ON THE INFLUENCE OF TEMPERATURE UPON THE
SOLUBILITY OF CASEIN IN ALKALINE SOLUTIONS.

BY T. BRAILSFORD ROBERTSON.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

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In previous papers I have suggested that a solution of a protein
may be regarded as a system of polymeric modifications of the
amphoteric electrolyte HXOH, the point of equilibrium being
shifted by any variation in the conditions, such as the addition
of acid, alkali, salts or the application of heat, which influences
the concentrations of the protein ions; just as the simplest pos-
sible amphoteric electrolyte, namely, water, consists of a mix-
ture of polymeric modifications of the molecule HOH, the point
of equilibrium being shifted by alterations in temperature. From
this point of view the process of heat-coagulation would be
regarded somewhat as follows; by repeated condensations of the
type

\[ HXOH + HXOH \rightarrow HXXOH + H_2O \]

larger and larger molecule-complexes are formed until the molecu-
lar aggregates assume the properties of matter in mass and the
solution assumes the character of a suspension which is usually
unstable, the protein particles being thrown out of solution in the
form of coagula or flocculi. If this point of view be correct then
it follows that one of the effects of applying heat to a protein
solution must be the shifting of an equilibrium of the type:

\[ HXOH + HXOH \rightleftharpoons HXXOH + H_2O \]
towards the right; were this so it would follow from van't Hoff’s
"principle of mobile equilibrium" that the hydrolysis of pro-

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teins is accompanied by the evolution of heat, which conclusion is in complete accord with experimental observation in so far as positive results have been obtained.\(^1\) The hypothesis is furthermore in accord with the view that heat-coagulation is accompanied by the withdrawal of water from the protein.\(^2\) Views in many respects similar to these have been expressed by a number of authors. Mann, indeed, states that in his opinion heat-coagulation is "brought about by one portion of the albumin molecule precipitating the remainder," a view which is essentially similar to that expressed above.\(^3\) In this connection it is also of interest to note that many authors have considered that the initial stages of protein hydrolysis consist in the "depolymerization" of the protein molecule.\(^4\) Sutherland has also pointed out that the weight of different proteins which combines with a gram equivalent of a heavy metal is a simple multiple of the lowest observed weight and he deduces therefrom that there is a large amount of "internal salt" formation in proteins; he has also expressed the view, practically identical with that put forward above, that coagulation of a protein is the result of polymerization through the neutralization of "valencies which are usually latent."\(^5\)

If the heat-coagulation of a protein consists in the polymerization of the amphoteric protein molecule with the elimination of water, according to the equation given above, then the influence of heat upon a compound of a protein with a base should be of the following type:

\[
\text{NaXOH} + \text{NaXOH} \rightarrow \text{NaXXOH} + \text{NaOH}
\]

\(^1\) The heat of reaction of protein hydrolysis is extremely small, observers have either failed to detect any change in the heat-content of the system or else have observed a very slight disengagement of heat. Tangl: Arch. f. d. ges. Physiol., cxv, p. 1, 1906; v. Lengyel: Ibid., p. 7; Hari: Ibid., p. 11, Ibid., cxxi, p. 459, 1908.


and the solution of the compound should become more alkaline on heating. The marked increase in the alkalinity of solutions of the caseinates of bases, which occurs on heating, has been observed by Osborne, but his interpretation of the phenomenon is quite different to that which is suggested above. He considers that the action of heat consists in increasing the hydrolytic dissociation of the caseinate. Since the free casein is very insoluble and, presumably, very slightly dissociated, an increase in the degree of hydrolytic dissociation of the salt would lead to an increase in the alkalinity of the solution, and furthermore, might be reasonably expected to lead to a marked opalescence of the solution or even to the precipitation of casein, since the free casein is insoluble in water. A marked increase in opalescence, on heating to 35° to 45° C. was observed by Osborne in solutions of calcium, barium, magnesium and lithium caseinates but it was not observed in solutions of sodium, potassium or ammonium caseinates; if the opalescence were due to undissociated casein being set free by hydrolytic dissociation it is difficult to see why it does not occur in solutions of sodium and potassium caseinate and especially in solutions of ammonium caseinate in which, as Osborne himself points out, since the ammonium hydroxide is a very weak base, hydrolysis might be expected to be especially intense. It is, however, possible to decide between the two hypotheses in a very simple way. I have shown in a previous paper that a given amount of alkali dissolves just sufficient casein to form the "neutral caseinate" of the base (such that 8 cc. of \( \frac{1}{10} \) alkali = 1 gram of casein) and that the resulting solution is neutral to litmus. If the influence of the application of heat upon this solution consisted in increasing the hydrolytic dissociation of the caseinate it would follow that the power of the given amount of alkali to bind casein is diminished by heat and therefore the solubility of casein in a given concentration of alkali would be diminished by an increase in temperature; on the contrary if the influence of the application of heat upon a solution of a neutral caseinate consists in the shifting of the equilibrium of the system in the direction:

\[
\text{NaXOH} + \text{NaXOH} \rightarrow \text{NaXXOH} + \text{NaOH}
\]

