

STUDIES IN THE ELECTROCHEMISTRY OF THE PROTEINS. I. THE DISSOCIATION OF POTASSIUM CASEINATE IN SOLUTIONS OF VARYING ALKALINITY

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I. Introduction

(a) The Object of the Investigation

The investigations of Hardy,¹ Sackur² myself³ and others have shown that the compounds of the proteins with inorganic acids and bases undergo quite extensive electrolytic dissociation when they are dissolved in water. The following investigation was undertaken with the prime object of ascertaining to what extent the protein salts, which are present in solutions of potassium hydroxide to which definite amounts of casein have been added, participate in the conduction of electricity through their solutions—in the hope that the information thus obtained would throw light upon the mode in which the proteins combine with bases and the mode in which these compounds dissociate.

In order to completely determine the share taken in the conduction of a current by the ions, derived from protein salts, which are present in a solution of a protein in alkali or in acid, two measurements are, as a rule, necessary. Thus, suppose we measure the depression of the conductivity of a KOH solution which is brought about by the addition to it of a definite amount of casein—let x_1 denote the conductivity (in reciprocal ohms per cubic centimeter) of the original KOH solution, and x its conductivity after the addition thereto of casein. Let b_1 denote the concentration of the

¹ W. B. Hardy: Jour. Phys., **33**, 251 (1905).

² Sackur: Zeit. phys. Chem., **41**, 672 (1902).

³ T. Brailsford Robertson: Jour. Phys. Chem., **11**, 437, 542 (1907); **12**, 473 and 542 (1908).

original KOH solution and b the amount of KOH which remains unneutralized after the introduction of the casein and c the equivalent-concentration of each of the ions derived from the protein salt. Let U denote the ionic velocity (in cm per sec. per volt-cm potential gradient) of the K^+ ion, V that of the hydroxyl ion and v_1, v_2, v_3, \dots those of the ions derived from protein salts, then from familiar electrochemical formulae we have:

$$\begin{aligned} x_1 &= 96.44(U + V)b_1 \\ x &= 96.44(U + V)b + 96.44(v_1 + v_2 + v_3 + \dots)c \\ \text{let } x_1 - x &\equiv \lambda, \text{ and } b_1 - b \equiv m. \end{aligned}$$

Then:—

$$\lambda = 96.44(U + V)m - 96.44(v_1 + v_2 + v_3 + \dots)c \dots (1)$$

In order, therefore, to determine the relative magnitudes of $(v_1 + v_2 + v_3 + \dots)c$ in various solutions, it is necessary to measure, not only λ , but also m , the amount of alkali bound by the protein. Of course in cases where, as in Sackur's experiments, alluded to above, and in some of my own previous experiments, the alkali is *completely* neutralized by the protein, no special separate measurement of m is necessary—but in many of the experiments herein described a considerable excess of alkali was present in the solutions, and hence a separate measurement of m was imperative.

In brief, therefore, the investigation herein described consisted in the measurement of the depression in the conductivity of KOH solutions due to the introduction of casein, and in the determination, by means of the gas-chain, of the proportion of the KOH in these solutions which was neutralized by the casein.

(b) The Apparatus Employed

In the gas-chain determinations two platinized platinum electrodes saturated with hydrogen were used—the one being dipped in the solution of unknown hydroxyl-concentration, the other in a solution of known hydroxyl-concentration; the latter being always the original KOH solution and the former the same solution with the addition thereto of a defi-

nite amount of casein. The electrodes which were employed were of a design due to Dr. F. G. Cottrell, which has previously been employed by me¹ and has been described in detail by Schmidt and Finger in this journal.² They were very kindly prepared for me by Dr. Cottrell himself, to whom I wish to take this opportunity of expressing my thanks.

The hydrogen was generated by the electrolysis of 6 percent (by volume) sulphuric acid, in an apparatus which has previously been described by Schmidt and Finger in the paper cited above. To guard against the possibility of any oxygen, ozone, or hydrogen peroxide being carried over with the hydrogen from the generator, the gas was passed through a heated glass tube which was loosely filled with platinized asbestos and which had, wrapped around it for a distance of about 20 cm, a coil of fine resistance wire, the internal diameter of the tube being about 0.5 cm. The hydrogen was completely cooled before it reached the electrodes, because, after leaving the heater, it was passed through a narrow glass tube about 70 cm long, leading to the water bath. The coil of the heater was heated by a portion of the same current (110 volt University Circuit) which generated the hydrogen, the current being led into the hydrogen-generator through 4 lamps in parallel (three 16 c. p. and one 32 c. p. 110 volt lamp), one of the 16 c. p. lamps being connected in series with the coil. In order to maintain the pressure of hydrogen which was necessary to drive it through the electrodes, the oxygen which came off from the generator was carried off by a tube which dipped into a column of water, the depth of the opening of the tube in the water being adjusted until the levels of the fluid in the inner and outer cylinders of the generator were approximately equal.

The complete chain was arranged as follows. The syphon-tube of the "half-element" containing the fluid into which the electrode dipped was immersed in a beaker filled with the same fluid—thus the "half-element" containing the

¹ T. Brailsford Robertson: *Jour. Phys. Chem.*, **11**, 437 (1907).

² C. L. A. Schmidt and C. P. Finger: *Jour. Phys. Chem.*, **12**, 406 (1908).

solution of unknown hydroxyl-concentration was in fluid connection with a beaker which contained the same solution, and the "half-element" containing the solution of known hydroxyl-concentration was in fluid connection with a beaker filled with that solution. The two beakers were then connected by a U-tube filled with agar saturated with KCl, thus effectively preventing any mixing of the two solutions and annihilating any contact-difference of potential between them.¹ The gas was passed through the electrode which dipped into the solution containing protein (the solution, that is, of unknown hydroxyl concentration) at the rate of from one to two large bubbles per second, and the excess of gas was permitted to pass through the other electrode. The whole chain was immersed in a small water bath placed inside an incubator which was maintained at a temperature between 31° and 32° (*vide infra*). It was thought necessary, at first, not to permit the hydrogen to escape into the incubator, lest it should be ignited, on opening the door of the incubator, by the flame beneath. Consequently the electrodes were inserted into the half-element through tightly-fitting rubber stoppers, and rubber tubes were attached to the side-tubes of the half-element and carried outside the incubator and the cupboard within which the incubator was set up. For reasons which will shortly be described, however, this procedure was, of necessity, abandoned, and the gas was permitted to escape into the incubator. As no explosion occurred during the progress of these experiments, this procedure was probably safe. The incubator was of the usual double-walled type employed by bacteriologists; its internal dimensions were 45 cm wide by 24 cm deep by 48 cm high. It was provided with two doors, the outer of the usual double-walled type, the inner a glass door through which thermometers, etc., could be read without disturbing the apparatus or causing fluctuations of temperature by currents of air. The inner chamber was provided at the top with two small air-exits.

The potentials between the electrodes of the chain were

¹ Bjerrum: Zeit. phys. Chem., **53**, 428 (1905).

measured on a 100 cm potentiometer bridge-wire, which had previously been standardized in the laboratory of physical chemistry. For the detection of the zero-point on the bridge-wire a D'Arsonval galvanometer provided with a damping-coil was employed, it gave a decided throw with the potential corresponding to 1 mm displacement on the bridge in all of these experiments. The constant fall in potential from end to end of the potentiometer wire was at first supplied by a storage-battery, but since none of the storage-batteries which were immediately available proved of sufficient capacity for the work required of them, this was replaced by an arrangement of four Gladstone-Lalande cells (model G-50), two in parallel and two in series, so as to obtain the voltage of two cells and the internal resistance of one. The potential derived from these was measured against a standard Weston cell, just before and just after every reading. The potential provided by the Gladstone-Lalande cells proved quite sufficiently constant, during the progress of an observation, provided they were always short-circuited across the bridge-wire for 15–20 minutes beforehand—this did not heat the bridge-wire in any degree which could be detected. The Weston cell was checked against another Weston cell which had been prepared in the department of physical chemistry, and the two were found to agree exactly.

The electrodes were platinized with Lummer and Kurlbaum's solution. They were very carefully washed, both within and without, between the observations, first in a stream of distilled water and then in the solution in which they were about to be immersed—every few days they were also washed in chromic and sulphuric acids solution and then, after thorough washing in a stream of distilled water, were allowed to soak for 12 hours in distilled water.

The conductivity-vessel which was employed was of the Kohlrausch-Holborn type, with a thermometer dipping into the fluid between the electrodes. This was immersed in the same water bath as the gas-chain, and the conductivities of the solutions were always measured at exactly 30°. The

capacity of the vessel was 0.1305 (measured by determining the resistance of an accurately N/50 KCl-solution). The electrodes were platinized with Lummer and Kurlbaum's solution. The same bridge-wire was employed for determining the conductivities as for determining the potentials of the gas-chain. A telephone was employed to detect the zero-point and the alternating current was supplied by an inductorium of the Ostwald type. The Rheostat was a "five dial bridge," manufactured by Nalder Bros. and certified to read correctly within 0.01 percent at 17° C. Its temperature-coefficient was only 0.025 percent per degree, so that I made no correction for the temperature of the rheostat. The resistance in the rheostat was always adjusted until the zero point was exactly in the middle of the bridge; the resistance in the rheostat was then, of course, exactly equal to that of the conductivity-vessel filled with the fluid under investigation. The arrangement of the wiring is represented in the following diagram.

