be ½ a. The amount lost from the anode portion will be (a - ½ a = ½ a. The amount lost from the cathode portion will be ½ a and the ratio of loss will be as 2:1. It is only because potassium caseinate was used that Robertson and Haas obtained these results. Had they used sodium caseinate, it is safe to say that they would have found an entirely different ratio of loss. It might be asked why the 2:1 ratio was obtained, since the transference number of the caseinate ion is 0.363 and not ½. The answer probably is that since no attempts were made to maintain a constant pH in the cathode compart-

² Landolt-Börnstein (14), p. 1104.
ment, hydroxyl ions accumulated which took part in carrying the current and thus lowered the transference number of the caseinate ion.

Another very important point is the high mobility calculated for the casein ion. This will be discussed in a second paper, where it is considered in relation to conductivity determinations made by us and by others with alkali caseinate solutions.

**SUMMARY.**

1. The deposition of casein on a platinum anode which takes place on the passage of a direct current through solutions of alkali caseinates was quantitatively studied, and it was found that: (a) the amount of casein which is deposited is directly proportional to the current, i.e. it obeys Faraday's law; (b) the amount of casein deposited is inversely proportional (within the limits studied) to the amount of alkali which is combined with the casein.

2. A method of determining the transport numbers of proteins insoluble at their isoelectric point has been developed.

3. A titration method for determining the amount of alkali in a casein solution is given.

4. Data from the results of transference experiments on sodium caseinate, potassium caseinate, cesium caseinate, and rubidium caseinate solutions are given. It is shown that the data are best explained on the assumption that in these solutions the carriers of the current are alkali metal cations and casein anions.

5. On the basis of our transference results an explanation is given of the results which were obtained by Robertson and by Haas in their migration experiments.

**BIBLIOGRAPHY.**