The influence of temperature upon solubility of casein then the alkali set free should be capable of dissolving more casein, or in other words, the solubility of casein in a given concentration of alkali would be increased by an increase in temperature. From either hypothesis the increase in the electrical conductivity of the solution upon heating, which was observed by Osborne, would necessarily follow. The following experiments were undertaken with a view to ascertaining which of the alternative hypotheses represents the facts more accurately.

EXPERIMENTAL.

It has been pointed out by Osborne, in the paper referred to above, that the influence of temperature upon solutions of caseinates is reversible, i.e., that the opalescence and increase in alkalinity which appear on heating disappear on cooling and reappear on heating again. This is probably true for all heat coagulations but where the protein is thrown down in coagula the hysteresis of the system (owing to the excessive internal molecular friction of large aggregates) prevents its reattaining equilibrium with a measurable velocity on cooling. That the equilibrium characteristic of low temperatures is rapidly regained if heat-coagulation is not pushed too far, so that the molecular aggregates which are formed are not too large, has been shown by Corin and Ansiaux who find that the first traces of coagulation disappear on quickly cooling and shaking the solution. We may, therefore, assume that a solution of a caseinate which has been heated regains, on cooling, its original condition and power of neutralizing bases. The method of procedure was as follows. Five, ten etc., cc. of \( \frac{N}{10} \) potassium or lithium hydrate or of a saturated solution of calcium hydrate (approximately \( \frac{N}{7} \)), were diluted to 100 cc. with distilled water, placed in tightly stoppered Erlenmeyer flasks and warmed in a thermostat to the desired temperature. Three times the amount of casein which would be dissolved by the given amount of alkali at room temperature (i.e., 3 grams to every 5 cc. of \( \frac{N}{10} \) alkali) was then introduced and the mixture left in the thermostat for from thirty to forty minutes, being vigorously shaken at frequent intervals. The resulting solution was then filtered in the thermostat (the filter and the receiving vessel having been previously warmed to the desired

1 Corin and Ansiaux. *Bull. de l'Acad. roy. de Belg.*, No. 21.
temperature) and the filtrate was allowed to cool or was cooled by immersing the containing vessel in tap-water. The temperatures were defined to within ±. An aliquot part (25 cc.) of the solution was then titrated against the alkali which had been used to dissolve the casein, phenolphthalein being used as indicator. Since 8 cc. of \( \frac{1}{10} \) alkali neutralize one gram of casein to phenolphthalein if the amount of alkali originally contained in the volume of solution titrated is known and the amount which it is necessary to add in order to secure neutrality to phenolphthalein is ascertained the amount of casein contained in the solution can immediately be deduced from the relation

\[ 1 \text{ cc.} \times 70 \text{ alkali} = \text{gram of casein}. \]

In a previous paper I have shown that this method of determination yields reliable results.\(^*\)

The following were the results obtained:

<table>
<thead>
<tr>
<th>Concentration of the alkaline solution</th>
<th>Grams of casein dissolved per 100 cc. solution at—</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21°</td>
</tr>
<tr>
<td>23×10^{-4} N KOH</td>
<td>0.46</td>
</tr>
<tr>
<td>46×10^{-4} &quot;</td>
<td>0.92</td>
</tr>
<tr>
<td>60×10^{-4} &quot;</td>
<td>1.38</td>
</tr>
<tr>
<td>92×10^{-4} &quot;</td>
<td>1.85</td>
</tr>
<tr>
<td>44×10^{-4} LiOH</td>
<td>0.89</td>
</tr>
<tr>
<td>88×10^{-4} &quot;</td>
<td>1.77</td>
</tr>
<tr>
<td>45×10^{-4} Ca(OH)_2</td>
<td>0.90</td>
</tr>
<tr>
<td>90×10^{-4} &quot;</td>
<td>1.80</td>
</tr>
</tbody>
</table>

It is evident that the power of the bases, potassium hydroxide and lithium hydroxide, to dissolve casein is greatly increased by increasing temperature. In all cases, the solutions, at the temperatures indicated, were acid to phenolphthalein and approximately neutral to litmus so that the effect of heating a solution of a caseinate cannot be to increase its hydrolytic dissociation. The facts are much more readily explained on the supposition that the effect of temperature consists in shifting the equilibrium:

\[ HXOH + HXOH \rightleftharpoons HXXOH + H_2O \]