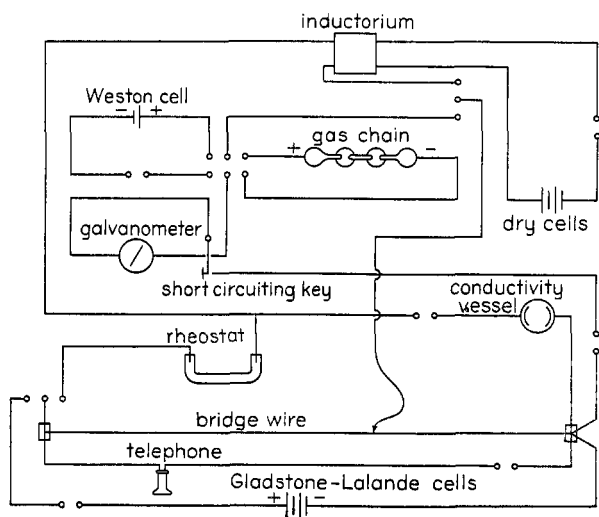


Fig. 1

The wires of the conductivity circuit were all "bell wire," so that their resistance could be neglected. The wires of the

potentiometer-circuit were somewhat thinner. All of the wires were insulated and carefully supported on glass, and were never allowed to touch the table. Where it was necessary to carry wires through the table, for example, or through the wall of the cupboard in which the incubator was placed, they were run through glass tubes. The wire connected with the slider on the bridge was encased in rubber tubing.

(c) The Preparation of the Casein

The casein employed was Eimer and Amend's C. P. casein "nach Hammarsten," specially purified in the following manner. Half a pound of the casein was triturated with about 12 liters of distilled water, the water being added in six successive portions. On each addition of water the casein was well stirred up in it in a porcelain mortar and then allowed to settle, then the supernatant water was poured off and fresh water was added. It was then washed in a similar manner in 5 kilos of Kahlbaum's C. P. alcohol, 99.8 percent, and then in 5 kilos of Kahlbaum's C. P. ether, "ueber Natrium destilliert." The mortar, containing the casein drained as free from superfluous ether as possible,¹ was then placed in an incubator over sulphuric acid at 40–50° C, the flame was turned out under the incubator and it was allowed to cool for about 24 hours. The casein is now found, if these operations have been conducted carefully, to be in the form of a dry, pure white powder, still containing, however, a considerable quantity of ether. The casein was now spread out, within the incubator, in a layer not over 1 cm deep, the flame under the incubator was lighted, fresh sulphuric acid was introduced if necessary, and it was allowed to stand for 24 hours at 40–50° C. The casein is then found to be free from appreciable water or ether.

I have previously shown² that the casein thus prepared

¹ At this point it is necessary to avoid exposing the mortar to the moist air of the room a minute longer than is necessary, otherwise the evaporating ether causes the condensation of sufficient moisture to spoil the product unless it is again treated with alcohol and ether.

² T. Brailsford Robertson: Jour. Biol. Chem., 2, 317 (1907).

gives every indication of being a pure product—it is insoluble in distilled water, save in traces which adhere to the casein particles, and it neutralizes to phenolphthalein and to litmus exactly the quantities of alkali determined by Söldner,¹ Lacquer and Sackur² and van Slyke and Hart.³

Casein which has been carefully prepared in the manner outlined above floats upon the top of and is not readily wetted by water or watery solutions of bases; if, however, it contains a mere trace of moisture, it is readily wetted by all save the most alkaline solutions. In order to successfully and completely dissolve perfectly anhydrous casein it is necessary to first add to it a very little of the solution in which it is to be dissolved, rub it up into a paste, and then add, while stirring, the remainder of the solution. This procedure was adopted in all of the experiments described below.

(d) The Experimental Procedure

I have pointed out in previous publications⁴ that it is difficult to obtain solutions of caseinates of much higher acidity than neutrality to litmus by merely shaking up casein in solutions of bases—not because casein will not form such solutions, but because, although it dissolves at first with considerable rapidity, after the excess of alkali is neutralized further casein dissolves with extreme slowness. Now it was found at an early stage in this investigation that the conductivities of the solutions containing casein must be measured as soon as possible after complete solution of the casein, for, otherwise, the hydrolysis which casein undergoes, the more rapidly the more alkaline its solution⁵ introduces a serious error into the determination. Hence it was imperative, not only that the measurement of the conductivities of these

¹ Söldner: *Landw. Versuchs.*, **35**, 351 (1881).

² Lacquer and Sackur: *Beitr. Chem. Physiol. u. Path.*, **3**, 196 (1906).

³ van Slyke and Hart: *Am. Chem. Jour.*, **33**, 461 (1905).

⁴ T. Brailsford Robertson: *Jour. Biol. Chem.*, **2**, 317 (1907); *Jour. Phys. Chem.*, **13**, 469; **14**, (1910).

⁵ T. Brailsford Robertson: *Jour. Biol. Chem.*, **2**, 317 (1907). "The Proteins," *Univ. of Calif. Publ. Physiol.*, **3**, 174 (1909) and foot-note.

solutions should be determined as soon as possible after complete solution of the casein, but, also, that the preparation of the solution, after the introduction of the casein, should consume as little time as possible. Hence all of the solutions containing casein which were acid to phenolphthalein were prepared by dissolving the casein in excess of alkali and then neutralizing this excess with hydrochloric acid. This procedure was found to have the further advantage that the absolute conductivities of the solutions thus obtained being higher than they would have been had they contained no KCl, the detection of the zero point on the bridge-wire by means of the galvanometer was rendered much easier and a less sensitive galvanometer could be employed than would otherwise have been required. In fact a greater excess of KCl was commonly present in the solution than was absolutely necessary. As a rule, save in the case of the most alkaline solutions, in a series of experiments in which only one concentration of casein was employed, the total concentration of potassium in each solution was kept constant and varying proportions of it were neutralized by HCl.

In this connection it is to be carefully noted that if the solution employed to dissolve the casein be too alkaline little or nothing is gained by the rapidity of its solution, because rapidity of its hydrolysis is also great. On the other hand, as I have said, if too small a proportion of free KOH is present solution is so slow that hydrolysis is extensive. Evidently an avoidance of both of these extremes will yield the most satisfactory results. I have found the proportion 10 cc of $N/10$ KOH to 1 gram of casein to be about the most satisfactory solvent for the casein. Save in the formation of solutions of higher alkalinity than this, therefore, part of the KOH was neutralized until the portion unneutralized stood in this proportion to the mass of casein undergoing solution, the casein was dissolved therein, and then the desired final proportion of KOH to casein was attained by the further addition of HCl. For example, it was desired to obtain a solution of 1 percent casein in 0.005 N KOH. Accordingly to 75 cc

0.1 N KOH were added 50 cc 0.1 N HCl and in this were dissolved 2.5 grams of casein; upon the attainment of complete solution, and while stirring, 12.5 cc of 0.1 N HCl were added and the whole solution was made up to 250 cc with distilled water; another solution was made up in precisely the same way but without the introduction of the casein; the conductivities of both solutions were then determined and their difference ($\equiv \lambda$) estimated.¹ The two solutions were arranged in the gas-chain in the manner described in (b) and the potential between the gas-electrodes immersed in them determined. Hence, of course, the OH concentration of the solution not containing casein being known, that of the solution containing casein was determined. The effect of the presence of KCl upon the dissociation of the KOH was, of course, negligible, since the KOH was always very dilute and even the KCl was always sufficiently dilute to be practically completely dissociated.

The extent of the error which is introduced into the determination of η by dissolving the casein in the first instance in a solution of too high alkalinity may be gauged from the following results:

Final solution 3 percent casein in 0.015 N KOH.

Amount of unneutralized KOH employed to dissolve 7.5 grams casein	$\lambda \times 10^8$
100 cc	305.0
75 cc	296.9

I have mentioned that the desired concentration of the KOH unneutralized by HCl in the solutions containing casein was attained by the addition, to the solutions of the casein in excess of KOH, of HCl *while stirring*. This is a matter of some importance. If acid be poured into a solution of a caseinate which is imperfectly mixed, the casein which is

¹ Since the concentration of KCl was the same in both solutions it would add the same amount to both conductivities and this would disappear in their difference; that is, assuming that the casein does not combine with or decompose the KCl and that the presence of excess of K^+ ions does not depress the dissociation of the potassium caseinate—as will be seen in the sequel no effects which could be attributed to any of these factors could be detected in these experiments.

precipitated in the acid portions of the fluid forms bulky coagula and is only with difficulty redissolved, even if the quantity of alkali still unneutralized by the acid is more than sufficient to hold in solution all of the casein that may be present. Consequently the solution of the caseinate must be rapidly stirred while the acid is being added. The same procedure, of course, considerably enhances the rapidity with which the casein dissolves in the alkali employed for its solution. I invariably placed the mixed fluid and casein in a beaker of squat form and 400 cc capacity—the mixture was then agitated by a flattened glass rod bent at right angles, so that the horizontal arm was about $2\frac{1}{2}$ cm long and as near as possible to the bottom of the beaker; this was rotated at the rate of about 1600 revolutions per minute by a small motor. As soon as the casein was completely dissolved the acid was delivered into the solution, a few drops at a time, from a pipette, the opening of which was held *at some depth below the surface of the liquid*. In some of the earlier experiments the acid was poured upon the surface of the solution, but all of these solutions foam to a certain extent and the foam is not agitated by the stirrer with the same rapidity as the fluid which lies below it, consequently, if the acid is poured upon the surface, casein is precipitated within the foam and is only with great difficulty redissolved, hence solutions prepared in this manner yielded very irregular results both in the gas-chain and conductivity determinations. All of the results reported in this paper were obtained with solutions prepared by delivering the acid into the agitated solution from a pipette dipped below the surface of the fluid. In this operation it is very necessary to avoid holding the opening of the pipette too close to the side of the beaker, as in that case a film of casein is precipitated on the glass and this film redissolves with great difficulty.

Since the conductivities of solutions of the caseinates must be determined as soon as possible after the introduction of casein into the solution employed to dissolve it, for otherwise hydrolysis introduces a considerable error, it is evident

that as little time as possible must be consumed in bringing the temperature of the solution to that at which its conductivity is to be determined. This could be achieved in either of two ways: Either a small volume of fluid could be employed, so disposed as to take up the temperature of the water bath very quickly. Or the water bath may be maintained at a somewhat higher temperature than that actually desired, and the conductivity of the fluid can be measured at the moment when it reaches the desired temperature. For reasons which will be sufficiently obvious the latter procedure was found to be the more convenient. The water bath was kept at a temperature lying between 31.5° and 32.5° , a preliminary measurement of the conductivity of the solution was made at a temperature between 29° and 29.5° and this preliminary determination was corrected at precisely 30° . In the first series of experiments upon 1 percent casein solutions this precaution was not taken and, consequently, the conductivity determinations were wholly irregular and unreliable. Of this series (cf. Table III), therefore, only the gas-chain determinations are reported.

Solution of 1 percent casein in 0.03 N KOH.

$\lambda \times 10^5$ determined immediately.....	348.8
$\lambda \times 10^5$ determined after allowing solution to stand at about 30° for 20 minutes.....	356.3

It is obvious, however, that in this procedure the gas-chain measurements are made at a temperature some 2° higher than the conductivity measurements, and it may be inquired to what extent this invalidates the comparison of the two sets of determinations. The error which is thus introduced, for the small difference of temperature concerned is, however, negligible, since it was found by actual trial that the difference between the potentials measured at 30° and those measured at 34° could barely, with certainty, be detected upon my potentiometer bridge. Nor is this fact surprising for, exclusive of any possible change in the equilibrium between protein and alkali, the potential, according to the Nernst formula, varies directly as the absolute tem-

perature and therefore only increases by 1/300th per degree centigrade at 30°. It has previously been observed by W. A. Osborne¹ and myself² that there is no evidence of an appreciable shift in the equilibrium between casein and alkali, as the temperature rises, until the temperature of 36° is reached. This temperature was never approached in these experiments.

I have mentioned, in describing the apparatus employed, that difficulty was encountered in leading off the hydrogen, after it had bubbled through the fluids, through the exit-tubes of the half-element to the outside of the incubator. The difficulty was this—all of the solutions containing casein foam upon passing the hydrogen through them, and to a greater extent the less the excess of alkali in the solutions. This foam, collecting in the exit and connected rubber tubes, gave rise to a pressure which drove the fluid out of the half-element and thus interrupted the continuity of the chain. Accordingly it was found necessary, not only to abandon the idea of conveying the waste hydrogen out of the incubator, but also to cut off short the exit-tube of the half-element and permit the foam to escape freely into the water bath (the lower end of the exit-tube was, of course, well above the surface of the water in the bath). After this plan had been adopted no further trouble was encountered from this source.

All observers who have endeavored to measure the conductivities of solutions containing proteins have encountered the difficulty involved in the precipitation which occurs at the electrodes, particularly, in the case of casein, in neutral or very faintly acid solutions. Whetham and Hardy,³ in order to minimize the error arising from this source, adopted the plan of heating the electrode, after platinization, to a dull red, thus clumping the platinum black and reducing the total surface of the electrode. I have hitherto tacitly

¹ W. A. Osborne: *Jour. Phys.*, **27**, 398 (1901).

² T. Brailsford Robertson: *Jour. Biol. Chem.*, **5**, 147 (1908).

³ W. B. Hardy: *Loc. cit.*

assumed this phenomenon to be connected in some way with the passage of the alternating current through the solution. In these experiments, however, I speedily found that in neutral or faintly acid solutions of potassium caseinate marked precipitation of casein occurs at the gas-electrode, without the passage of any current, usually after but sometimes even before the passage of the hydrogen. At first I was inclined to attribute this to gases collected, in the interval between experiments, in the tubes conveying the hydrogen, to impurities in the hydrogen, etc.—but after very careful exclusion of all of these possibilities the precipitation still took place, and it occurred to me that it might be due to the hydrogen ions dissolved in the platinum itself. When hydrogen is passed through a platinized platinum electrode, the potential measured across the chain represents, at first, a higher acidity of this electrode than it does later on, when the electrode has come into equilibrium with the solution; in other words, the hydrogen ions dissolved in the platinum have not yet come into equilibrium with those in the solution, an excess of hydrogen ions is still present in the platinum. It therefore appeared possible that this initial acidity of the platinum itself might be responsible for the precipitation of casein at its surface, and this idea found confirmation in the fact that on prolonged passage of hydrogen the film of protein deposited on the electrode slowly redissolved. It occurred to me that it might be possible to avoid this precipitation altogether by bringing the electrode nearly to equilibrium with a low concentration of hydrogen ions before introducing it into the solution at all; accordingly, before making a gas-chain determination with a caseinate solution of very low hydroxyl concentration (neutral or acid to litmus) the electrode which was to be dipped into the protein solution was immersed in distilled water and gas was passed through it for an hour or more—it was then immediately washed in the protein solution and used. The device was found to work excellently, and in all of the experiments described herein precipitation at the electrodes was avoided entirely, at least

so far as the eye could perceive. A similar device was found effective in avoiding precipitation at the electrodes of the conductivity-vessel. The vessel was simply filled with distilled water and allowed to stand in the water bath for some hours before making a determination.

In order to ensure a correct determination of the potential of the chain in these experiments it was always found necessary to pass the gas for three hours, taking a reading every hour, generally for four hours and, in the neutral or faintly acid solutions, for as much as six hours. If two successive readings on the bridge, taken an hour apart, did not differ by more than 1 mm the result was considered correct. Difficulty in obtaining constant readings was, of course, only encountered in the chains yielding the highest potentials, in which, as the apparatus was arranged, 1 mm on the bridge-wire made a difference of only about $\frac{1}{2}$ percent to the calculated value of the potential. In the chains of low potential greater accuracy was desired, but no difficulty was encountered in attaining it, since in these, successive readings were nearly always identical, or if they were not, the difference could invariably be traced to some obvious source of error which was eliminated in a repetition of the experiment.

Since, however, prolonged exposure of the protein solution in the gas-chain to the temperature of the water bath was essential, the question arises whether the accuracy of the determinations may not have been invalidated by hydrolysis of the casein. The answer to this question is in the negative, the change in the hydroxyl concentration of a protein solution, due to hydrolysis, is negligible in comparison with the change in its conductivity. Moreover it has been shown¹ that the displacement of the neutral point on the potentiometer-wire, due to hydrolysis of the casein in an alkaline solution of a caseinate is opposite in sense to the displacement due to the coming to equilibrium of the electrode with the solution in which it is dipped. Were appreciable change in

¹ T. Brailsford Robertson and C. L. A. Schmidt: *Jour. Biol. Chem.*, 5, 31 (1908).

hydroxyl concentration due to hydrolysis occurring, therefore, the position of the neutral point on the bridge should indicate at first a diminishing and later increasing potential. No trace of this was discovered save in one or two of the most alkaline solutions (1 percent casein in 0.03 N KOH; 3 percent casein in 0.05 N KOH), in these the displacement due to hydrolysis was never more than 1 mm and the minimum value of the potential was taken as the true one. It is, however, to be recollected that the hydrolysis was probably most rapid in the period of time preceding the attainment of this minimum, so that even the minimum potential may be considerably in error, especially as in the chains containing these solutions the potential was low and 1 mm displacement on the bridge introduced a considerable error into the determination of the potential. This error is diminished in its percentage magnitude in the calculation therefrom of m , the amount of alkali neutralized by the protein, but, nevertheless, the determination of m , in the solutions mentioned, is not to be considered trustworthy. In the remainder of the solutions no effect upon the hydroxyl concentration of the caseinate solutions, due to hydrolysis, could be discovered; had such an effect been present to any appreciable extent it would, of course, have been detected in these solutions much more readily than in those in which such an effect was detected, since the potentials of the chains containing these solutions were higher, so that a smaller change in hydroxyl concentration of the solution would have produced a greater (absolute) displacement of the neutral point upon the bridge-wire.