\(^2\) T. Brailsford Robertson: This Journal, ii, p. 317, 1907.
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towards the right so that a given amount of alkali, since it is
associated with a molecule of nearly double the weight, neutral-
izes nearly twice as much casein at 66° as it does at room tem-
perature (21°). The marked diminution in the solubility of
casein in calcium hydrate solutions which occurs on raising the
temperature can be explained by supposing that the salt
Ca(XXOH), is insoluble while the salt Ca(XOH), is soluble, and
this explains also why Osborne obtained an increase in opal-
escence upon heating solutions of calcium, magnesium and ba-
rium caseinates but not upon heating solutions of potassium,
sodium and ammonium caseinates. Since calcium hydrate
solutions do not fail, at any of the temperatures investigated, to
dissolve some casein it is evident that the conversion of the casein
molecule HXOH into double, triple or higher polymers cannot
be complete but that an equilibrium between the two forms exists
at every temperature. Since calcium hydrate solutions of dif-
ferent concentrations dissolve different amounts of casein at the
same temperature (as can be deduced by interpolation from the
above table) the amount which is dissolved by any giver con-
centration of calcium hydrate cannot represent, merely, satu-
ration of the solution with the salt Ca(XXOH), or Ca(XXXOH),
but must represent also the solution of the calcium salt of unpoly-
merized molecules in equilibrium with a saturated solution of the
calcium salt of the polymerized molecules. It appears probable
in the light of these results, that the view which I have pre-
viously expressed is the correct one, namely, that the influence
of heat upon proteins consists, among other effects, in shifting
the equilibrium among the polymeric modifications of the am-
photeric protein molecule in the direction of higher complexes.

An alternative hypothesis, which would cover the above facts,
is that casein acts a dibasic acid and that at room temperatures
salts of the type Na₂X(OH)₂ are formed while at higher tempera-
tures acid salts of the type NaHX(OH)₂ are formed. The fact that
solutions of both the neutral and “basic” caseinates obey Ostwalt’s
dilution-law for a salt of a monobasic acid, however, excludes
this possibility.¹ A possible source of error in the above experi-

¹ T. Brailsford Robertson: *Journ of Physical Chem.*, xi, p. 542, 1907;
mental determinations may be mentioned. Solutions of the neutral caseinates undergo fairly rapid auto-hydrolysis, about one-third being hydrolyzed in twelve hours at 37°. This effect would of course be negligible in the short period during which the solutions are being prepared, but at higher temperatures the velocity of hydrolysis would probably be increased and this might conceivably vitiate the accuracy of the titrations. Special determinations made with a view to estimating the magnitude of the error thus introduced showed that it was nearly inappreciable. Thus 100 cc. of \( 46 \times 10^{-4} \text{ N KOH} \) at 88° dissolves 1.13 gram of casein, the solution having been allowed to stand in the thermostat for one-half hour. After three hours in the thermostat the titration indicated that 1.25 gram had been dissolved. The error at 88° in half an hour would therefore be, in this solution, about .04 gram and at lower temperatures and in more dilute solutions it must, of course, be considerably less. It may here be noted that in none of the solutions in which more casein was dissolved at higher temperatures than would be dissolved at room temperature was any appreciable tendency towards precipitation of casein on cooling observed, although in many cases there was a marked increase in the opalescence of the solution. This is, however, not surprising since an appreciable amount of acid may be added to a solution of alkali "saturated" with casein at room-temperature before precipitation occurs. Such solutions are possibly to be regarded as being "super-saturated" with casein and in a condition of unstable equilibrium.

CONCLUSIONS.

(1) The solubility of the casein in alkaline solutions is considerably augmented by carrying out the process of solution at temperatures above 40° C.

(2) It is pointed out that this fact is not in harmony with the view that a rise in temperature increases the degree of hydrolytic dissociation of solutions of the caseinates.

(3) In explanation of this fact and of the increase in alkalinity and electrical conductivity of caseinate solutions upon heating,

1 T. Brailsford Robertson: This Journal, ii, p. 317, 1907.
which were observed by Osborne, it is suggested that the influence of heat upon proteins consists, among other effects, in shifting equilibria of the type:

\[ \text{HXOII} + \text{HXOII} \rightleftharpoons \text{XXXOII} + \text{II}_2\text{O} \]

in the direction of higher complexes, and that heat-coagulation is a result of repeated condensations of this type.

(4) The solubility of casein in solutions of various concentrations of potassium hydroxide, lithium hydroxide and calcium hydroxide at various temperatures has been determined.
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