In concluding these prefatory remarks, it may be stated that unless all of the precautions which I have described are observed with the utmost fidelity the results of experiments such as these, and conducted in this manner, will be found to be wholly irregular and, save in a qualitative sense, untrustworthy.

II. The Experimental Results

In the following tables, in which the results of these experiments are shown, the symbols employed have the following significance:

$b_1 \equiv$ The concentration of the KOH solution in which casein was dissolved.

$\pi \equiv$ The potential of the chain in volts = $0.0601 \log_{10} \frac{b_1}{b}$

$b \equiv$ The hydroxyl concentration of the solution containing casein.

$m \equiv b_1 - b \equiv$ the concentration of alkali neutralized by the casein.

$x_1 \equiv$ The conductivity, in reciprocal ohms, of the solution containing no casein.

$x \equiv$ The conductivity, in reciprocal ohms, of the solution containing casein.

$\lambda \equiv x_1 - x \equiv$ the alteration of the conductivity of the alkaline solution which is brought about by the addition of casein.

$r \equiv$ The total concentration of potassium in the solution.

$z = 96.44 (U + V) m - \lambda =$ the conductivity of the KOH *neutralized* by the casein minus the depression in the conductivity of the alkaline solution due to the presence of the casein = the conductivity of the caseinate itself.

$A \equiv \frac{z}{m} =$ the "apparent" equivalent-molecular conductivity of the caseinate.

Referring to equation 1 (vide introduction) it is evident that z is the portion of the conductivity of its solution which is due to the transport of electricity by the ions of the caseinate itself. The ratio z/m , which we have denoted by A , therefore, is the "apparent" equivalent-molecular conductivity of the caseinate, that is, the equivalent-molecular conductivity which it would possess were, in every solution, the sum of the valencies of the positive ions of the caseinate (*mutatis mutandis*, the negative ions) equal to the number of KOH molecules bound up in one molecule of the caseinate, or, in other words, were the true equivalent-molecular concentration of the caseinate equal to that of the KOH bound up in

it. As this supposition is not necessarily correct for the more and is, as we shall see, very improbably correct for the less alkaline solutions, the values of this ratio cannot be regarded as affording the *true* values of the equivalent-molecular conductivities of the caseinates.

The equivalent-molecular conductivities, at 18° of the KOH neutralized by the casein in the various solutions were computed by interpolation from the results cited in Kohlrausch and Holborn's "Leitvermögen der Elektrolyte" (1898), p. 160. These values were then multiplied by 1.235 in order to obtain the molecular conductivities at 30° (cf. Kohlrausch and Holborn, p. 199). In order to avoid ambiguity the entire table of molecular conductivities which was employed is reproduced here (Table I). The values of the *conductivity* of the KOH neutralized by the casein in any given solution was obtained from this table by multiplying the molecular conductivity lying opposite the concentration nearest to that neutralized by the casein by the concentration of KOH which was actually neutralized by the casein.

It must be recollected that in all except the most alkaline solutions the casein was first dissolved in alkali of such concentration that 10 cc 0.1 N KOH = 1 gram casein, and the desired, final ratio of potassium hydrate to casein was procured by the addition of acid. In the above definitions of my notation such phrases as "concentration of the KOH solution in which the casein was dissolved," "conductivity in reciprocal ohms of the solution containing no casein," etc., refer, not to the solution actually employed to dissolve the casein, but to the solution prepared in exactly the same manner as the *final* solution of potassium caseinate, but with the omission of the casein.

The conductivity of the distilled water is not subtracted from the conductivities of the solutions, because it simply adds the same small amount to each of the quantities x_1 and x and this disappears in their difference ($\equiv \lambda$). It was not, however, above 8×10^{-6} reciprocal ohms.

The value of b and therefore of m was estimated from the

Nernst formula, $\pi = \frac{RT}{F\eta} \log_{\text{nat}} \frac{C_1}{C_2}$ where R and T are, respectively, the gas-constant and the absolute temperature, η is the valency of the ion (in this instance I) and F the Faraday constant. This yields the formula $\pi = 0.0601 \log_{10} \frac{b_1}{b}$ at 30° , being the potential of the chain in volts, b_1 the known concentration of alkali in which the casein was (finally) dissolved, and b the hydroxyl concentration of the solution of the caseinate.

TABLE I
Molecular conductivities of KOH solutions at 30° . After Kohlrausch and Holborn

Concentration in G-mols per litre	Molecular con- ductivity in re- ciprocal ohms per cc per gramme-mol. per litre $\times 10^3$	Concentration in G-mols per litre	Molecular con- ductivity in re- ciprocal ohms per cc per gramme-mol. per litre $\times 10^3$
0.0010	289	0.0250	276
0.0015	288.5	0.0260	276
0.0020	288	0.0270	275
0.0025	287	0.0280	275
0.0030	286.5	0.0290	274
0.0040	285	0.0300	274
0.0050	284	0.0310	274
0.0060	284	0.0320	273.5
0.0070	283	0.0330	273
0.0080	283	0.0340	273
0.0090	282	0.0350	273
0.0100	282	0.0360	273
0.0110	282	0.0370	272.5
0.0120	281	0.0380	272
0.0130	281	0.0390	272
0.0140	280	0.0400	272
0.0150	280	0.0410	272
0.0160	280	0.0420	271.5
0.0170	279	0.0430	271
0.0180	279	0.0440	271
0.0190	278	0.0450	271
0.0200	278	0.0460	271
0.0210	278	0.0470	271
0.0220	277	0.0480	271
0.0230	277	0.0490	271
0.0240	276	0.0500	270.5

Concentration of the casein 0.5 percent

b_1	π	b	m	$x_1 \times 10^5$	$x \times 10^5$	$\lambda \times 10^5$	r	$z \times 10^5$	$A \times 10^3$	Remarks
0.02000	0.0160	1.683×10^{-2}	0.00917	—	—	—	0.020	—	—	Hydrolysis too rapid to permit the determination of x .
0.01000	0.0462	1.701×10^{-3}	0.00830	410.4	260.5	149.9	0.020	85.0	102.4	
0.00750	0.0708	4.987×10^{-4}	0.00700	379.4	255.9	123.5	0.020	74.6	106.6	
0.00500	0.1181	5.429×10^{-5}	0.00495	350.8	262.6	88.2	0.020	52.4	105.9	
0.00250	0.2600	1.181×10^{-7}	0.00250	320.6	280.0	40.6	0.020	31.2	124.8	
0.00150	0.2710	4.648×10^{-8}	0.00150	310.7	287.4	23.3	0.020	20.0	133.3	

TABLE III

Concentration of the casein 1.0 percent (first series)

b_1	π	b	m	r	Remarks
0.03028	0.0239	1.213×10^{-2}	0.01815	0.03028	
0.02907	0.0273	1.021×10^{-2}	0.01886	0.03028	
0.02422	0.0320	7.111×10^{-3}	0.01711	0.02422	
0.01817	0.0538	2.314×10^{-3}	0.01586	0.01817	
0.01212	0.0937	3.351×10^{-4}	0.01178	0.01817	
0.01091	0.1042	2.015×10^{-4}	0.01071	0.01817	
0.00970	0.1332	5.907×10^{-5}	0.00964	0.01817	
0.00849	0.1736	1.096×10^{-5}	0.00848	0.01817	
0.00728	0.2276	1.191×10^{-6}	0.00728	0.01817	
0.00607	0.2542	3.583×10^{-7}	0.00607	0.01817	
0.00486	0.2758	1.251×10^{-7}	0.00486	0.01817	
0.00486	0.2753	1.276×10^{-7}	0.00486	0.01817	Av. value of $m = 0.2756$
0.00365	0.3034	3.265×10^{-8}	0.00365	0.01817	
0.00244	0.3130	1.514×10^{-8}	0.00244	0.01817	

TABLE IV
Concentration of the casein 1.0 percent (second series)

b_1	π	b	m	$x_1 \times 10^5$	$x \times 10^5$	$\lambda \times 10^5$	r	$z \times 10^5$	$\Lambda \times 10^3$
0.03000	0.0231	1.237×10^{-2}	0.01763	795.7	446.9	348.8	0.030	143.1	81.2
0.02500	0.0313	7.523×10^{-3}	0.01748	729.1	386.1	343.0	0.030	144.7	82.2
0.02000	0.0471	3.292×10^{-3}	0.01671	669.2	347.1	322.1	0.030	144.1	86.2
0.01750	0.0603	1.736×10^{-3}	0.01576	641.3	341.6	299.7	0.030	141.6	89.9
0.01500	0.0741	8.782×10^{-4}	0.01412	609.8	341.6	268.2	0.030	127.2	90.1
0.01250	0.0954	3.233×10^{-4}	0.01218	580.0	349.9	230.1	0.030	112.2	92.1
0.01000	0.1299	6.892×10^{-5}	0.00993	548.3	361.5	186.8	0.030	93.2	93.9
0.00750	0.2265	1.276×10^{-6}	0.00750	519.9	378.3	141.6	0.030	70.7	94.3
0.00500	0.2596	2.398×10^{-7}	0.00500	488.8	396.7	92.1	0.030	49.9	99.8
0.00250	0.2903	3.699×10^{-8}	0.00250	459.5	415.6	43.9	0.030	27.9	111.6

TABLE V
Concentration of the casein 1.5 percent

b_1	π	b	m	$x_1 \times 10^5$	$x \times 10^5$	$\lambda \times 10^5$	r	$z \times 10^5$	$\Lambda \times 10^3$
0.03000	0.0439	5.578×10^{-3}	0.02442	790.9	314.5	476.4	0.030	197.6	80.9
0.02000	0.0881	6.842×10^{-4}	0.01932	665.8	299.3	366.5	0.030	170.6	88.3
0.01500	0.1280	1.113×10^{-4}	0.01489	604.1	320.6	283.5	0.030	133.4	89.6
0.01000	0.2523	6.337×10^{-7}	0.01000	546.0	357.5	188.5	0.030	93.5	93.5
0.00750	0.2897	1.136×10^{-7}	0.00750	519.9	374.9	145.0	0.030	67.3	89.7
0.00500	0.3128	3.119×10^{-8}	0.00500	488.8	393.4	95.4	0.030	46.6	93.2

TABLE VI
Concentration of the cascin 2.0 percent

b_1	π	b	m	$x_1 \times 10^5$	$\lambda \times 10^5$	r	$z \times 10^5$	$\Lambda \times 10^3$
0.05000	0.0311	1.522×10^{-2}	0.03478	1254.8	593.2	0.050	287.9	82.8
0.03000	0.0778	1.525×10^{-3}	0.02847	781.4	242.1	0.030	243.6	85.6
0.02000	0.1343	1.167×10^{-4}	0.01988	790.9	410.4	0.040	172.2	81.6
0.01500	0.2425	1.383×10^{-6}	0.01500	733.1	446.9	0.040	133.8	89.2
0.01000	0.3000	1.020×10^{-7}	0.01000	676.2	486.9	0.040	92.7	92.7
0.00500	0.3152	2.846×10^{-8}	0.00500	615.6	519.9	0.040	46.3	92.6

TABLE VII
Concentration of the cascin 3.0 percent

b_1	π	b	m	$x_1 \times 10^5$	$\lambda \times 10^5$	r	$z \times 10^5$	$\Lambda \times 10^3$	Remarks
0.05000	0.0733	3.011×10^{-3}	0.04699	1254.8	873.2	0.050	401.3	85.33	Average value of m 0.04703
0.05000	0.0739	2.943×10^{-3}	0.04706	—	—	0.050	—	—	
0.03000	0.1438	1.217×10^{-4}	0.02988	912.6	579.7	0.040	239.0	67.30	
0.02500	0.2343	3.156×10^{-6}	0.02500	858.6	488.9	0.040	201.1	80.4	
0.02000	0.2823	4.017×10^{-7}	0.02000	795.7	391.7	0.040	164.3	82.2	
0.01500	0.3172	7.920×10^{-8}	0.01500	741.5	296.9	0.040	123.1	82.1	
0.01000	0.3413	2.096×10^{-8}	0.01000	686.8	266.6	0.040	75.4	75.4	

It will be observed that the "apparent" equivalent-molecular conductivity of the caseinate increases somewhat rapidly (cf. the increase in the equivalent-molecular conductivity of KOH as its concentration diminishes, in Table I) as the combining-capacity of the casein decreases.

Regarding the probable accuracy, unavoidable hydrolysis, etc., apart, of the above determination, the following may be said: In the determination of the potentials of the chains, an error of 1 mm in the determination of the position of the neutral point upon the bridge would result, under the experimental conditions employed, in an error of 0.0015 in the value of π . This error, as a simple calculation serves to show, would lead to an error of 6 percent in the determination of the ratio $\frac{b_1}{b}$. Since the value of b_1 was much more accurately defined than this, we may assume that an error of 1 mm in the determination of the neutral point on the bridge-wire would lead to an error of 6 percent in the estimated value of b . The actual error, due to error in the determination of the position of the neutral point upon the bridge-wire, was certainly not more than half this in the more alkaline solutions, but was possibly two or three times this in the least alkaline solutions. Since b was always less than m , however, and usually very much less, the error in m , arising from this source, may for all practical purposes, be neglected save in one or two of the most alkaline solutions, in which it might possibly amount to 3 percent. The resistance of the conductivity-vessel filled with solution was always determined to within 0.1 of an ohm. The specific resistance-capacity of the vessel employed was 0.1305. A simple calculation shows that an error of 0.1 ohm in the determination of the resistance of the conductivity-vessel filled with fluid would lead to an error of $\frac{x^2}{1.305 + x}$ in the determination of x ; since x is always negligible in comparison with 1.305, we may write this $\frac{x^2}{1.305}$, the corresponding error in the determination of

x_1 being, of course, $\frac{x_1^2}{1.305}$. The possible error in the determination of λ will, of course, be the sum of the errors in x_1 and x , that is, $\frac{x^2 + x_1^2}{1.305}$. Thus, in the first experiment cited in Table IV ($b_1 = 0.030$, percent of casein = 1.0) the possible error in the determination of x_1 is 4.87×10^{-5} and in the determination of x is 1.54×10^{-5} , hence the possible error in the determination of λ is 6.4×10^{-5} , or 1.8 percent of its value. In the last experiment cited in Table IV ($b_1 = 0.00250$, percent of casein = 1) the possible error in the determination of x_1 is 1.63×10^{-5} , and in the determination of x it is 1.33×10^{-5} , hence the possible error in the determination of λ is 3.0×10^{-5} , or 6.8 percent of its value. The actual error, due to instrumental sources, was probably not more than half this. Tables of the possible errors in the values of λ , calculated in the above manner, will be found in the theoretical part of the paper.

Theoretical

(i) The Dissociation of Potassium Caseinate Is Not Appreciably Affected by the Presence, in Its Solution, of KCl

As I have already mentioned, in passing, the presence of KCl might be expected to alter the depression of the conductivity of a solution of KOH, which is brought about by the addition of casein, in either of the three following ways:

(a) Through decomposition of the KCl by the casein with the liberation of HCl and the binding of potassium. As might be anticipated, under the conditions of the investigation, no evidence of the occurrence of such a reaction was obtained.

(b) Through direct combination of the casein with the entire KCl molecule. From the results of Bugarszky and Liebermann¹ and of Hardy² it would appear evident that such combination, at such low concentrations of KCl as those employed in these experiments, could only occur to an im-

¹ Bugarszky and Liebermann: *Arch. ges. Physiol.*, **72**, 51 (1898).

² W. B. Hardy: *Loc. cit.*

measurably small extent, and, in fact, as we shall very shortly see, no evidence of the occurrence of such combination was obtained.

(c) Through depression of the dissociation of the potassium caseinate owing to the presence of excess of potassium ions. That the value of λ might be affected in this manner by the presence of the KCl appeared very likely, and was fully anticipated when this investigation was undertaken. That it nevertheless does not occur in any appreciable degree is clearly revealed by the following facts:

Two and a half grammes of casein were dissolved in solutions containing varying amounts of KOH of which, in each instance, so much was neutralized by 0.1 N HCl as to leave the equivalent of 25 cc of 0.1 N KOH unneutralized by the acid. These solutions were then each diluted to 250 cc, so that the final solutions consisted of 1 percent casein dissolved in 0.01 N KOH plus varying amounts of KCl. The conductivities of these solutions (at 30°) were then determined, and also the conductivities of solutions similarly made up without the introduction of casein, and from these the values of λ were computed. The capacity of the resistance-vessel employed was 0.1251, so that the possible error in the determination of λ , due to instrumental sources alone, was:

$$\pm \frac{x_1^2 + x^2}{1.251}$$

In the following table are given the results of these experiments. In the first column are given the concentrations of KCl in the solutions, in the second the conductivity, in reciprocal ohms, of the solution containing no casein ($\equiv x_1$), in the third the conductivity, in reciprocal ohms, of the solution containing casein ($\equiv x$), in the fourth the value of λ ($\equiv x_1 - x$), in the fifth the deviation of λ from its average value ($\equiv \Delta$), in the sixth the possible (instrumental) error in the determination of λ ($\equiv \varepsilon$).

It is evident that the values of — thus obtained were appreciably constant, the deviation (Δ) from the average

TABLE VIII

Concentration of KCl	κ_1	κ	λ	Δ	ϵ
0.00 N	277.4×10^{-5}	81.2×10^{-5}	196.2×10^{-5}	0.5×10^{-5}	0.7×10^{-5}
0.01 N	417.0×10^{-5}	222.6×10^{-5}	194.4×10^{-5}	1.3×10^{-5}	1.8×10^{-5}
0.02 N	548.7×10^{-5}	360.5×10^{-5}	188.2×10^{-5}	7.5×10^{-5}	3.4×10^{-5}
0.03 N	698.4×10^{-5}	494.5×10^{-5}	203.9×10^{-5}	8.2×10^{-5}	5.9×10^{-5}
Av.			195.7×10^{-5}		

being less or only very slightly greater than the purely metrical error of the determination. I refrain from describing in detail a number of similar experiments which yielded similar results. It will be recollected (Eqn. 1, Introduction) that λ represents the molecular conductivity of the neutralized base plus the share taken by the ions of the caseinate in transporting electricity through its solution. Since in these solutions the KOH was nearly completely neutralized, and the excessively minute concentration of free KOH which was present must have been, in all of the solutions, completely ionized, it is evident that the influence, if any, of increasing the *total* concentration of potassium in the solutions from 0.01 N to 0.04 N upon the dissociation of the potassium caseinate could not have been greater than 5 percent. Now even assuming that an increase in the potassium-concentration of the solutions from 0.01 N to 0.04 N only multiplied the concentration of potassium *ions* four times (which is, of course, probably a gross understatement of the case) then, calling A the concentration of protein-ions in the solution containing 0.01 N potassium, K the dissociation-constant of the potassium caseinate, and X the concentration of the undissociated caseinate, and assuming that the caseinate dissociates into one potassium and one casein ion, we have, for the solution containing 0.01 N potassium:

$$0.01 \times A = KX$$

and for the solution containing 0.04 N potassium:

$$0.04(A - \frac{A}{20}) = K(X + \frac{A}{20})$$

whence $A = 56X$, or the caseinate must, in the first solution, have been $\frac{56}{57}$ ths dissociated. If we suppose that the caseinate splits off two or more potassium ions, then the first solution must have been still more highly dissociated. If we assume that only one potassium ion is split off but two or more casein ions, then the degree of dissociation of the first solution would be about $\frac{52}{53}$ rds for two casein ions, $\frac{48}{49}$ ths for three ions and so forth. Hence, if we assume that the potassium caseinate splits off potassium ions we are forced to the conclusion that it is, at the concentrations employed in this investigation, very completely dissociated. Since, as we shall see, the very definite relation which subsists between λ and the alkalinity of the solution to which the casein was added *is independent of the concentration of KCl in the solutions*, this conclusion applies to all of the solutions dealt with in this investigation. Nor, at first sight, does this conclusion appear to be inconsistent with the physical magnitude of the conductivities observed in these solutions. The velocity of the potassium ion at 18° and 0.01 N concentration is about 61×10^{-5} cm-sec under 1 volt per cm potential gradient. Assuming that the velocity of the casein ion is about 15×10^{-5} in the same units,¹ the equivalent-molecular conductivity of the potassium caseinate, if it were completely dissociated, would be $\frac{(61 + 15) \times 10^{-5}}{1.037 \times 10^{-2}} = 73.1 \times 10^{-3}$, which, allowing for the difference in the temperatures at which the two estimates are made, approximates very closely to that actually observed (cf. Table IV).

A closer examination of our data, however, reveals a number of facts which are inconsistent with the view that

¹ Since the velocities of very large ions tend to approach this limit (Bredig: Zeit. phys. Chem., **13**, 191 (1894); cf. also W. B. Hardy: Loc. cit., on the velocity of the serum-globulin ion.

potassium caseinate dissociates, in the solution under consideration, practically completely into potassium and casein ions. The value which we have calculated of the theoretical equivalent-molecular conductivity of potassium caseinate upon the supposition that it splits into potassium and casein ions (about 90×10^{-3} at 30° ¹) is the *maximum* value which it could attain at any concentration or combining-capacity of the casein; for in obtaining it we assumed that the caseinate was *completely* dissociated, and the velocity of the casein ion at 18° cannot well be appreciably greater than 15×10^{-5} cm-sec. That is, upon this supposition, it is very improbable that the equivalent-molecular conductivity of a potassium caseinate solution at 30° could ever exceed 90×10^{-5} . Now, as we have seen, the "apparent" equivalent-molecular conductivities of the potassium caseinate solutions, which are given in the tables in the experimental part of the paper, in many cases considerably exceed 90×10^{-3} , reaching, indeed, in solutions in which the combining-capacity of the casein is low, as high a magnitude as 120×10^{-3} , while, as we shall see, extrapolating from the experimental results, it is very probable that could solutions in which casein possesses a still lower combining capacity have been conveniently worked with much higher "apparent" equivalent-molecular conductivities than these would have been observed. This being the case, then potassium caseinate in 1 percent solution in 0.01 N KOH cannot be nearly so highly dissociated as we have concluded it must be ($\frac{56}{57}$ ths) on the supposition that it dissociates into potassium and protein ions, for, in that case, its equivalent-molecular conductivity on approaching "saturation" of the alkali, could only increase by 1/57th and, as we have seen, it increases much more than this.

The only hypothesis which would appear to satisfactorily

¹ I may mention in passing that although I have not made any endeavor to measure it accurately, I have observed that the temperature-coefficient of the conductivity of caseinate solutions is of the normal order of magnitude, that is, the conductivity increases about 2 percent per degree rise in temperature.

reconcile these facts and at the same time harmonize with other features of the behavior of casein and of the proteins in general is that *in its solution in water potassium caseinate does not yield potassium ions, but dissociates into complex ions in which the casein is bound up in a non-dissociable form*—upon which supposition, of course, the presence of KCl might be expected to exert little or no influence upon the dissociation of the caseinate. In this connection it is of interest to note that Bugarszky and Liebermann,¹ by direct measurement with concentration-cells, showed that the depression in the Cl^- ion concentration of an HCl solution, due to the addition of proteins, is almost exactly equal to the depression of the H^+ ion concentration, which would appear to indicate that in the HCl-salts of proteins the Cl^- ion is bound up in a complex ion (since the salts which are formed are presumably, judging by the analogy afforded by casein, quite extensively dissociated). I have pointed out, also, in a previous communication,² that although solutions of "neutral" ammonium caseinate obey Ostwald's dilution-formula for a binary electrolyte, yet the sum of the velocities of the two ions into which this salt presumably dissociates, computed by interpolation from the formula, is less than that of the ammonium ion itself, and I have suggested that this phenomenon is due to the formation of complex ions in which the ammonium is bound up in a non-dissociable form.

It is to be noted, however, that should the above assumption turn out to be correct, then *the sum of the valencies of the positive ions which the caseinate yields upon dissociation cannot be equal to or less, but must be greater than the number of KOH molecules bound up in one molecule of caseinate*—for otherwise the apparent equivalent-molecular conductivities given in the tables in the experimental part of the paper would be equal to or less than the true equivalent-molecular conductivities. But the true equivalent-molecular conductivity at 18° , upon the supposition that the caseinate splits

¹ Loc cit.

² T. Brailsford Robertson: Jour. Phys. Chem., **11**, 542 (1907).

up only into protein ions, cannot well be greater than $\frac{(15 + 15) \times 10^{-5}}{1.037 \times 10^{-2}} = \text{about } 30 \times 10^{-3}$, and at 30° it cannot be much in excess of 40×10^{-3} . In other words, what the ions of the caseinate lose, upon this supposition, in migration-velocity they must gain or more than gain in the number of atomic charges which they transport, for otherwise the observed conductivities could not be attained.

(ii) The Combining Capacity of Casein

A number of investigators have shown,¹ by direct titration to neutrality to litmus, that the combining capacity of casein, at absolute neutrality, is constant, that is, is independent of the total concentration of the system. The potentiometric determinations, cited in the above tables, enable us to confirm these observations. It will be recollected that the OH^- concentration in the solutions containing casein was determined by measuring the potential between two hydrogen electrodes, the one dipped in the solution containing a given concentration of casein, the other in an exactly similar solution to which no casein had been added. Plotting a curve in which the reaction of the solutions containing no casein form the abscissae and the potentials between the two solutions the ordinates, the reaction ($= x$) of the solution to which the given concentration of casein had to be added in order to procure an exactly neutral solution is given by the intersection of this curve with the curve defined by the formula: $y = 0.4107 - 0.0601 \log_{10} x^2$.

The points of intersection of these curves were found in the following way: the values of y in the above curve corresponding to x (alkalinity of solution in which casein was dissolved) = 0.0025, 0.005, 0.0075, 0.010, 0.015 and 0.020 were computed and these points were marked upon 10×10 standard

¹ Soldner: *Landw. Versuchs.*, **35**, 351 (1888). Lacqueur and Sackur: *Beitr. chem. Physiol. und Pathol.*, **3**, 196 (1903). Van Slyke and Hart: *Am. Chem. Jour.*, **33**, 461 (1905).

² Taking the H^+ concentration at absolute neutrality at 30° as 1.47×10^{-7} ; cf. Kohlrausch and Heydweiller: *Wied. Ann.*, **53**, 209 (1894).

cross-section paper and joined by straight lines. Experimental values of π lying upon each side of its value at neutrality, in each solution, were then also marked off upon the paper and joined by straight lines. The abscissae of the point of intersection of the two straight lines yielded the number of gramme-equivalents of KOH which were bound by the given percentage of casein in the production of an absolutely neutral solution.¹ Dividing the number of gramme-equivalents of KOH neutralized by the casein by the percentage concentration of the casein we obtain the number of gramme-equivalents of KOH neutralized by 1 gramme of casein at absolute neutrality. The following are the results:

Concentration of casein per cent	Gramme-equivalents KOH neutralized by 1 gramme of casein at absolute neutrality at 30°
0.5	52×10^{-5}
1.0 (First series)	50×10^{-5}
1.0 (Second series)	43×10^{-5}
1.5	53×10^{-5}
2.0	54×10^{-5}
3.0	56×10^{-5}
	Av. 51×10^{-5}

The values of the combining-capacities of the casein at neutrality to litmus computed in this manner are seen to be appreciably constant, especially when it is recollected that in solutions so nearly neutral the possible error in the estimation of π is somewhat large (cf. discussion of the experimental errors at the end of the experimental section). The average value is 51×10^{-5} equivalent-gramme-molecules per gramme of casein, which is in excellent agreement with that obtained

¹ At first it was thought necessary to pass the curve $y = ax^3 + bx^2 + cx + d$ through four of the points of the experimental curve $\pi = f(b_1)$ and to determine algebraically the point of intersection of this curve with the curve $y = 0.4107 - 0.0601 \log_{10} x$; this was, however, found to be an unnecessary refinement, as the results obtained did not differ appreciably from those obtained by the above, simpler, method.

by titration (50×10^{-5} equivalent-gramme-molecules per gramme).

The *maximum* combining capacity of casein, in the presence of considerable excess of alkali, is seen to be surprisingly large—in the neighborhood of 180×10^{-5} equivalent-gramme-molecules per gramme. Were only one $-\text{COOH}$ group of the casein molecule concerned, in these solutions, in the neutralization of alkali, the molecular weight of casein would be only 556 which is, of course, having regard to the number of amino-acid groups which must be linked together in the casein molecule, an impossibly low value. Hence, in the presence of excess of alkali, casein must behave as a pluribasic acid.

In this connection it is of interest to refer to the results of Spiro and Pemsel.¹ These observers dissolved casein in excess of alkali, obtaining solutions which, in the light of the results which I have described, must have contained a considerable excess of unneutralized alkali. They then precipitated the caseinate (as they thought, unaltered) by an excess of ammonium sulphate. The precipitate was then filtered off and washed and the quantity of alkali which had been carried down by the casein was determined by titration of the filtrate. The following were their results:

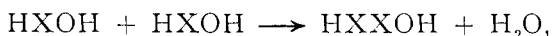
Solution	Mg of NaOH bound by one gramme of casein	Gramme- equivalents of NaOH bound by 1 gramme of casein
1.14 grams casein in 10 cc N/5 NaOH	26.1	65×10^{-5}
1.41 grams casein in 10 cc N/5 NaOH	29.2	73×10^{-5}
1.24 grams casein in 10 cc N/5 NaOH	33.1	82×10^{-5}
1.31 grams casein in 10 cc N/5 NaOH	34.3	86×10^{-5}

Since in all of these solutions the amount of alkali which was actually bound by 1 gram of casein *while* in solution must have been at least 160×10^{-5} gramme equivalents, it is clear that *ammonium sulphate does not precipitate the caseinates*

¹ Spiro and Pemsel: Zeit. phys. Chem., **26**, 233 (1898-9).

in an unaltered condition. The amount of alkali actually carried down by the casein was approximately that which it binds at neutrality to phenolphthalein.

We thus see that while in the presence of excess of alkali 1 gramme of casein will bind about 180×10^{-5} equivalent-gramme-molecules of KOH, in solutions which are neutral to litmus it will only bind 50×10^{-5} equivalent-gramme-molecules of KOH, while, as I have previously shown, in solutions which are "saturated" with casein, that is, solutions which contain only just sufficient alkali to hold the casein in solution, 1 gramme of casein will only bind 11.4×10^{-5} equivalent-gramme-molecules of KOH. This remarkable change in combining-capacity with decreasing alkalinity could only, *since free casein, uncombined with acid or alkali, is insoluble in water*, be achieved in one or both of two ways, namely, (i) by a decrease in the number of —COOH groups which are concerned in the neutralization of the KOH as "saturation" is approached and (ii) by polymerization of the protein through a series of reactions of the type



so that as "saturation" is more and more nearly approached the molecules of casein become heavier and heavier, so that 1 gram of casein necessarily binds less KOH than it could at reactions more distant from that at "saturation."

The relation between m , the amount of alkali neutralized by the casein, and b_1 , the alkalinity of the solution in which the casein was dissolved, is shown graphically, for all of the concentrations of casein employed, in Fig. 2. It will be understood, of course, that the curves only represent this relation for alkalinities of the original solution in excess of that necessary to dissolve all of the casein. For an ordinary acid, forming only one salt with the base, which did not undergo hydrolytic dissociation, the curve would, of course, be a straight line parallel with the axis of x . It will be seen that as the proportion of base to casein declines, the combining capacity of the casein tends to become directly pro-

portional to the concentration of the base, but that as the proportion of base to casein (and the excess of unneutralized base) becomes large, the combining capacity of the casein

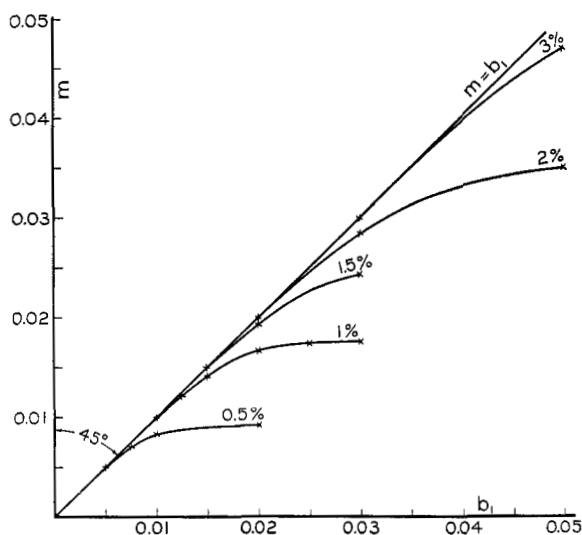


Fig. 2

The ordinates = m = the concentration of KOH neutralized by 0.5%, 1%, 1.5%, 2.0% and 3% casein.

The abscissae = b_1 = the concentration of the KOH solution in which the casein was dissolved.

tends towards constancy, *i. e.*, in comparatively strongly alkaline solutions the behavior of casein approximates more and more to that of an ordinary acid.

(iii) The Relation between λ and b_1

An inspection of the tables in the experimental part of the paper reveals a remarkably simple relation between the alkalinity of a solution of KOH and the depression of its conductivity which results from the addition to it of a given amount of casein. This relation is of the form

$$\lambda \times 10^5 = Ab_1 - Bb_1^2 - D,$$

where A, B and D are constants for a given concentration of casein.

Applying this formula to the results cited in Table IV and determining the constants A, B and D from all of the experimental data by the method of least squares, we find:

$$\lambda \times 10^5 = 26880 b_1 - 475800 b_1^2 - 28.98$$

In the accompanying table the experimental values of $\lambda \times 10^5$ for 1 percent solutions of casein in KOH of various concentrations ($\equiv b_1$) and those calculated from the above formula are compared. In the first column are given the alkalinities of the solutions to which the casein was added ($\equiv b_1$), in the second are given the values of $\lambda \times 10^5$ experimentally ascertained, in the third the calculated values of $\lambda \times 10^5$, in the fourth the difference ($\equiv \Delta$) between the experimental and the calculated values of $\lambda \times 10^5$, and in the fifth the possible metrical error ($\equiv \varepsilon$) in the experimental determination of $\lambda \times 10^5$.

TABLE IX

b_1	$\lambda \times 10^5$ Experimental	$\lambda \times 10^5$ Calculated	Δ	ε
0.03000	348.8	349.3	+0.5	± 8.0
0.02500	343.0	345.6	+2.6	± 6.5
0.02000	322.1	318.3	-3.8	± 5.5
0.01750	299.7	295.9	-3.8	± 5.1
0.01500	268.2	267.2	-1.0	± 4.7
0.01250	230.1	232.8	+2.7	± 4.4
0.01000	186.8	192.3	+5.5	± 4.1
0.00750	141.6	145.8	+4.2	± 4.0
0.00500	92.1	93.5	+1.4	± 3.8
0.00250	43.9	35.4	-8.5	± 3.7
			$\Sigma \Delta = -0.2$	

It will be seen that the deviation of the calculated from the experimental values of $\lambda \times 10^5$ are nearly always less than the possible error, due to instrumental sources alone, in the experimental determination of $\lambda \times 10^5$, while the algebraic sum of these deviations is negligible. The formula therefore represents, in a highly satisfactory manner, the

relation between b_1 and λ for 1 percent solutions of casein in KOH-solutions.

A further inspection of the tables in the experimental part of the paper reveals the fact that the relation between λ and b_1 for *all* of the concentrations of casein investigated can be represented by the more general formula:

$$\lambda \times 10^5 = \alpha b_1 - \frac{\beta}{C} b_1^2 - \gamma C \dots \dots \dots (2)$$

where C is the percentage concentration of casein and α , β and γ are constants, the values of which we have already determined for 1 percent casein. For a 0.5 percent solution of casein this equation, therefore, becomes:

$$\lambda \times 10^5 = 26880 b_1 - 951600 b_1^2 - 14.49$$

in the following table the experimental and calculated values of $\lambda \times 10^5$ are compared—the symbols have the same meaning as in Table IX.

TABLE X

b_1	$\lambda \times 10^5$ Experimental	$\lambda \times 10^5$ Calculated	Δ	ϵ
0.01000	149.9	159.2	+ 9.3	± 2.3
0.00750	123.5	133.6	+ 10.1	± 2.0
0.00500	88.2	96.1	+ 7.9	± 1.8
0.00250	40.6	37.6	— 3.0	± 1.8
0.00150	23.3	24.2	+ 0.9	± 1.8

For a 1.5 percent solution of casein equation (2) becomes:

$$\lambda \times 10^5 = 26880 b_1 - 317200 b_1^2 - 43.47$$

in the following table the experimental and calculated values of $\lambda \times 10^5$ are compared:

TABLE XI

b_1	$\lambda \times 10^5$ Experimental	$\lambda \times 10^5$ Calculated	Δ	ϵ
0.03000	476.4	477.5	+ 1.1	± 7.0
0.02000	366.5	367.3	+ 0.8	± 5.1
0.01500	283.5	288.4	+ 4.9	± 4.5
0.01000	188.5	193.6	+ 5.1	± 4.1
0.00750	145.0	140.4	— 4.6	± 3.9
0.00500	95.4	83.0	— 12.4	± 3.8

For a 2 percent solution of casein equation (2) becomes:

$$\lambda \times 10^5 = 26880 b_1 - 237900 b_1^2 - 57.96$$

in the following table the experimental and calculated values of $\lambda \times 10^5$ are compared:

TABLE XII

b_1	$\lambda \times 10^5$ Experimental	$\lambda \times 10^5$ Calculated	Δ	ϵ
0.05000	661.6	691.4	+29.8	± 18.5
0.03000	539.3	534.4	— 4.9	± 11.5
0.02000	380.5	384.5	+ 4.0	± 7.6
0.01500	286.2	291.7	+ 5.5	± 7.1
0.01000	189.3	187.1	— 2.2	± 6.7
0.00500	95.7	70.5	—25.2	± 6.2

For a 3 percent solution of casein equation (3) becomes:

$$\lambda \times 10^5 = 26880 b_1 - 158600 b_1^2 - 86.94$$

in the following table the experimental and calculated values of $\lambda \times 10^5$ are compared:

TABLE XIII

b_1	$\lambda \times 10^5$ Experimental	$\lambda \times 10^5$ Calculated	Δ	ϵ
0.05000	873.2	860.7	—12.5	± 16.4
0.03000	579.7	576.8	— 2.9	± 9.0
0.02500	488.9	486.0	— 2.9	± 8.4
0.02000	391.7	387.3	— 4.4	± 7.6
0.01500	296.9	280.6	—16.3	± 7.2
0.01000	206.6	166.1	—40.5	± 6.8

(iv) The "Apparent" Equivalent-molecular Conductivity of Potassium Caseinate at "Saturation"

We have seen that the relation between λ , b_1 and the concentration of casein ($\equiv C$) is very accurately represented by the formula:

$$\lambda \times 10^5 = \alpha b_1 - \frac{\beta}{C} b_1^2 - rC$$

Putting, in this equation, $\lambda \times 10^5$ equal to zero we find.

$b_1 = 0.00114$ C or 0.05536 C. Considering, for the present, only the smaller value of b_1 , we see that when $\lambda = 0$, that is, when the change in the conductivity of an alkaline solution which is brought about by dissolving a given percentage of casein therein is zero, then the proportion of alkali to casein is such that 1 gram of casein is combined with 11.4×10^5 equivalent-gramme-molecules of alkali. *This is precisely the combining-capacity of casein at "saturation" of the base with casein, that is, when the base has dissolved the maximum amount of casein which it will dissolve.* The exact coincidence of the two numerical values, especially when we consider that the above is computed by least squares from a large number of determinations which are apparently not connected with estimates of the *solubility* of casein, is surprising, and leaves no room for question that the magnitude of the two quantities is determined by identical factors. This result is probably to be interpreted as follows: in order that λ may be negative it will be seen, referring to equation 1 in the introduction, that the amount of electricity transported by the ions of the caseinate through 1 cm in 1 sec under a potential gradient of 1 volt per cm must be greater than that which would be transported, under the same conditions, by the KOH which is neutralized in the formation of the caseinate. It is hardly to be considered possible that this could be accomplished unless free protein, uncombined with KOH, were present in the solution. But free casein is, as is well known, insoluble in water. Hence the point at which λ changes from positive to negative (*i. e.* = 0) marks the point at which further casein cannot go into solution, and the above data may be regarded as affording confirmation of my previous estimate of the alkali-equivalent of casein at "saturation," namely, 11.4×10^5 equivalent-gramme-molecules per gramme.¹ Conversely, however, we cannot escape the conclusion that the conductivity (and, of course, the "apparent" equivalent-molecular conductivity) of a solution of caseinate in which

¹ T. Brailsford Robertson: Jour. Phys. Chem., **13**, 469 (1909).

the proportion of casein to base is 1 gramme to 11.4×10^{-5} equivalent-gramme-molecules is equal to that of the KOH neutralized by it, that is, at 30° , to about 280×10^{-3} . But, as we have seen, the true equivalent-molecular conductivity of a solution of potassium caseinate, assuming that it dissociates into potassium and casein ions, could not exceed 90×10^{-3} at 30° . The only mechanism, it would appear, by which so high a conductivity of its solution could be attained is that of dissociation of the caseinate into *two or more polyvalent protein ions*. We cannot, I think, on reviewing the evidence contained in the lack of influence of KCl upon the conductivity of solutions of potassium caseinate, in my previous observation that the sum of the velocities of the ions into which ammonium caseinate dissociates is less than that of the ammonium ion itself, in the high conductivity of solutions of the caseinates which approach "saturation," and in the light of the analogy afforded by the behavior of the salts of the proteins with HCl (cf. the results of Bugarszky and Liebermann, cited above) avoid the conclusion that *potassium caseinate does not dissociate potassium ions as such but that the potassium is bound up in complex, probably polyvalent ions*.

In the light of this conclusion, the rapid increase in the "apparent" equivalent-molecular conductivity of the caseinate as "saturation" of the base is approached finds a simple explanation in the supposition that as the combining capacity of casein diminishes its degree of polymerization increases and, consequently, the number of terminal —COO^- and —NH_3^+ groups which may be furnished by 1 molecule is proportionately increased, so that the valencies of the ions into which the salt dissociates also increase. On the assumption, also, that only two such ions are split off by the "neutral" and "basic" caseinates, respectively, my previous results, showing that the Ostwald dilution-law for a binary electrolyte holds good for these solutions are readily understood.

The fact that λ is again zero at high values of b_1 ($= 0.05536$ C, cf. above) is probably to be interpreted simply as meaning that the addition of a given amount of casein to a solution

containing a great excess of alkali does not appreciably affect its conductivity. It is to be observed, however, that this portion of the $\lambda - b_1$ curve was never actually approached in these experiments (since the error in the determination of λ at such high alkalinities is so great as to render the determination of very little value), so that any conclusions concerning it must rest upon a very extended and probably unjustifiable extrapolation from the experimental data.

Conclusions

(1) The "apparent" equivalent-molecular conductivity of potassium caseinate in aqueous solution, that is, the equivalent-molecular conductivity calculated on the assumption that the equivalent-molecular concentration of the caseinate is equal to that of the KOH bound by it, rises somewhat rapidly as the combining capacity of the casein and the alkalinity of its solution decrease.

(2) The numerical value of the "apparent" equivalent-molecular conductivity of potassium caseinate at 30° rises from 80×10^{-3} reciprocal ohms per cc per equivalent-gramme-molecule per liter in solutions containing excess of alkali to that of KOH itself, the latter being determined by extrapolation from the actual observations

(3) The presence of KCl, in the concentrations employed, is without appreciable effect upon the dissociation of potassium caseinate in the solutions investigated.

(4) It is suggested, in explanation of these and previous results, that potassium caseinate does not, when in solution, split off potassium ions, but dissociates into complex polyvalent ions containing the potassium bound up in a non-dissociable form, the sum of the valencies of the positive (or of the negative) ions of the caseinate being greater than the number of molecules of KOH bound up in 1 molecule of caseinate.

(5) The combining capacity of casein (determined by gas-chain measurements) tends to become proportional to the concentration of the alkali in which it is dissolved, at low

alkalinities, but at high alkalinities it tends to approach a constant maximum value.

(6) The maximum value of the combining capacity of casein is about 180×10^{-5} equivalent-gramme-molecules per gram.

(7) It is pointed out that ammonium sulphate does not precipitate the caseinates of the alkalis in an unaltered condition.

(8) The combining capacity of casein at neutrality to litmus (*i. e.*, at absolute neutrality) was found to be independent of the total concentration of the system and its average value (6 determinations) was found to be 51×10^{-5} equivalent-gramme-molecules per gramme. This agrees well with the value which other observers have found by titration (50×10^{-5}).

(9) The depression in the conductivity of a solution of KOH (at 30°) ($\equiv \lambda$) which is brought about by the introduction of a given percentage of casein ($\equiv C$) is connected with its alkalinity ($\equiv b_1$) by the equation:

$$\lambda \times 10^5 = 2680 b_1 - \frac{475800}{C} b_1^2 - 28.98 C$$

(10) The depression in the conductivity of a solution of KOH which is brought about by the introduction of a given percentage of casein is zero when the solution contains just sufficient KOH (11.4×10^{-5} equivalent-gramme-molecules per gramme) to hold the casein in solution.